9.3.3. Fed-Batch Operation

What is a Fed-Batch Operation?

A Variable-Volume Semi-Batch Operation with Feed but Withdrawal only at the End

A Semi-Batch Operation in Which the Feed Containing Sources of Carbon, Nitrogen, and Others are Fed either Intermittently or Continuously During the Course of Otherwise a Batch Operation

Why a Fed-Batch Operation?

- To Overcome Substrate Inhibition
- To Overcome Glucose Effect
- To Overcome Catabolite Repression
- To Utilize Auxotrophs
- To Achieve High Cell and Metabolite Density
- To Extent Operational Period
- To Alleviate High Broth Viscosity
- To Make Up for Water Loss by Evaporation

To Increase Reaction Rates and/or Yields

and Overcome Physical Difficulties

Products Using Fed-Batch Operation

- Antibiotics
- Baker`s Yeast
- Enzymes
- Microbial Cells
- Natural Lipids
- Nucleotides
- Organic Acids
- r-DNA Products
- Solvents
- Vitamins
- Yeasts

Specific Growth Rate

$$\mu = \frac{1}{XV}\frac{d(XV)}{dt}$$

- Batch $$\mu = \frac{1}{X}\frac{dX}{dt}$$

- Fed-Batch $$\mu = \frac{1}{X}\frac{dX}{dt} + \frac{1}{V}\frac{dV}{dt}$$

Growth Yield

$$Y = \frac{\Delta X}{\Delta S}$$

$$= -\frac{dX}{dS}$$

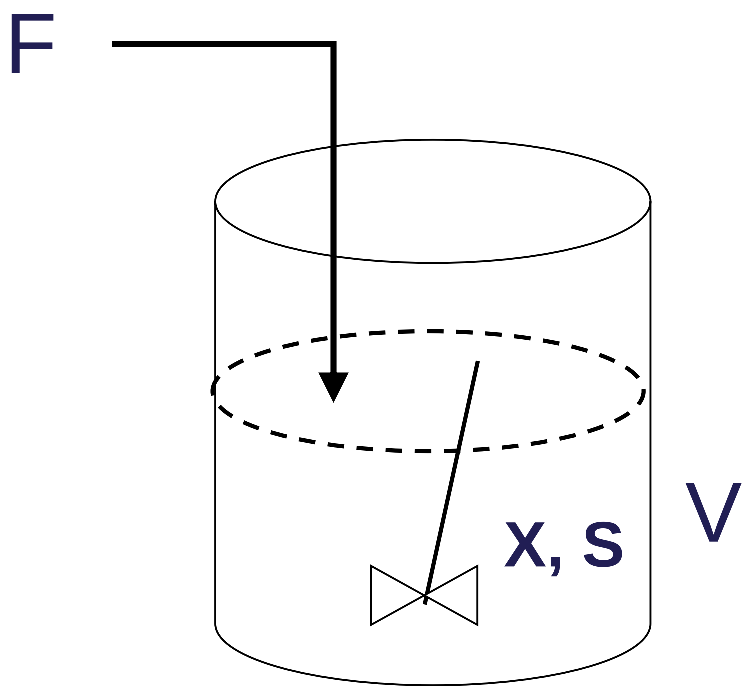
Mass Balance

Acc = In – Out + Gen – Con

Accumulation rate = Input rate – Output rate + Generation rate – Consumption rate

$$\frac{d(\quad)}{dt} =$$

Fed-Batch Operation



F (L/h) : Flow Rate
V (L) : Working Volume
X (g/L) : Cell Concentration
S (g/L) : Substrate Concentration

Mass Balances

- cell

$$\frac{d(XV)}{dt} = \mu XV -----(1)$$

- substrate

$$\frac{d(SV)}{dt} = S_F F - \frac{1}{Y}\mu XV ---(2)$$

- Total

$$\frac{dV}{dt} = F ------(3)$$

Determination of Feed Rate
(using mass balance equations)

- cell

$$\frac{d(XV)}{dt} = \mu XV \rightarrow \frac{dX}{dt} = (\mu - \frac{F}{V})X -----(1)$$

- substrate

$$\frac{d(SV)}{dt} = S_F F - \frac{1}{Y}\mu XV \rightarrow \frac{dS}{dt} = (S_F - S)\frac{F}{V} - \frac{\mu X}{Y} ---(2)$$

- Total $\frac{dV}{dt} = F ------(3)$

With $\frac{dS}{dt} = 0$

(2) $\longrightarrow$ $F = \frac{\mu X}{Y}\frac{V}{S_F - S}$ ....(4)

Determination of Feed Rate

(using mass balance equations)

- cell

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

$$XV = X_0 V_0 e^{\mu t} \text{.......(5)}$$

(4) → $$F = \frac{\mu X}{Y}\frac{V}{S_F - S} = \frac{\mu X_0 V_0}{Y(S_F - S)} e^{\mu t}$$

↑ (5)

E. coli Fed-Batch Operation with Exponential Feeding

Productivity (g product/L hr)

- **Productivity**

$$Pd = \frac{\frac{CellMass}{Volume}(X) \times SpecificExpression(Ps)}{CultureTime}$$

- **Necessary condition**
  - **High cell density culture**
  - **High specific expression**

To Maximize Productivity

- **High cell density culture**
  - **By-product: acetic acid**
    - **Synthetic media: $\mu = 0.35\ hr^{-1}$**
    - **Complex media: $\mu = 0.2\ hr^{-1}$**
- **High specific expression**
  - **Expression vs. specific growth rate**
    - **Low μ, high product**
  - **Proteolytic degradation**

