[Case Study]

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

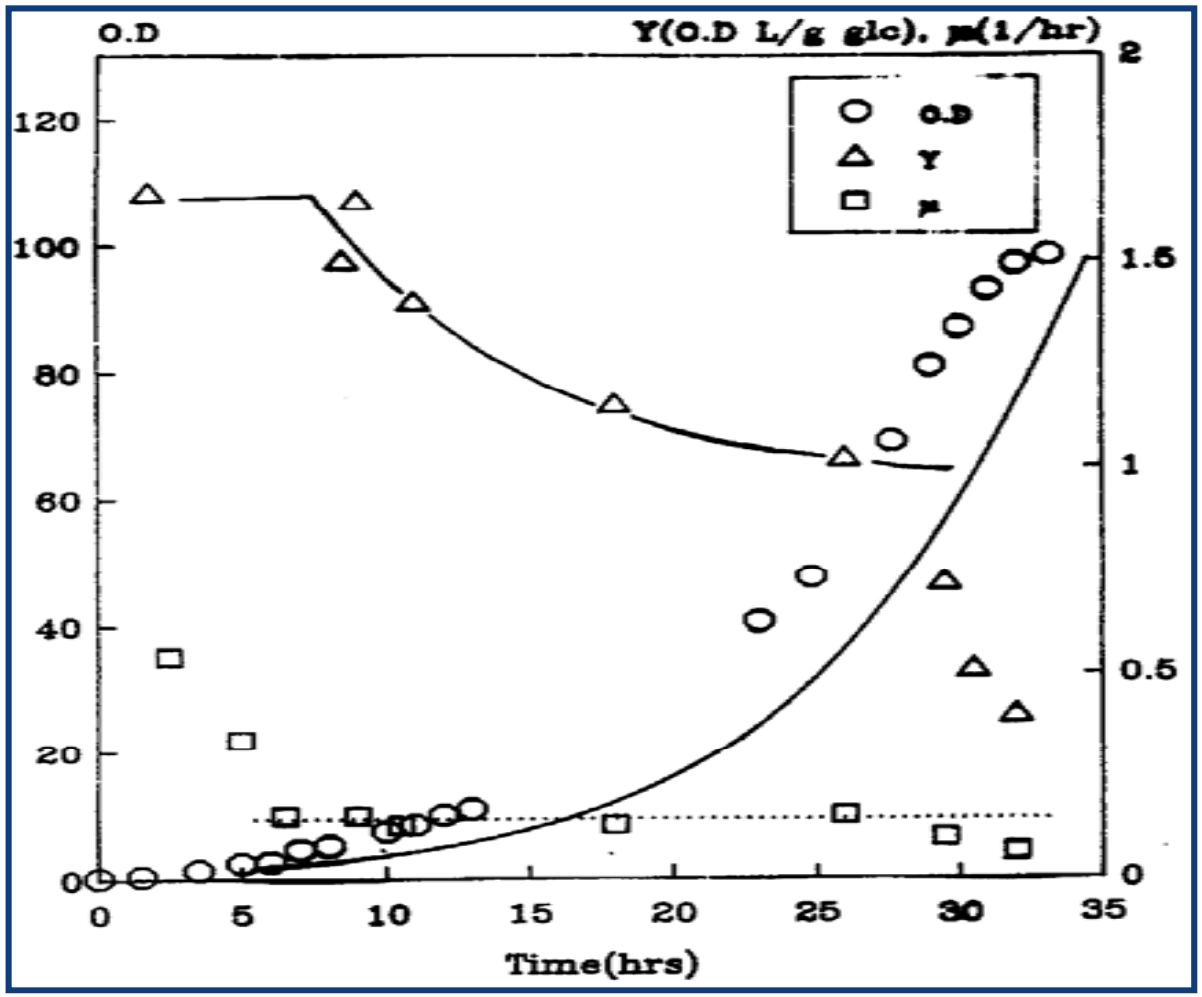
- **Feedback Control**
- **Feed Forward Control**
  - **μ↑, acetic acid ↑, growth↓, expression ↓**

