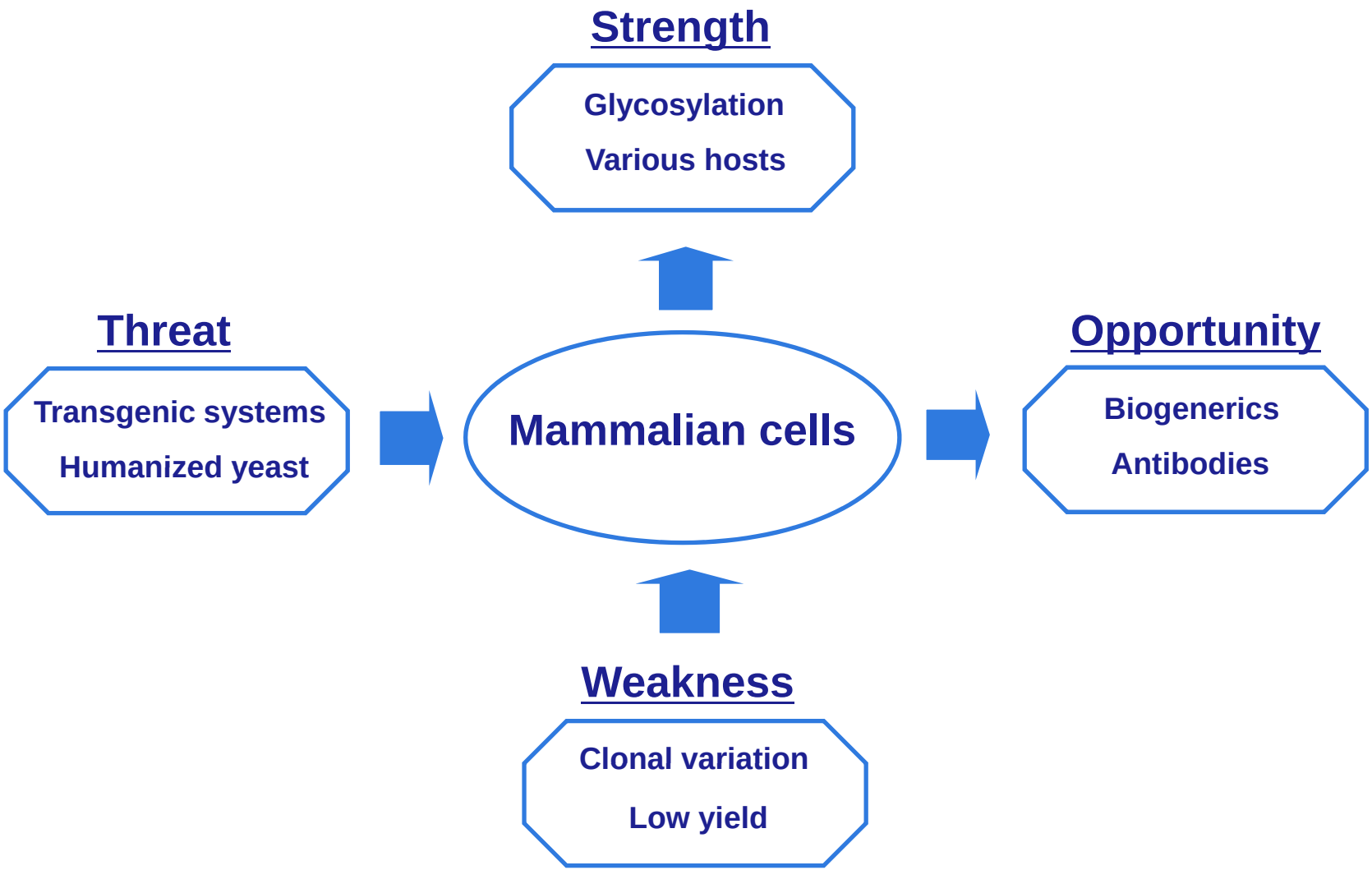
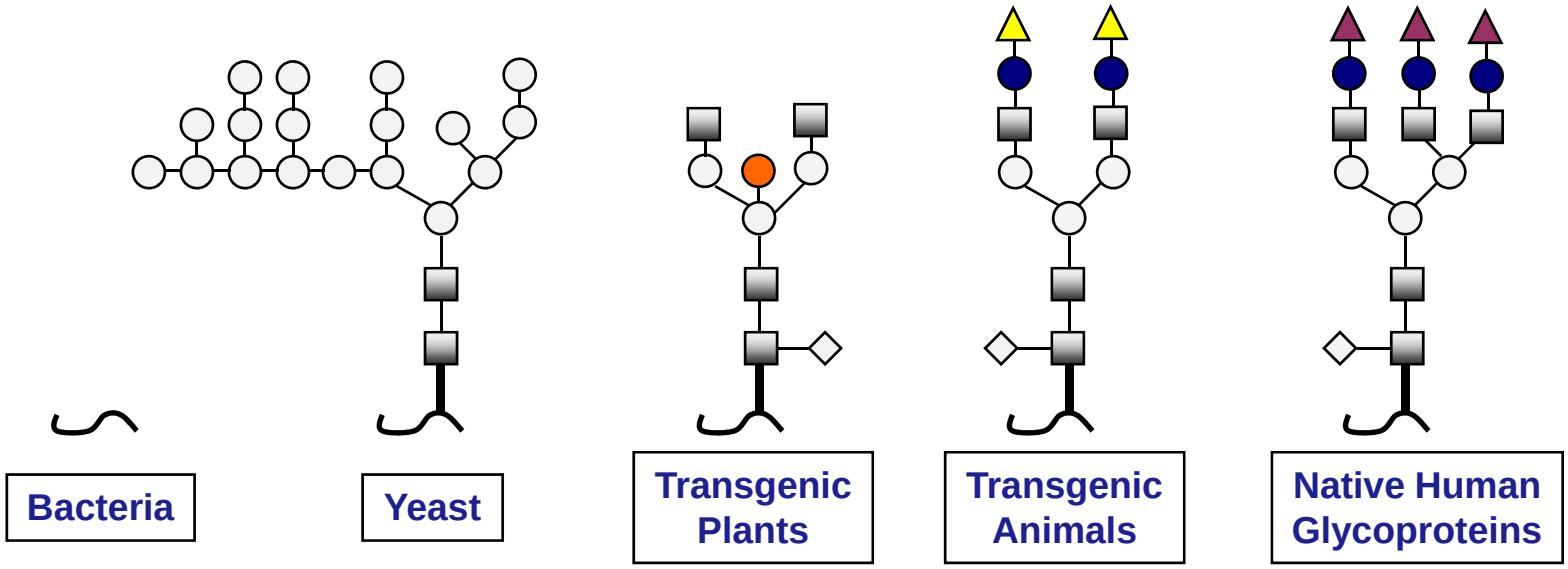
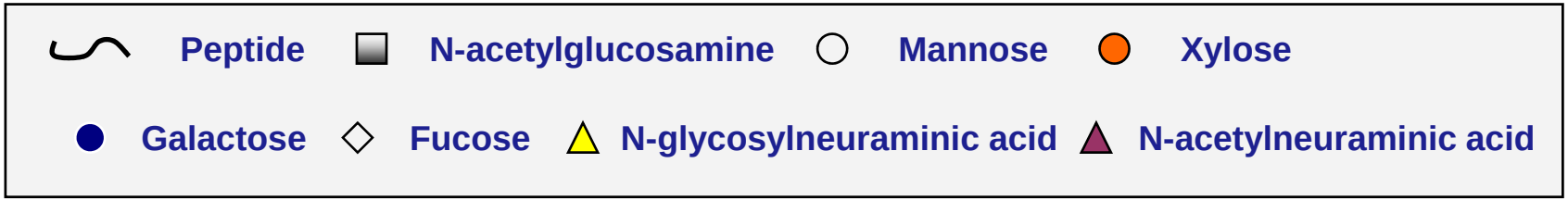
Mammalian Cell Culture

