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 Extend 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

\[ \frac{d\left(\right)}{dt} = \]
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
\[ \frac{d(XV)}{dt} = \mu XV \]  

- substrate
\[ \frac{d(SV)}{dt} = S_F F - \frac{1}{Y} \mu XV \]  

- Total
\[ \frac{dV}{dt} = F \]
Determination of Feed Rate
(using mass balance equations)

- cell

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

- substrate

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

- Total

\[ \frac{dV}{dt} = F \]  

With \[ \frac{dS}{dt} = 0 \]

\( (2) \rightarrow F = \frac{\mu X}{Y} \frac{V}{S_F - S} \)
Determination of Feed Rate
(using mass balance equations)

- cell

\[
\frac{d(XV)}{dt} = \mu XV
\]

\[
XV = X_0 V_0 e^{\mu t} \quad \ldots \ldots (5)
\]

(4) \quad F = \frac{\mu X}{Y} \frac{V}{S_F - S} = \frac{\mu X_0 V_0}{Y(S_F - S)} e^{\mu t} \quad \ldots \ldots (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
High cell density culture
- By-product: acetic acid
  - Synthetic media: $\mu = 0.35 \text{ hr}^{-1}$
  - Complex media: $\mu = 0.2 \text{ hr}^{-1}$

High specific expression
- Expression vs. specific growth rate
  - Low $\mu$, high product
- Proteolytic degradation
Feed Flow Rate Control
in Fed-Batch Operation

- Feedback Control
- Feed Forward Control
  - $\mu \uparrow$, acetic acid $\uparrow$, growth $\downarrow$, expression $\downarrow$

$$\mu = \frac{\mu_m S}{K_s + S}$$
Exponential Feeding

\[ F = \frac{X_0 \times V_0 \times \mu \times e^{\mu t}}{S_F \times Y} \]
Fed-batch culture of E. coli W3110 with a constant yield coefficient (0.714). Dotted line is the set point of specific growth rate.
Growth Yield

Yield

Time Variable

\[ Y = f \left( \text{time} \right) \]

- \( Y_0 \)
- \( Y_m \)
- \( t_0 \)

Time
Growth Yield

Yield

Time Variable

\[ \frac{dY}{dt} = \frac{1}{ty} (Y_m - Y), t = t_0, Y = Y_0 \]

\[ Y = 0.7 \exp \left\{ - (t - 8) / 8 \right\} + 0.95 \]
Fed-batch culture of E. coli W3110 with time-variable yield coefficient. Dotted line is the set point of specific growth rate. Solid lines are calculated optical density and yield coefficient.
Fed-Batch Operation

Time course of production of the target protein.
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. MgSO₄ limitation
7. Plasmid instability
8. Degradation by protease

- Low μ,
- Slow feeding,
- Pure oxygen
- Ammonium phosphate feeding
- MgSO₄ 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
<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Starting medium</td>
</tr>
<tr>
<td>Yeast Extract (g/L)</td>
<td>1.0</td>
</tr>
<tr>
<td>KH$_2$PO$_4$ (g/L)</td>
<td>15.0</td>
</tr>
<tr>
<td>MgSO$_4$·7H$_2$O (g/L)</td>
<td>4.0</td>
</tr>
<tr>
<td>Glucose</td>
<td>2.0</td>
</tr>
<tr>
<td>FeSO$_4$·7H$_2$O (mg/L)</td>
<td>-</td>
</tr>
<tr>
<td>CaCl$_2$·2H$_2$O (mg/L)</td>
<td>-</td>
</tr>
<tr>
<td>MnSO$_4$·5H$_2$O (mg/L)</td>
<td>-</td>
</tr>
<tr>
<td>CoCl$_2$·6H$_2$O (mg/L)</td>
<td>-</td>
</tr>
<tr>
<td>ZnSO$_4$·7H$_2$O (mg/L)</td>
<td>-</td>
</tr>
<tr>
<td>CuCl$_2$·5H$_2$O (mg/L)</td>
<td>-</td>
</tr>
<tr>
<td>Na$_2$MoO$_4$·2H$_2$O (mg/L)</td>
<td>-</td>
</tr>
</tbody>
</table>
Fed-Batch Operation with YE
Fed-batch culture with intermittent feeding of yeast extract.
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

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

\[ Pd = \frac{XP_{s,max}}{t} \]

\[ [ XV = X_0V_0e^{\mu t} \]

\[ = \frac{X\mu P_{s,max}}{\ln\left(\frac{XV}{X_0V_0}\right)} \]

• Maximization of Pd

\[ \text{Maximization of } (\mu P_{s,max}) \]

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

Optimum \( \mu = 0.23 \text{ (1/hr)} \)
Optimum Specific Growth Rate for Maximum Productivity