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

Exponential Feeding

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

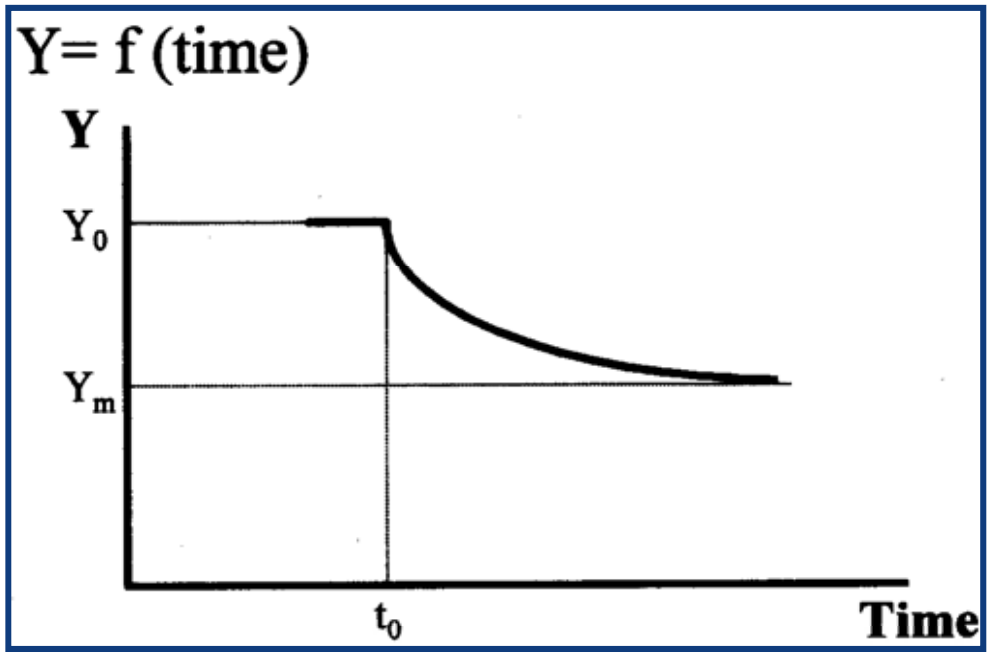
Fed-Batch Operation



Growth Yield