Feed Flow Rate Control in Fed-Batch Operation

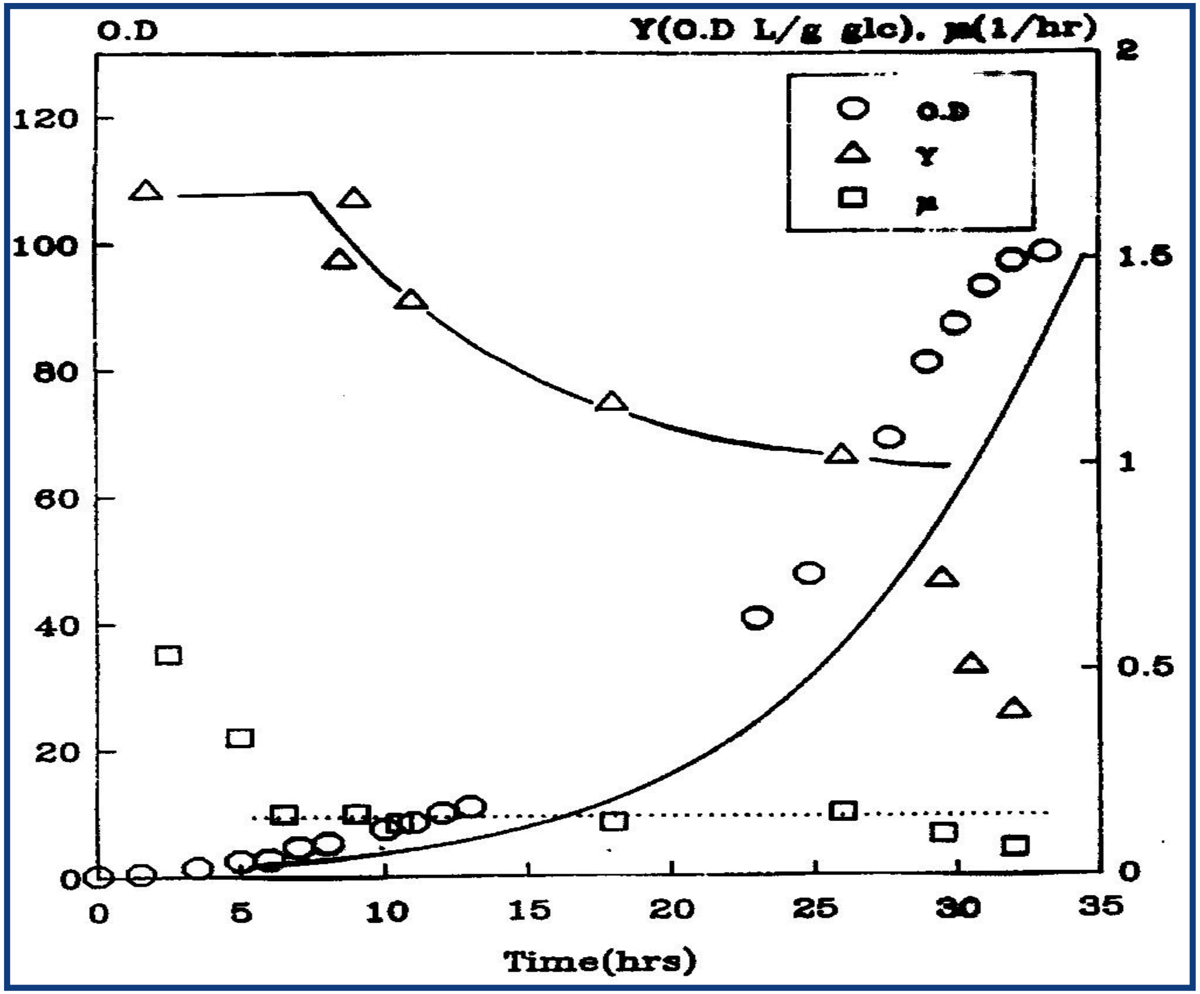
- **Feedback Control**
- **Feed Forward Control**
  - **μ↑, acetic acid ↑, growth↓, expression ↓**