Mammalian Cell Culture



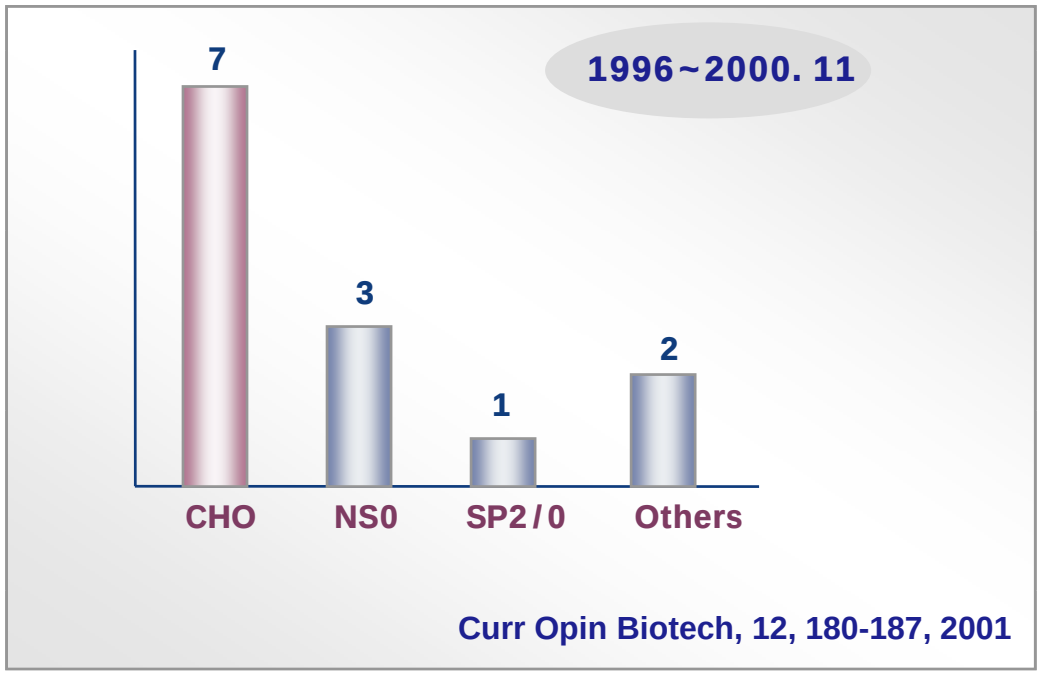
Glycosylation





Host Cells

- **Recombinant therapeutics approved in the US using mammalian cell lines**



CHO, NS0, PER.C6 …

Clonal Variation (weakness)

- Clonal variation
  - Random integration of foreign gene into host chromosome
  - Difficulty in the development of general process

Low Yield (weakness)

- Low yield of foreign proteins
  - Slow growth : low cell density
  - Physiological constraints:
    -- osmolality, shear resistance
  - Necessity of complex media
  - Low product titer : ~ mg/L level

Yield Improvement

- Optimization of culture process
  - Culture media
  - Environmental conditions
  - Culture modes
- Host cell
  - Cellular engineering
  - Anti-apoptosis engineering

Culture Media

❑ **Serum-containing media**

- Basal medium with 5-10% FBS (v/v)
- Contamination
- in 1990s

❑ **Serum-free media**

- Devoid of Animal-derived material
- Protein-free or Chemically defined media
- in 2000s

❑ Source
- Commercially available media
- Proprietary
- Contract

❑ Clone-specific

ex) Insulin-dependency

Cell Type

- **Anchorage dependent cells**
  - Cell culture dish, T-flask
  - Roller bottle
  - Microcarrier culture

