Department of Biochemical Engineering & Biotechnology

Indian Institute of Technology, Delhi

BE – 314P (Bioprocess Engineering Laboratory)

Experiment –8

OBJECTIVE: To study the cell growth in lactic acid fermentation under controlled pH and to compute $Y_{x/s}$, $Y_{p/s}$ and product in the 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 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 cell begins to die.

The cell growth in lactic acid fermentation is inhibited by the product lactic acid (un-dissociated form). With the on set of exponential phase, pH begins to go down with time due to lactic acid production. At very low pH, the cell growth rate ceases. Therefore it is essential to control the pH in lactic acid fermentation.

MATERIALS & METHODS:

Microorganism: *Lactobacillus rhamnosus*

Shaker: at 40^0 C and 80 rpm for the growth of inoculum in seed medium.
Inoculum Culture age: 18-24h

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

a)	Yeast Extract	3.0	g/l
b)	K_2HPO_4	0.2	g/l
c)	KH_2PO_4	0.2	g/l
d)	Na_2SO_4	2.0	g/l
e)	Sodium Succinate	2.0	g/l
e)	Glucose	10.0	g/l
f)	$MgSO_4.7H_2O$	0.3	g/l
g)	$MnSO_4.H_2O$	0.03	g/l
h)	$FeSO_4.7H_2O$	0.03	g/l

Medium Preparation & sterilization:

Stock solutions of Glucose, $MnSO_4.H_2O$, $MgSO_4.7H_2O$ and $FeSO_4.7H_2O$ are prepared in distilled water (having the maximum micro salinity of 100 and pH 6.6 – 6.9).

Glucose	20 % (w/v)
$MgSO_4.7H_2O$	10 % (w/v)
$FeSO_4.7H_20$	1 % (w/v)
$MnSO_4.H_2O$	10 % (w/v)

The stock solutions are sterilized separately and added to the fermentor containing sterile media under aseptic conditions.

Stock %age denotes the amount of respective component in and volume make upto 100ml by distilled water.

Stock solutions of Glucose, $MgSO_4$ and $MnSO_4$ are sterilized separately in an autoclave at 15psi for 20 minutes. The $FeSO_4$ stock is filter sterilized in a sterile test tube, in order to prevent its oxidation at higher temperature.

Quantities of media components required for the working volume are dissolved in distilled water, contained in conical flask, one after the other in the order of KH_2PO_4, K_2HPO_4, Na_2SO_4, Sodium Succinate and Yeast Extract. Now the flasks' contents are transferred to the fermentor containing some distilled water. The volume of media initially kept in the fermentor prior to sterilization is equal to the working volume required minus the volume of the stock solutions that are to be added and the inoculum volume.

Fermentor is sterilized at 121^0 C for 25minutes. After the sterilization cycle is over i.e. heating, holding and cooling of the fermentor is done, respective glucose and salts stock solutions are added to it through a transfer bottle.

The medium volume in the fermentor is adjusted finally with sterilized distilled water through a transfer bottle just before inoculation. Now the volume in the fermentor should be equal to the working volume minus the inoculum volume.

Fermentor operating conditions & inoculation:

Operating temperature	= 40^oC
pH	= 6.2 (controlled by 5N NaOH)
Run time duration	= 12h
Expected max $OD_{600, 1\ cm}$	= 1.4
Agitation	= 400
Dissolved oxygen (DO)	= < 1.0 % (maintained by N_2 sparging)

Before inoculation set operating conditions as above

Inoculate the 3L fermentor (@2% of 2.2 L) with 44 ml culture from 20 -24h old inoculum (given). (Total medium volume in the 3L fermentor=2.2L)

OBSERVATIONS:

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

RESULTS: Plot OD, pH, glucose and specific growth rate vs time. Also plot specific growth arte vs pH. Compute $Y_{x/s}$, $Y_{p/s}$ and production of lactic acid.

DISCUSSION: Write a discussion on the results