Yield

Time Variable



Growth Yield

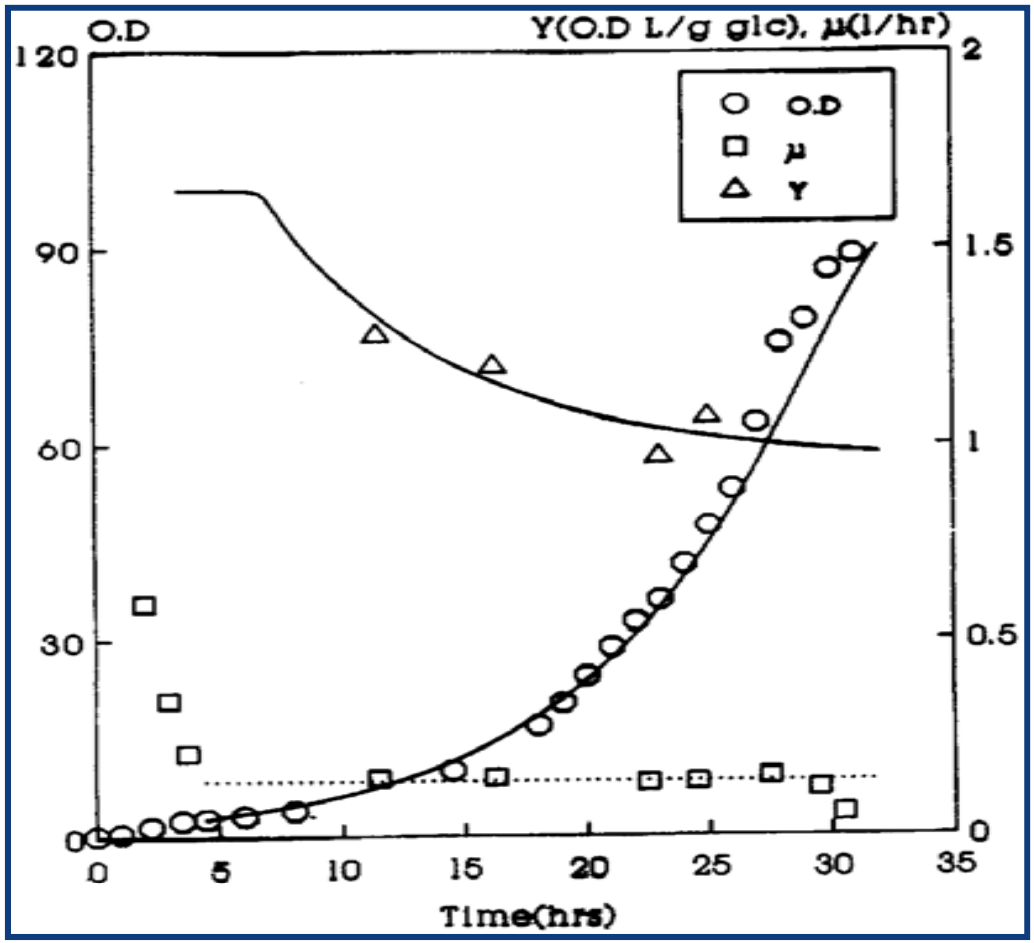
Yield

Time Variable

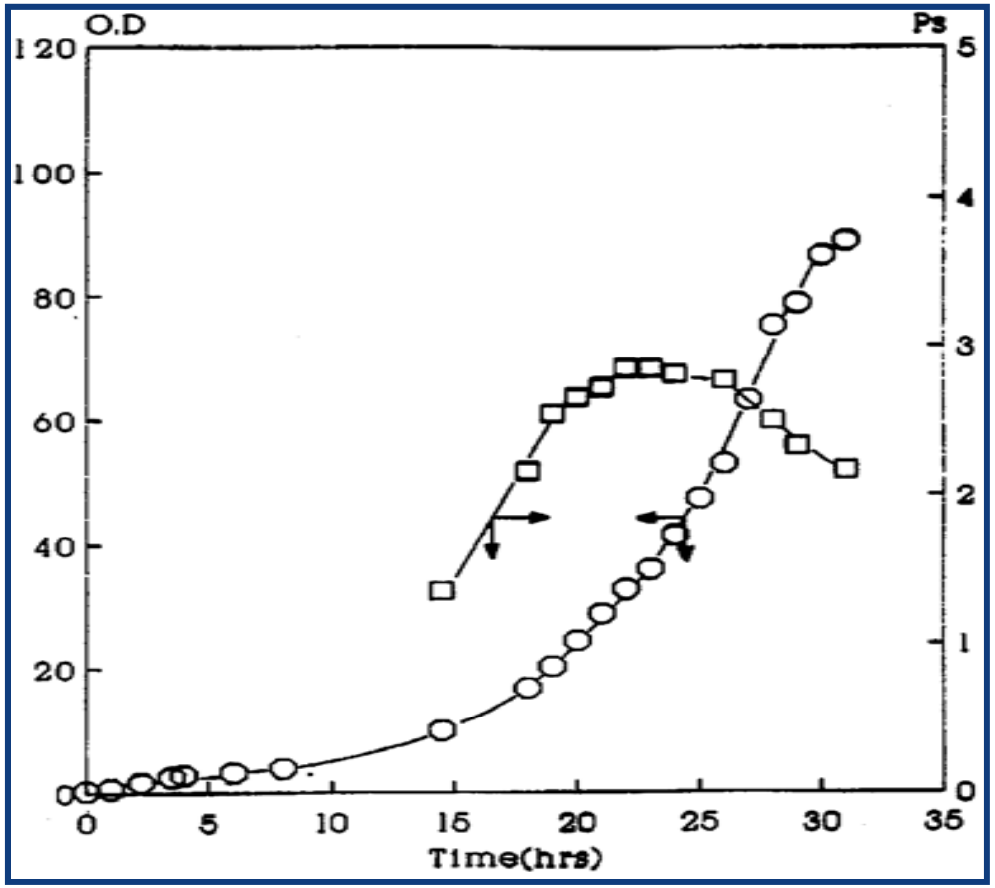
$$\frac{dY}{dt} = \frac{1}{ty}(Ym - Y), t = t_0, Y = Y_0$$