- **Anchorage independent cells**
  - Cell culture dish, T-flask
  - Suspension culture

Environmental Conditions

Temperature

- Optimal temp. for cell growth: 36-37ºC
- Optimal temp. for production : 36-37ºC ?

pH

- pH affects many cellular functions.
- Optimal pH for cell growth: pH 7.0-7.4

Little is known about optimal pH for q_p

Osmolality

- Osmolality affects many cellular functions.
- Optimal osmolality for cell growth:

  --- 280-320 mOsm/kg
- Fed-batch culture
- Hypo- & Hyper-osmolality

  for increasing production

CO_2

- Physiological concentration :

  ~ 35 mmHg
- Elevated CO_2 concentration affects cell growth and production

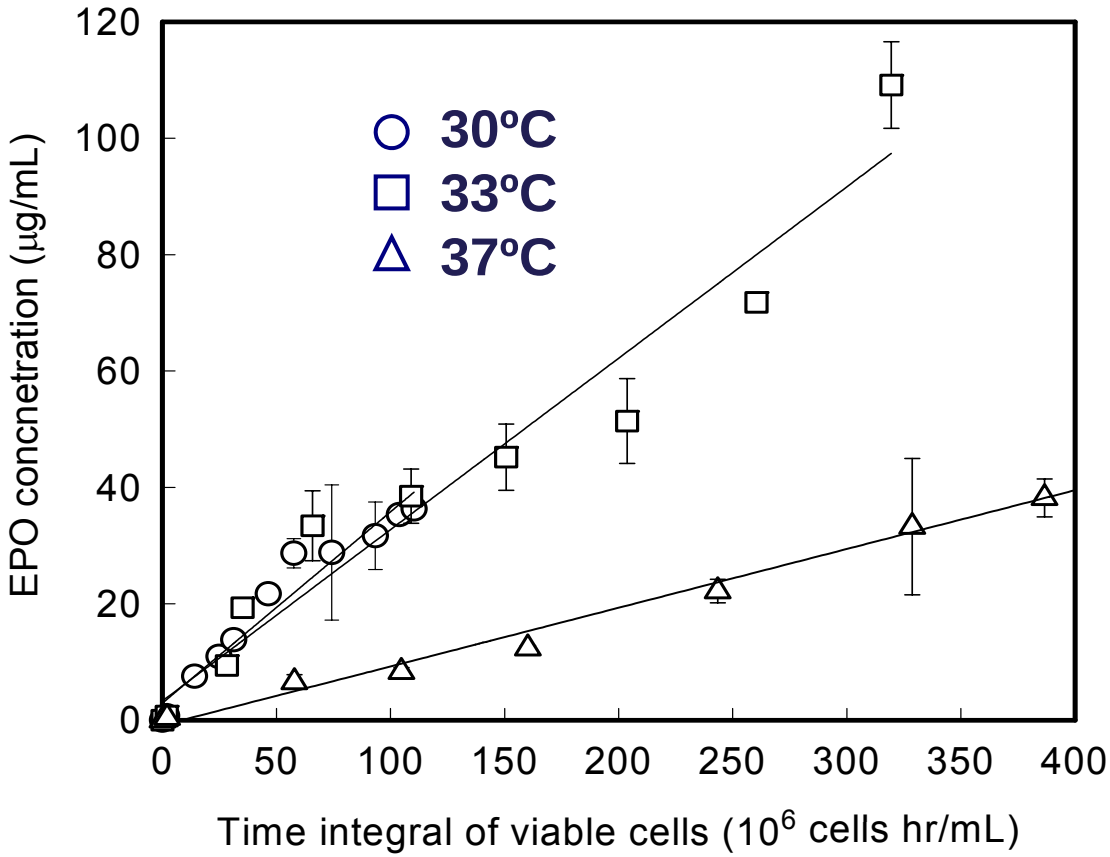
Temperature

- Easy to control

Cell growth rate	30-33 ºC	<	37 ºC
Viability	30-33 ºC	>	37 ºC
$q_{nutrient}$	30-33 ºC	<	37 ºC
q_{O_2}	30-33 ºC	<	37 ºC
Shear resistance	30-33 ºC	>	37 ºC
$q_{r\text{-}protein}$	30-33 ºC	?	37 ºC