$$\mu = \frac{\mu_m S}{Ks + S}$$

Exponential Feeding

$$F = \frac{X_0 \times V_0 \times \mu \times e^{\mu t}}{S_F \times Y}$$

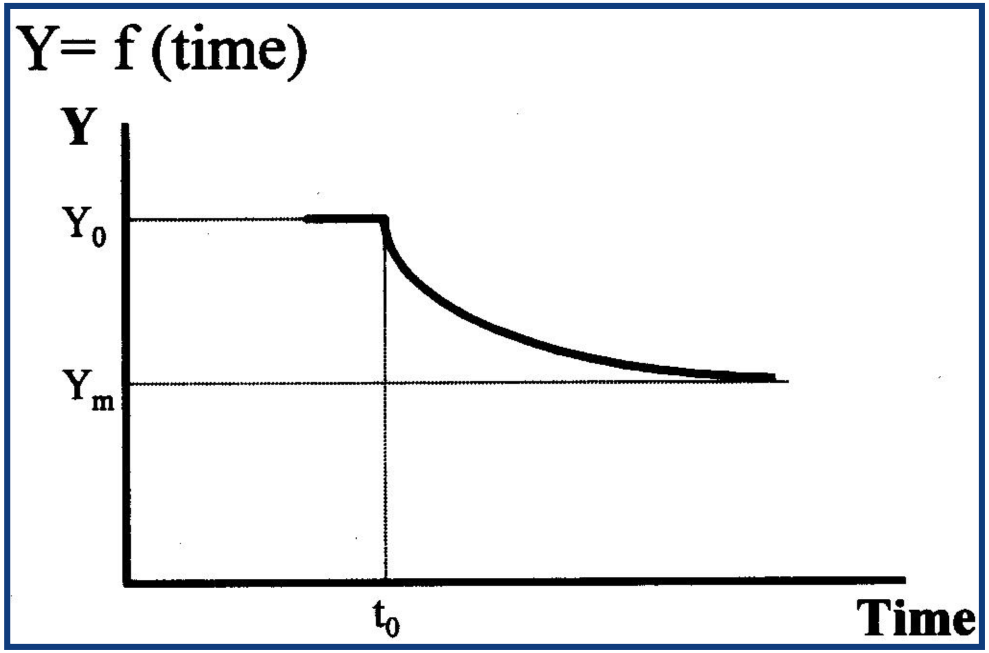
Fed-Batch Operation



Growth Yield

Yield

Time Variable



Growth Yield

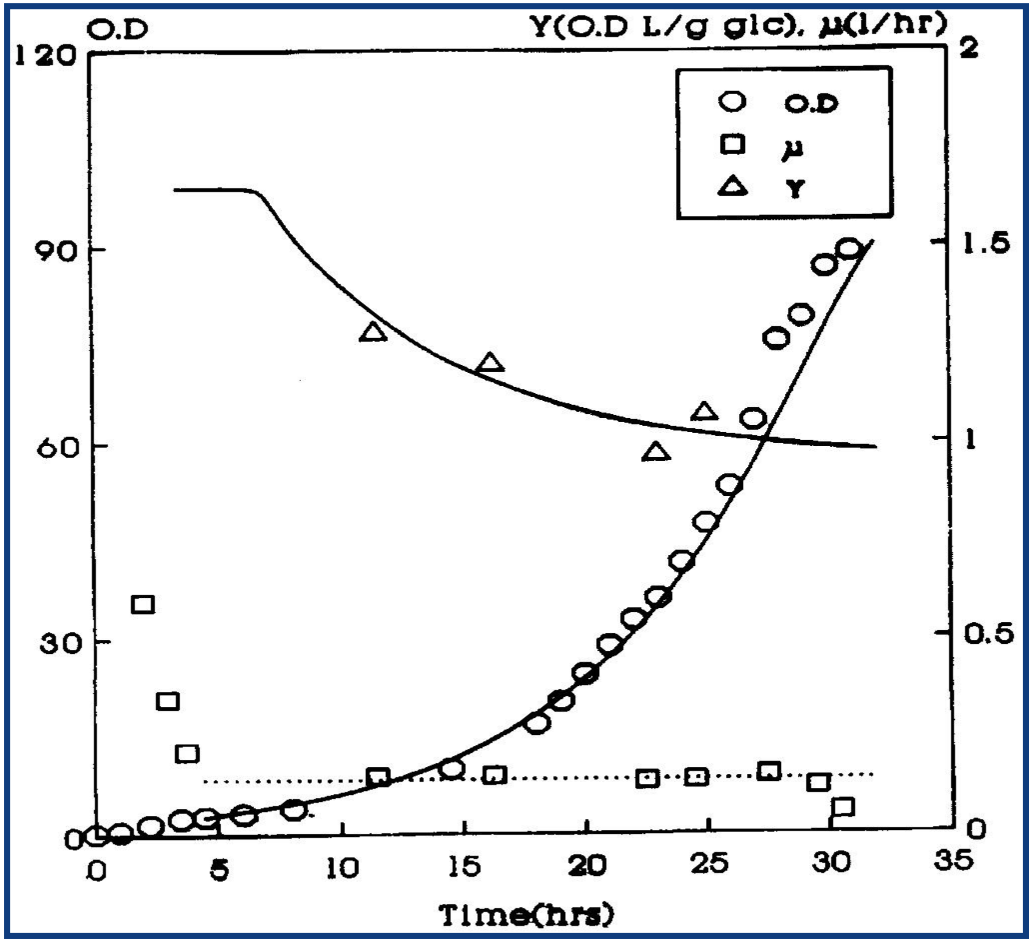
Yield

Time Variable

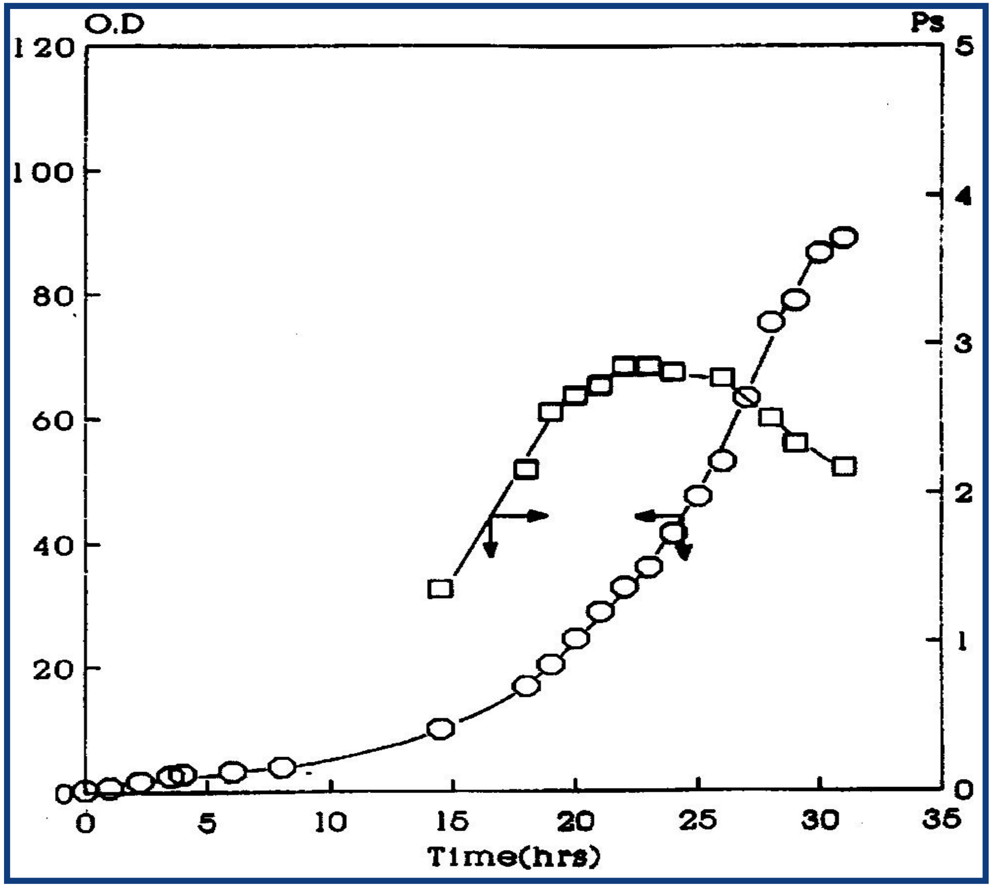
$$\frac{dY}{dt} = \frac{1}{ty}(Ym - Y), t = t_0, Y = Y_0$$