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

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

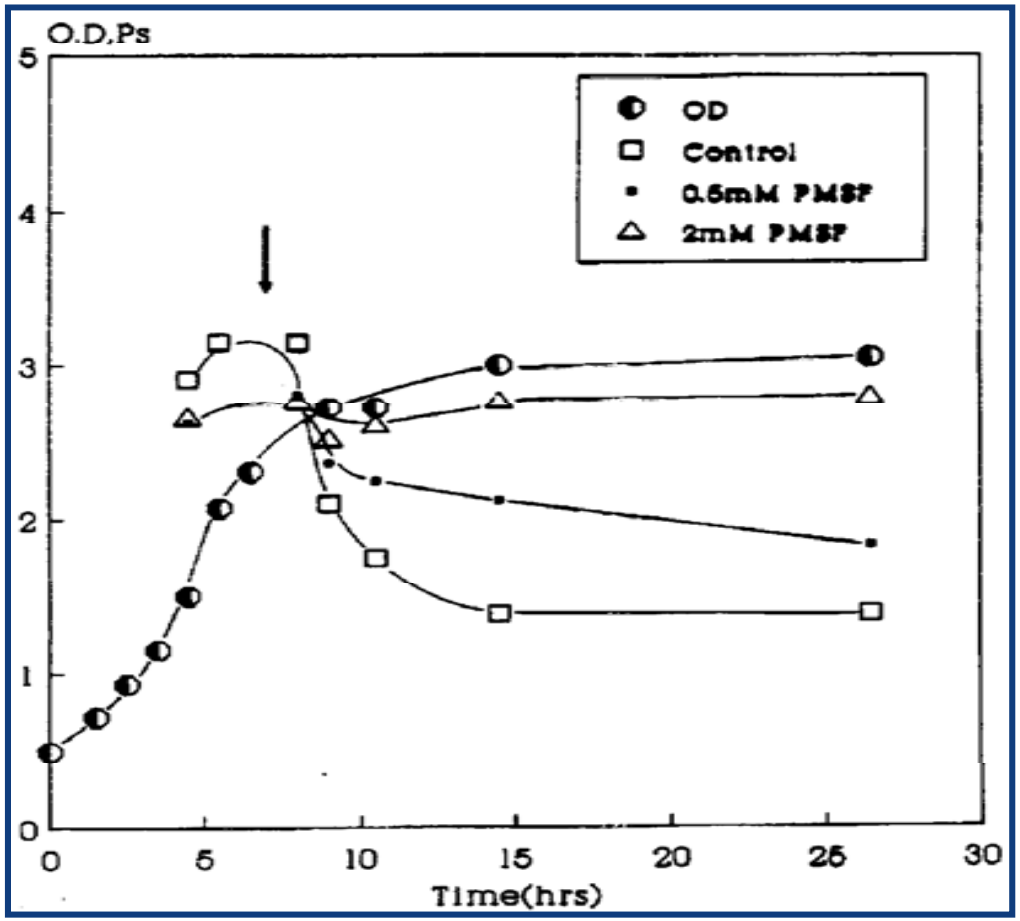
} Low μ, Slow feeding, Pure oxygen

4. N source limitation
5. P source limitation

} Ammonium phosphate feeding

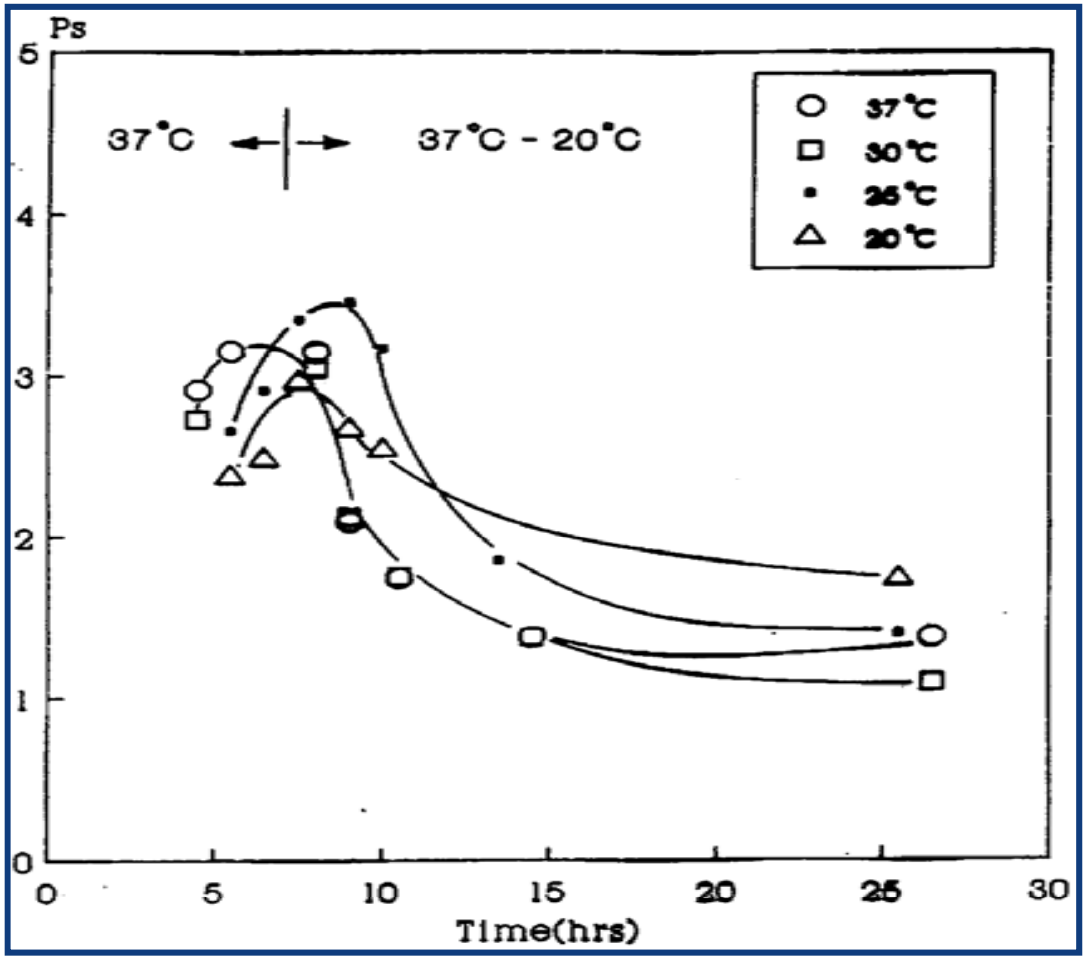
6. $MgSO_4$ limitation → $MgSO_4$ feeding
7. Plasmid instability
8. Degradation by protease

Effect of PMSF on Protein Degradation.