Temperature

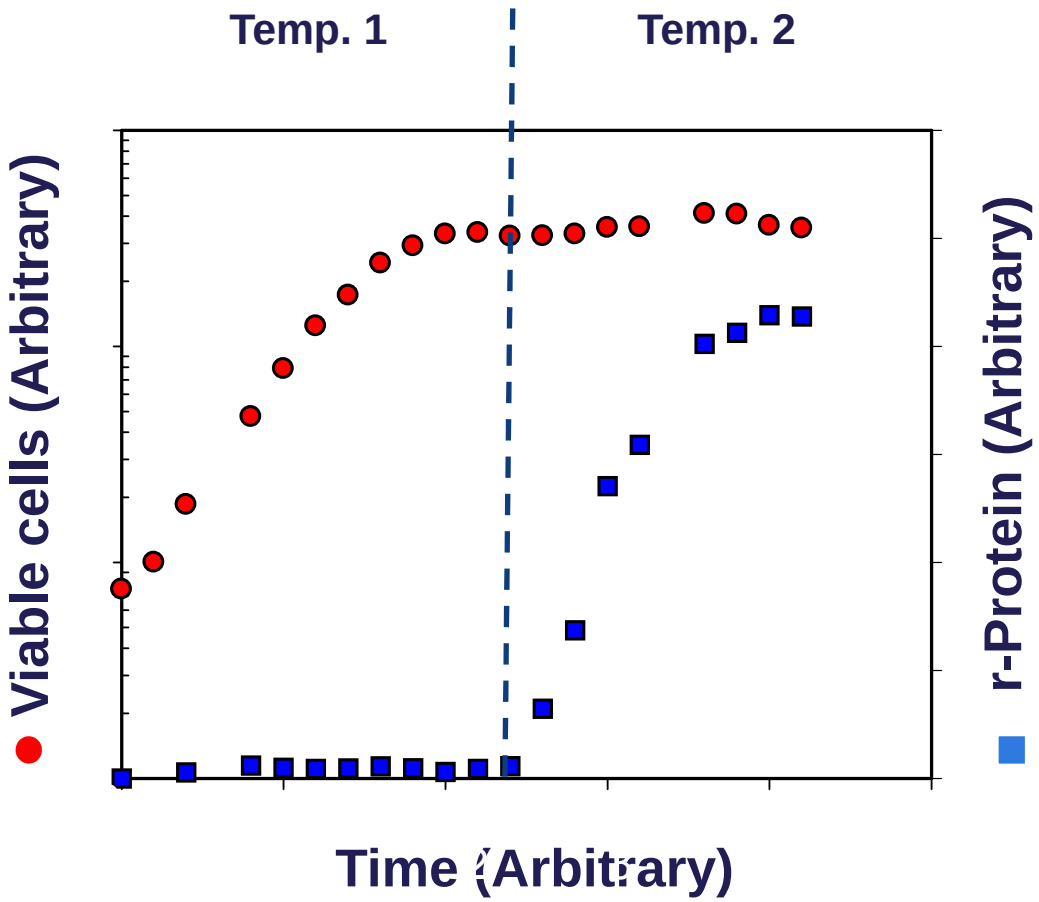
Specific EPO Productivity, $q_{EPO} = P_{EPO} / \int Xdt$



Slope = q_{EPO} (mg/10^6 cells/hr)