$$Y = 0.7 Exp \ \{-(t - 8)/8\} + 0.95$$

Fed-Batch Operation



Fed-Batch Operation



Why does the specific expression rate decrease with the increase of cell density?

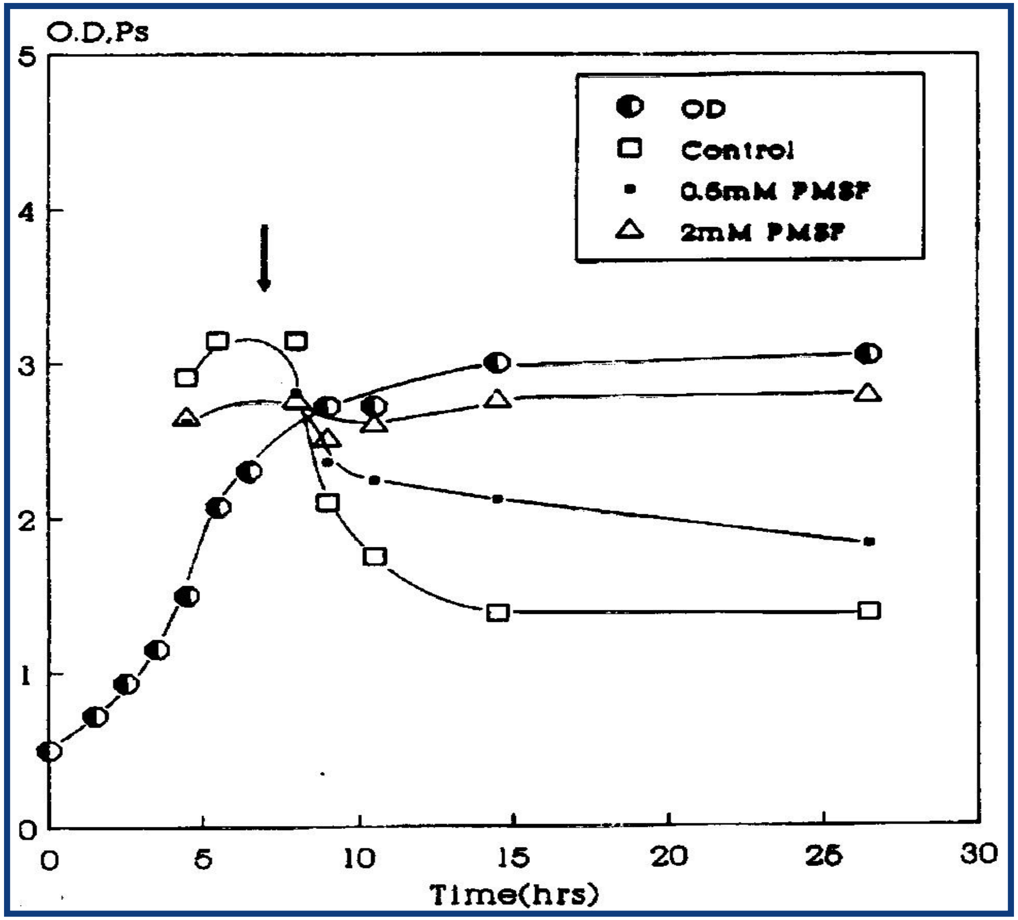
1. Po2 limitation
2. Glucose accumulation
3. Acetic acid accumulation
4. N source limitation
5. P source limitation
6. $MgSO_4$ limitation
7. Plasmid instability
8. Degradation by protease