PMSF (Phenyl Methane Sulfonyl Fluoride)

Effect of Culture Temperature on Protein Degradation



Product Degradation by Protease

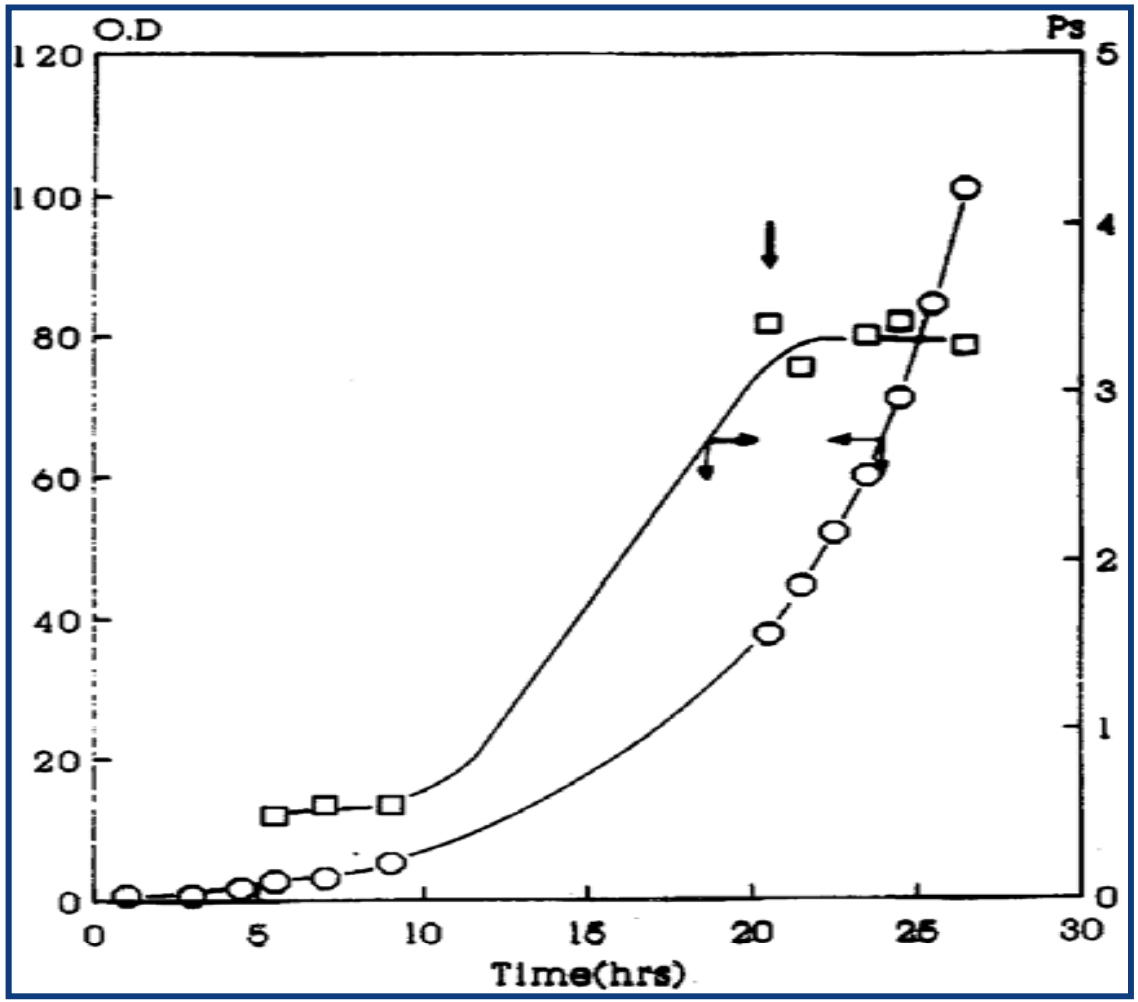
- Protease is induced under stressful conditions