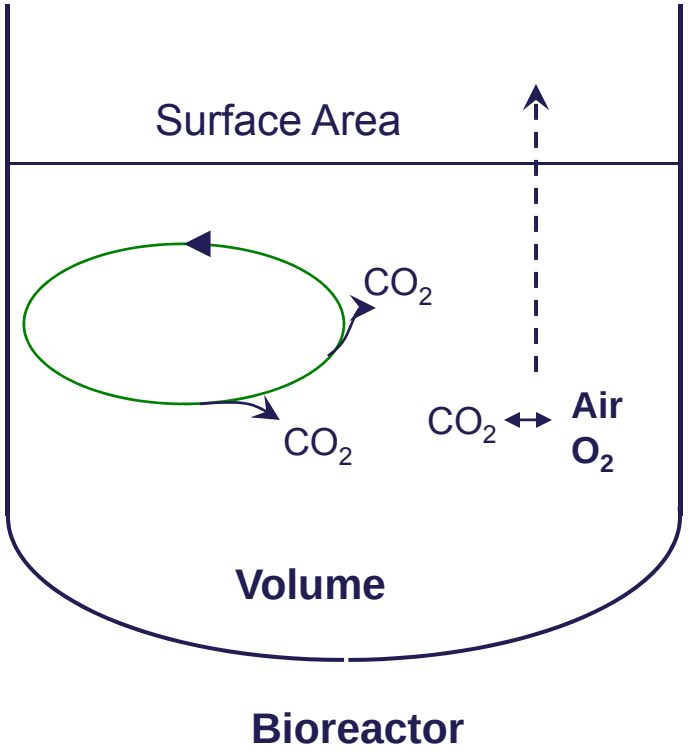
Yoon et al. (2003), Biotechnol Bioeng 82:289-298

Temperature



Environmental Conditions – CO_2

- Generation of CO_2 from TCA cycle in high cell density culture
- As the reactor scale increases, surface area to volume ratio decreases.