1–3 → Low μ, Slow feeding, Pure oxygen

4–5 → Ammonium phosphate feeding

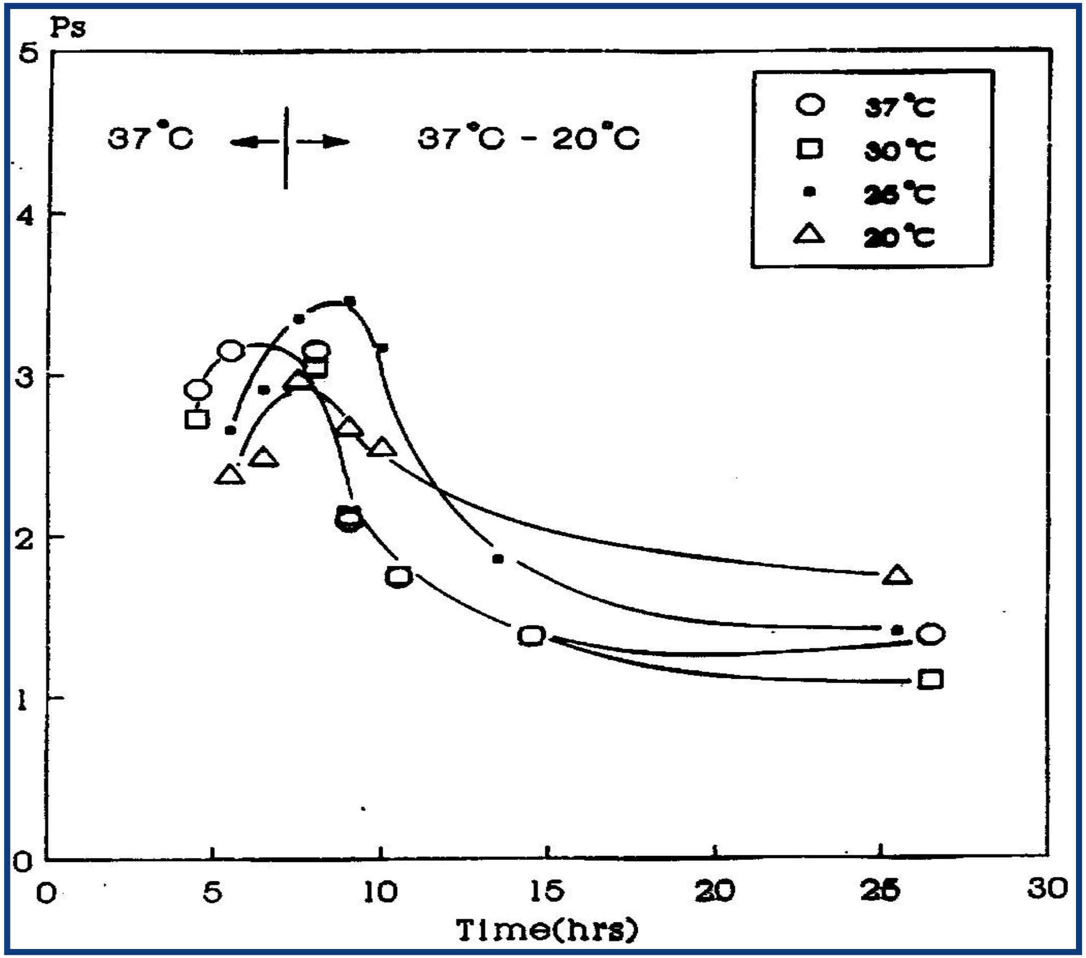
6 → $MgSO_4$ feeding

Effect of PMSF on Protein Degradation.



PMSF (Phenyl Methane Sulfonyl Fluoride)

Effect of Culture Temperature on Protein Degradation



Product Degradation by Protease

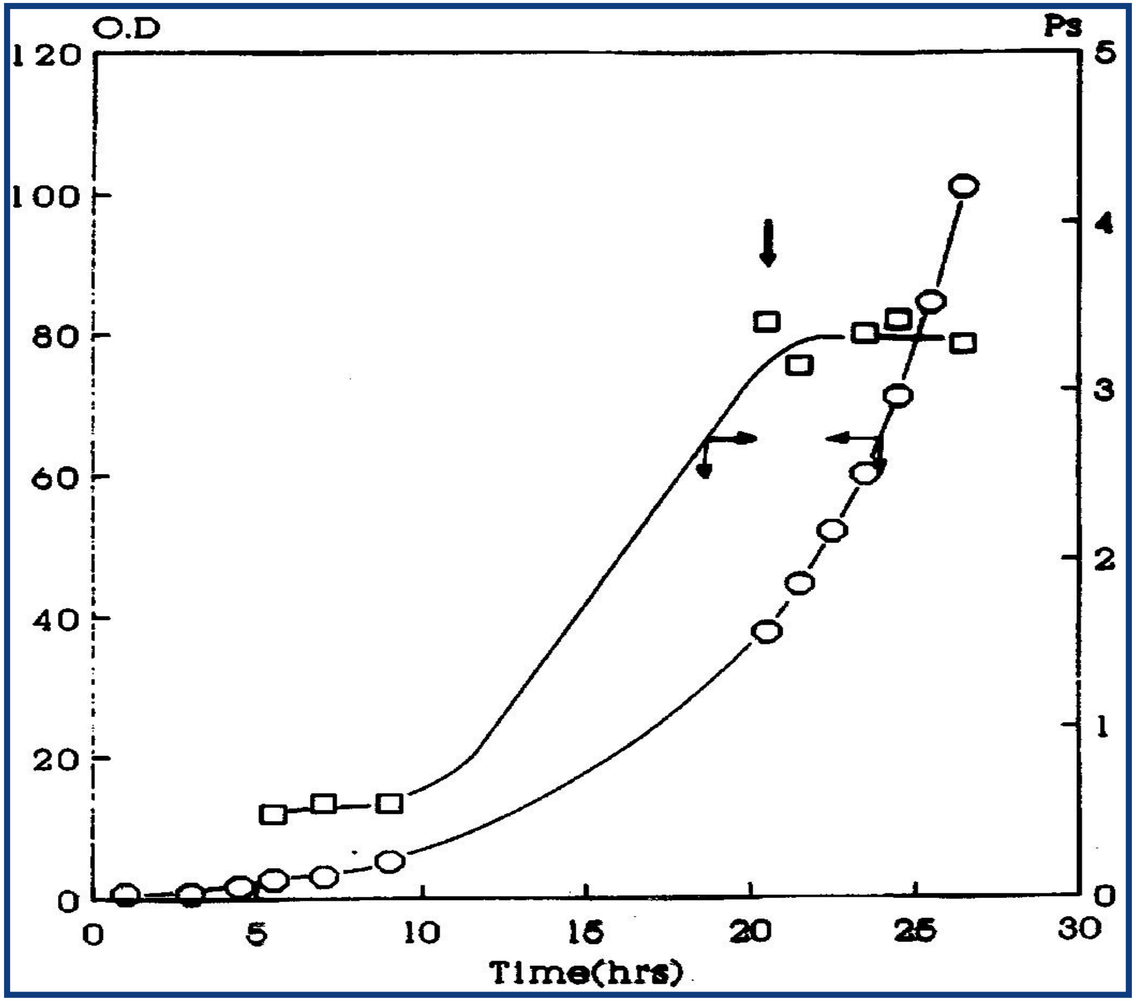
- Protease is induced under stressful conditions