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

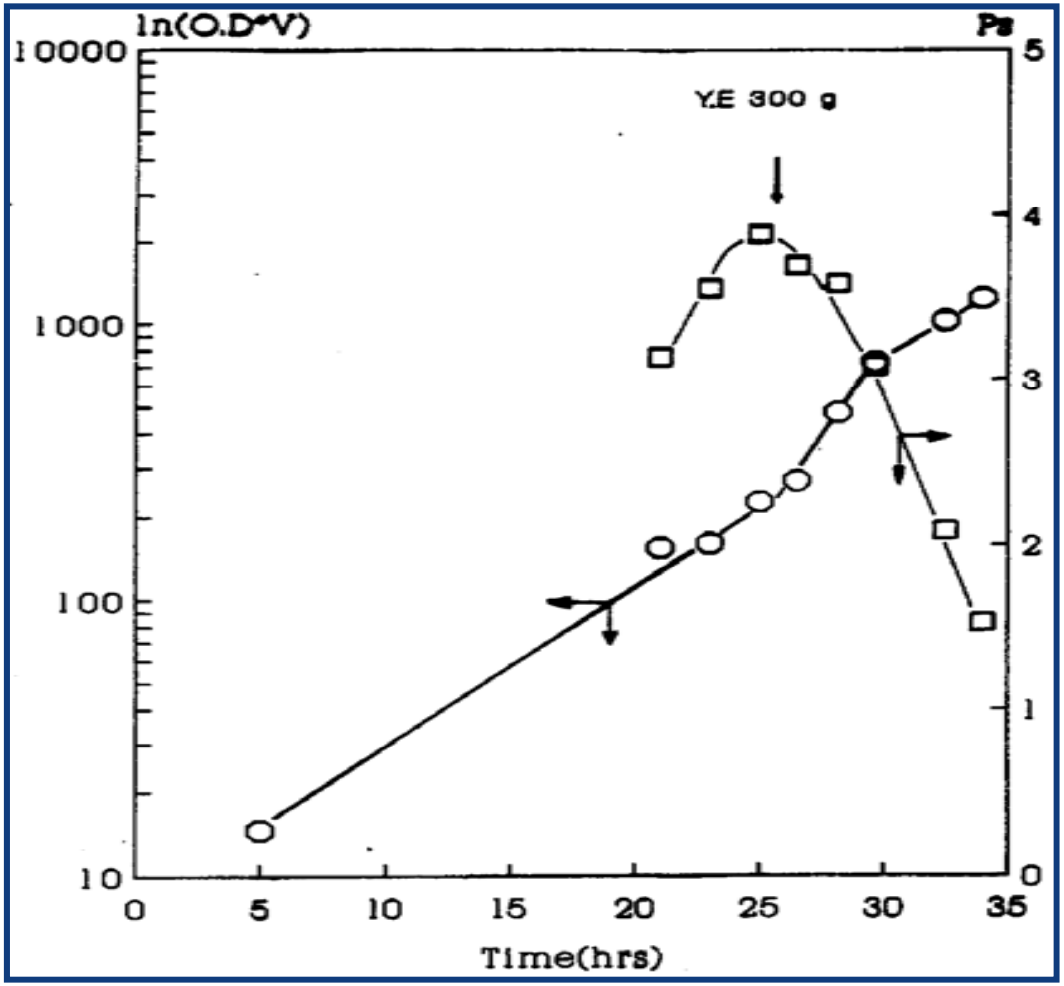
Composition of Modified Medium

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

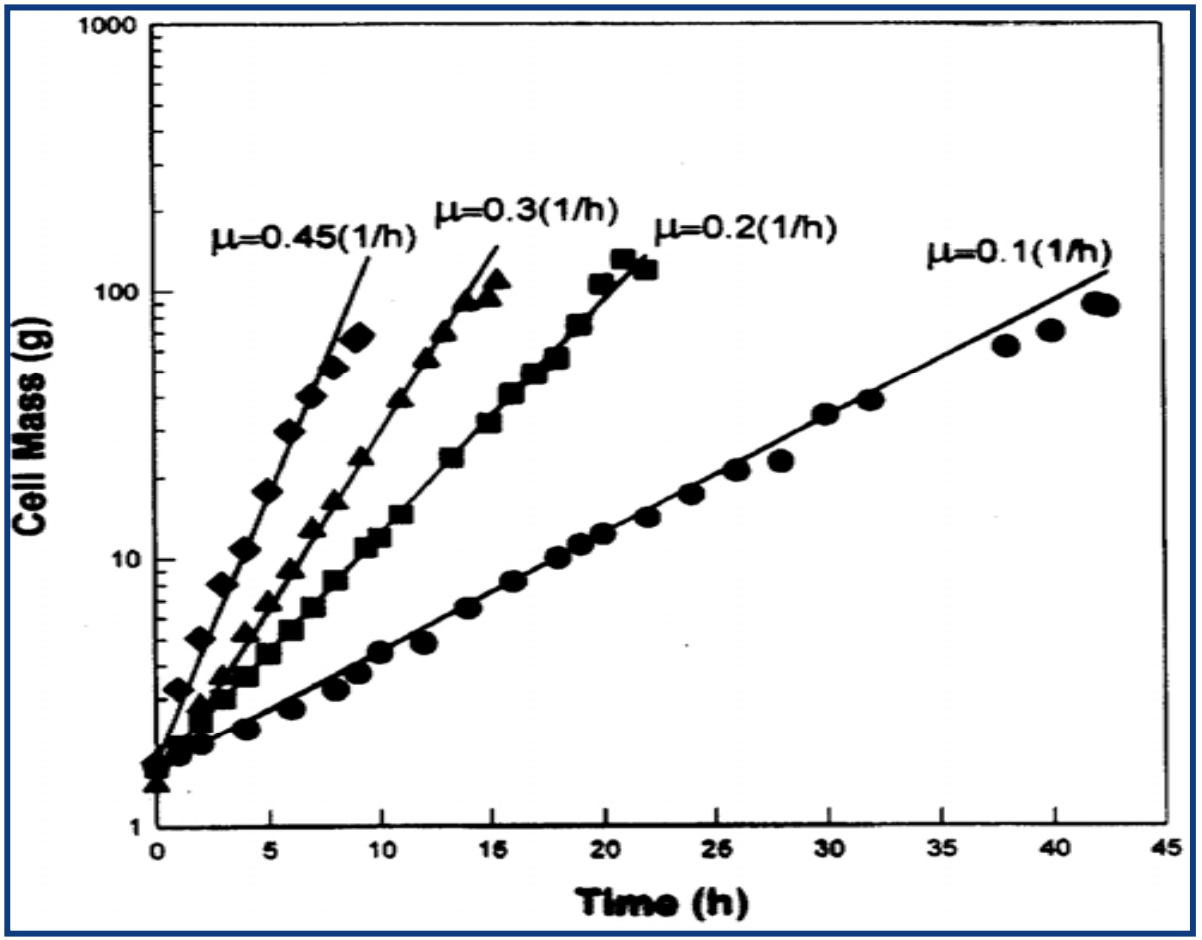
Fed-Batch Operation with YE



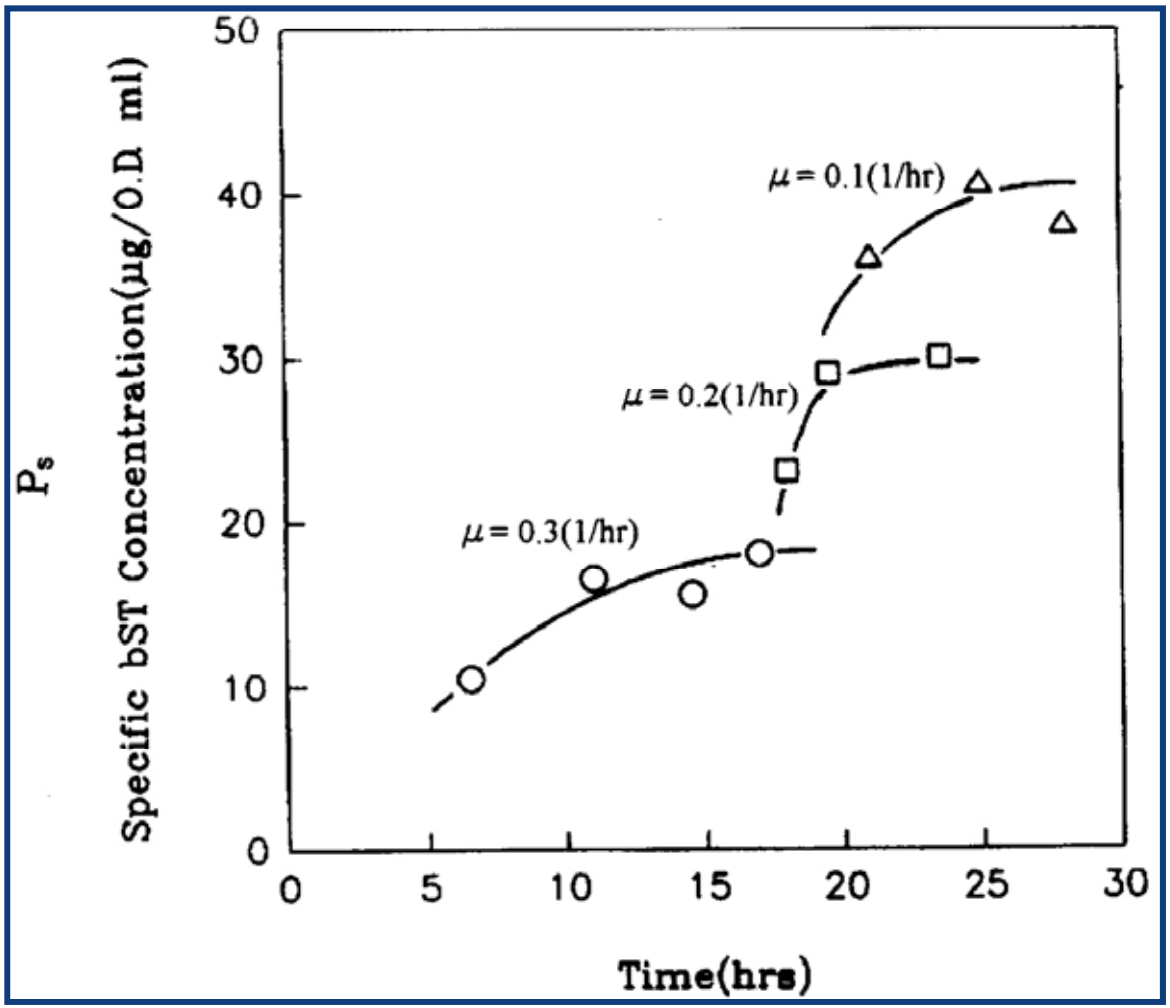