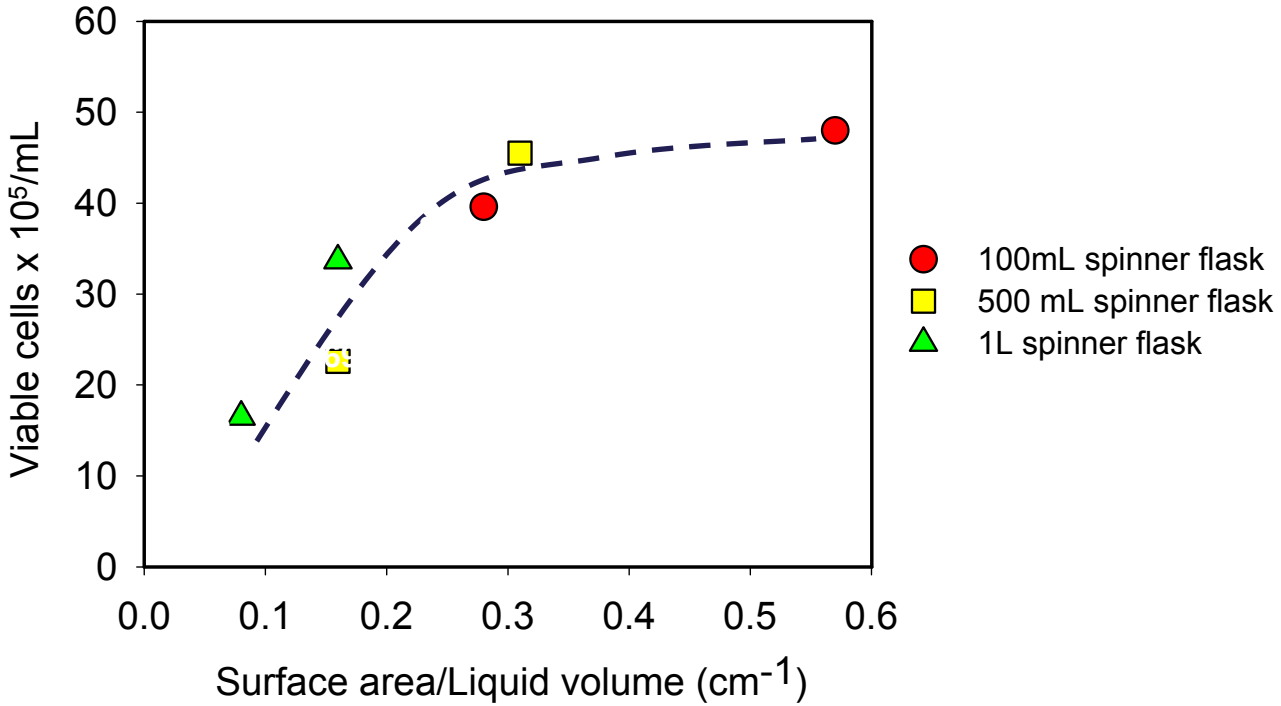


Accumulation of CO_2

- Suppression of cell growth
- Decrease in protein quality
- Decrease in production

Environmental Conditions – CO_2

Ex) Spinner flask



Culture Modes

- **Production of recombinant protein in CHO cells**

	High level expression	Low level expression
Recombinant Protein	EPO Antibody	β-IFN Factor VIII FSH
Specific Production Rate μg /10^6cells/day	> 10	< 1
Culture Mode	Batch, Fed-batch (Stable protein), Perfusion (Labile protein)	Perfusion