{ Depletion of Carbon or Nitrogen source
High Temperature
Expose to ethanol, UV

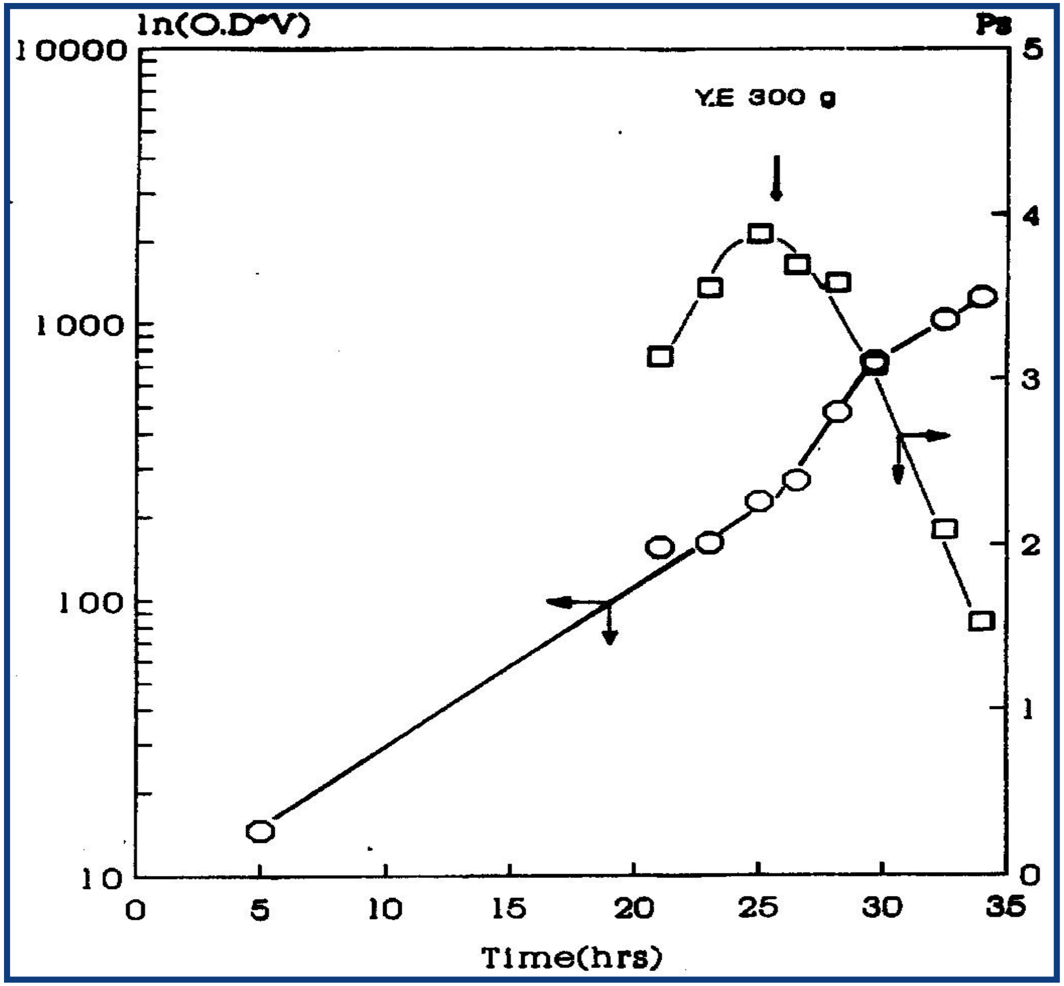
Composition of Modified Medium

Component	Concentration		
	Starting medium	Feeding medium 1	Feeding medium 2
Yeast Extract (g/L)	1.0	-	100
KH_2PO_4 (g/L)	15.0	-	-
$MgSO_4 \cdot 7H_2O$ (g/L)	4.0	-	15.5
Glucose	2.0	400	400
$FeSO_4 \cdot 7H_2O$ (mg/L)	-	200	200
$CaCl_2 \cdot 2H_2O$ (mg/L)	-	1300	1300
$MnSO_4 \cdot 5H_2O$ (mg/L)	-	67	67
$CoCl_2 \cdot 6H_2O$ (mg/L)	-	13	13
$ZnSO_4 \cdot 7H_2O$ (mg/L)	-	67	67
$CuCl_2 \cdot 5H_2O$ (mg/L)	-	13	13
$Na_2MoO_4 \cdot 2H_2O$ (mg/L)	-	13	13