Fed-Batch Operation with YE



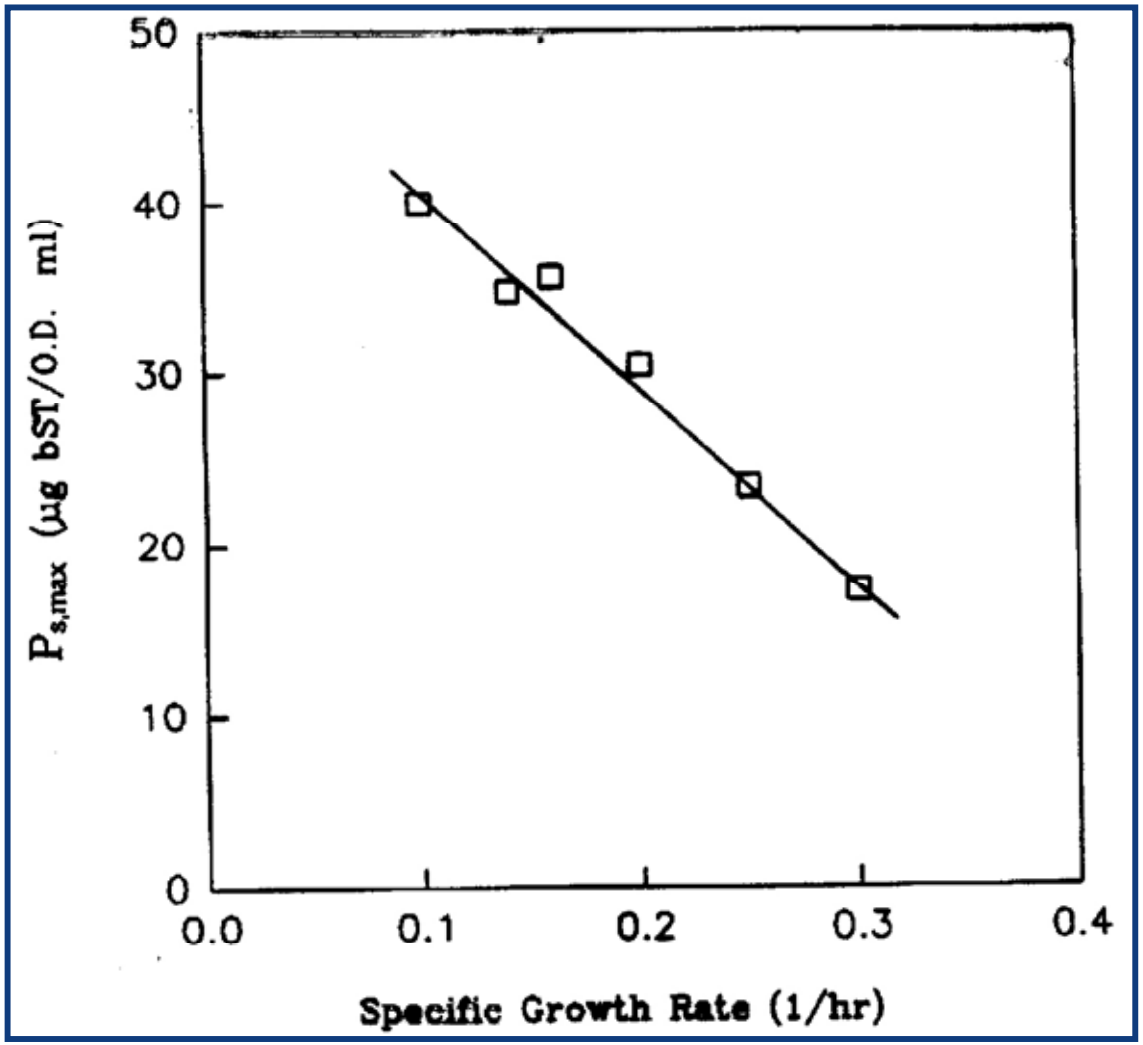
Fed-Batch Operation with Controlled Specific Growth Rate



Fed-Batch Operation with Controlled Specific Growth Rate



Effect of Specific Growth Rate on Expression



Productivity Maximization

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

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

$$[\ XV = X_0 V_0 e^{\mu t} \qquad t = \frac{1}{\mu}\ln(\frac{XV}{X_0 V_0})]$$

$$= \frac{X\mu P_{s,\max}}{\ln(\frac{XV}{X_0 V_0})}$$

- *Maximization of Pd*

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