Hydrophobicity
Structural unstability
Complexity, etc

Perfusion System

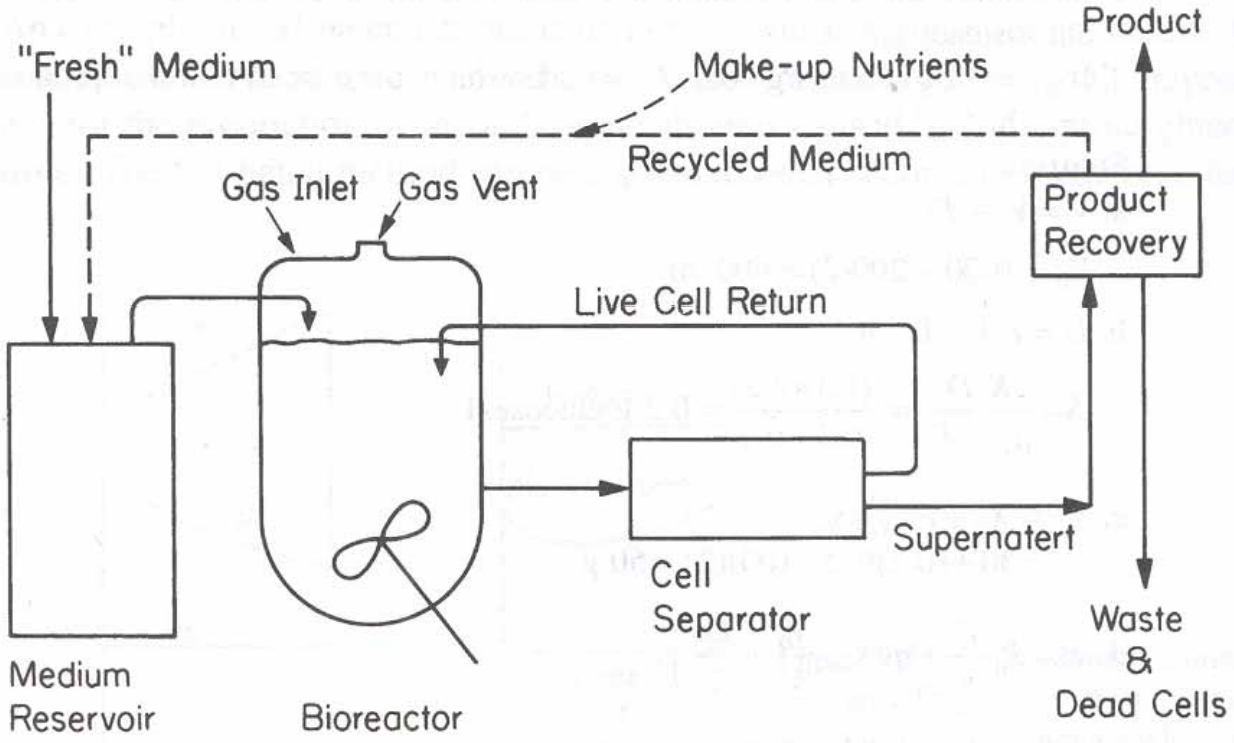
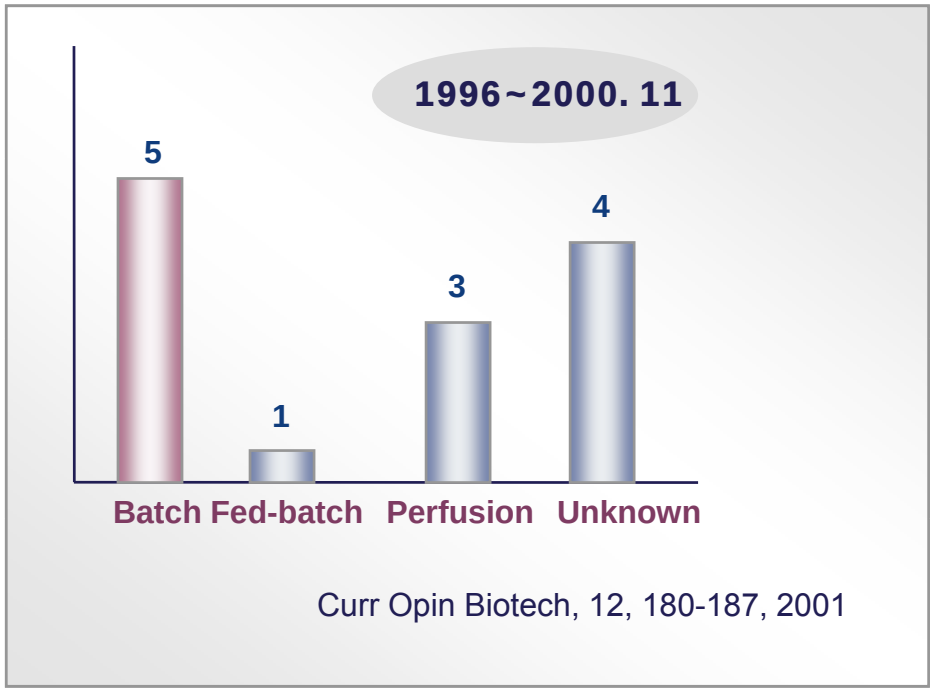


Fig. 9.10. Schematic of a perfusion system with external centrifugation and return of cells. Internal retention of cells is also possible. Return of spent medium is optional.

Culture Modes



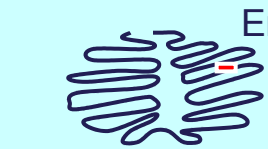
Curr Opin Biotech, 12, 180-187, 2001

Batch → **Fed-batch (Ab), Perfusion**

Culture Modes

	Batch	Fed -batch	Perfusion
Cell density	Low ~ 4 x 10^6/mL	High ~ 1.5 x 10^7/mL	High 3~4 x 10^7/mL
Labor intensive	Severe, More frequent turnaround	Less Severe	Less severe
Operation time	5- 7 days	15- 25 days	30-180 days
Others	Low risk in contamination	Stable protein production Accumulation of by-product	High risk in contamination Labile protein production Removal of by-product

Cellular Engineering



Engineered gene (New function)

CHO chromosome

- ❑ Enhanced cellular machinery
- ❑ Facilitation of process optimization
- ❑ High productivity
- ❑ Reduced culture scale