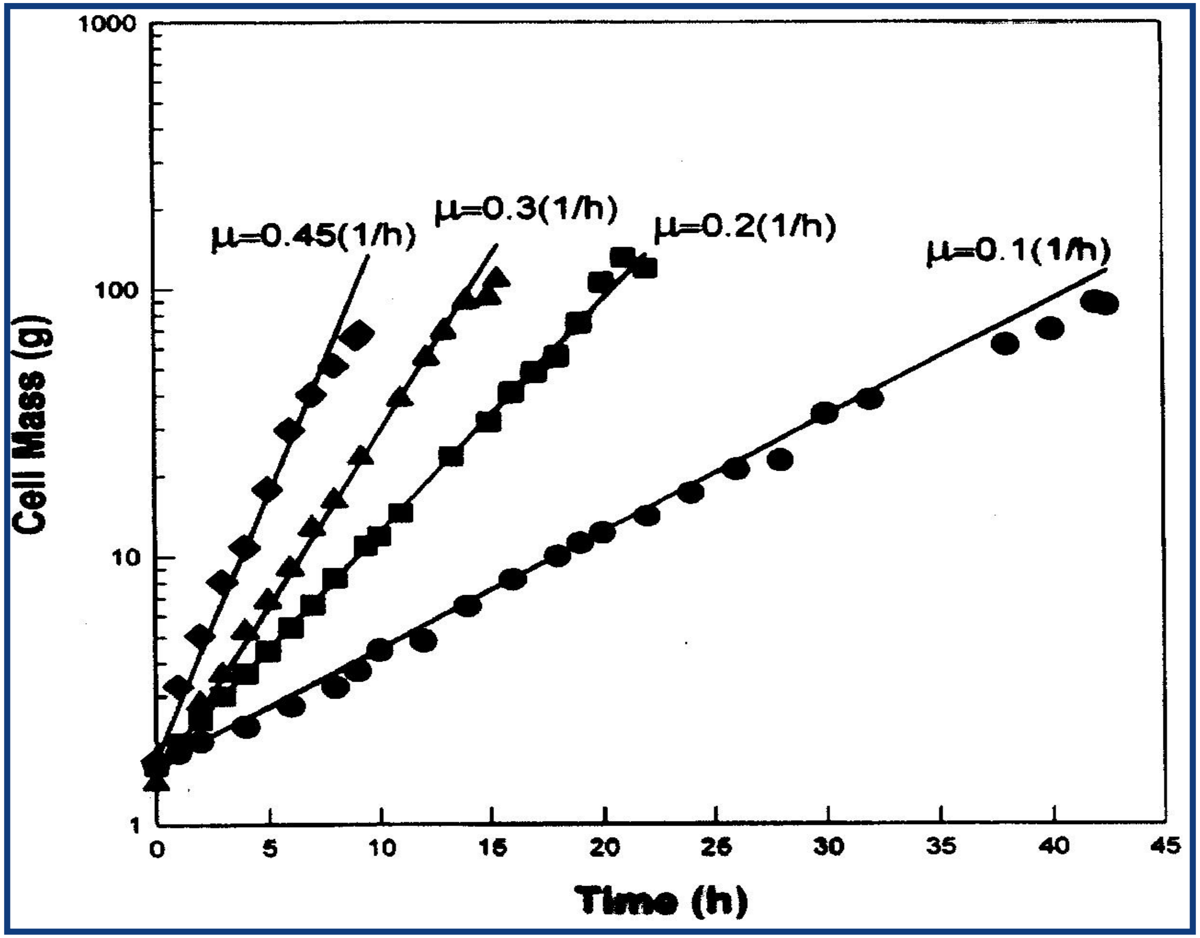
Fed-Batch Operation with YE



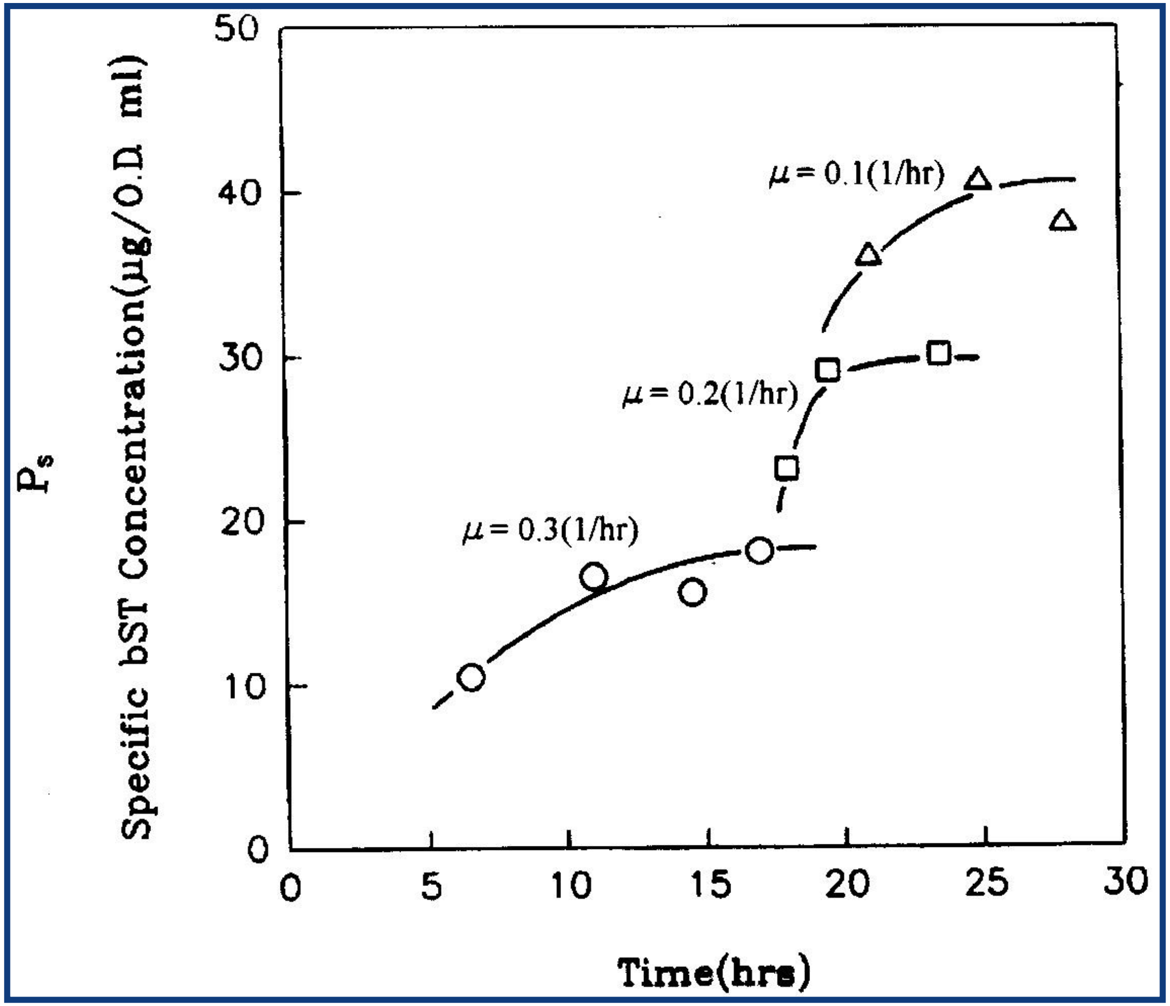
Fed-Batch Operation with YE



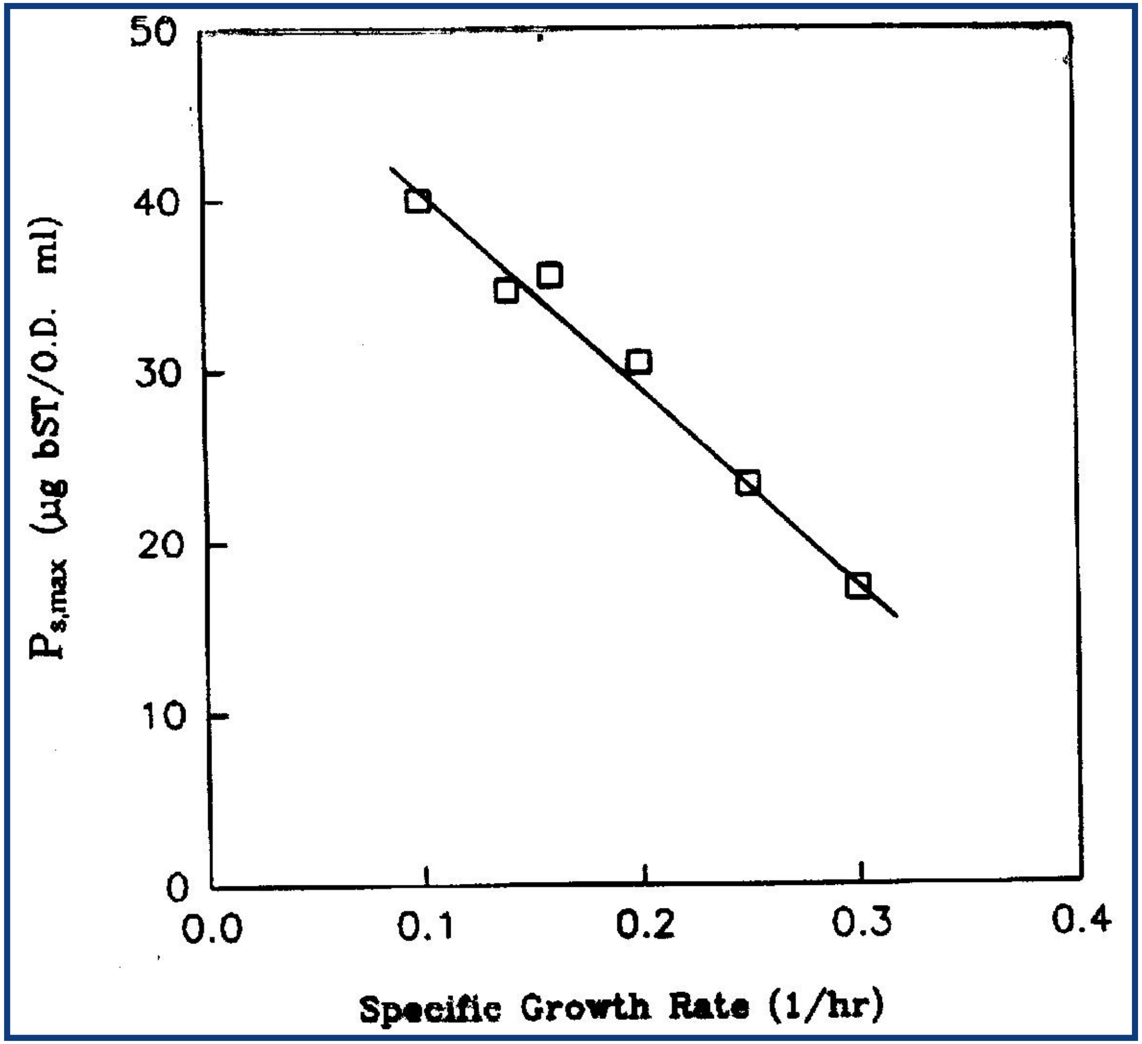
Fed-Batch Operation with Controlled Specific Growth Rate



Fed-Batch Operation with Controlled Specific Growth Rate



Effect of Specific Growth Rate on Expression



Productivity Maximization

$P_{s,max}$ = -13.2μ + 5.96

$$Pd = \frac{XP_{s,\max}}{t}$$

$$[\ XV = X_0V_0e^{\mu t} \longrightarrow t = \frac{1}{\mu}\ln(\frac{XV}{X_0V_0})]$$

$$= \frac{X\mu P_{s,\max}}{\ln(\frac{XV}{X_0V_0})}$$

- *Maximization of Pd*

$\longrightarrow$ *Maximization of* $(\mu P_{s,max})$

$$\mu P_{s,max} = -13.2\mu^2 + 5.96\mu$$

Optimum μ = 0.23 (1/hr)

Optimum Specific Growth Rate for Maximum Productivity