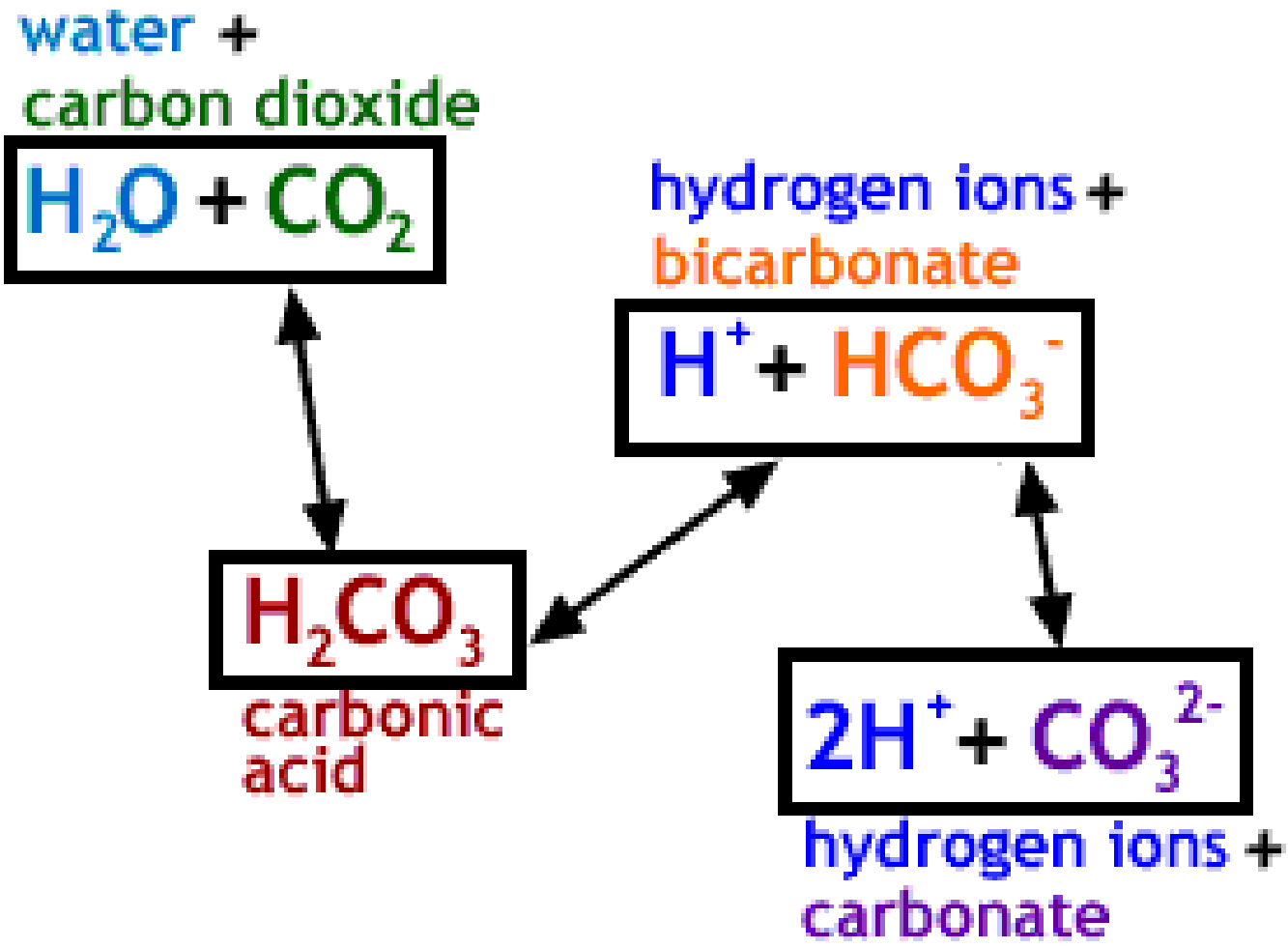
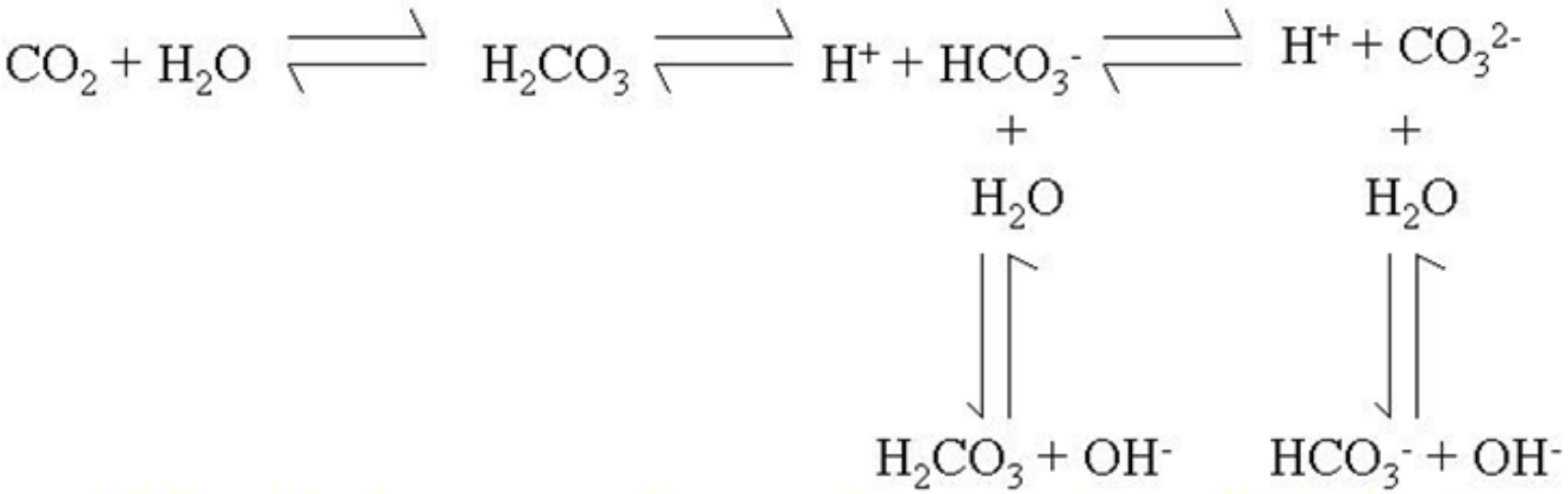
Growth Conditions of Animal Cells

- Mammalian cells
  - 37°C, pH ≈ 7.3
  - Doubling time: 12 to 20 h
  - Carbonate buffer ($HCO_3^{2-}/H_2CO_3^-$) using 5% CO_2-enriched air
  - HEPES (*N*-[2-hydroxyethyl]piperazine-*N'*-[2-ethanesulfonic acid]) buffer can be used without CO_2.
- Insect cells
  - 28°C, pH ≈ 6.2
- Fish cells
  - 25~35°C, pH = 7.0 ~7.5

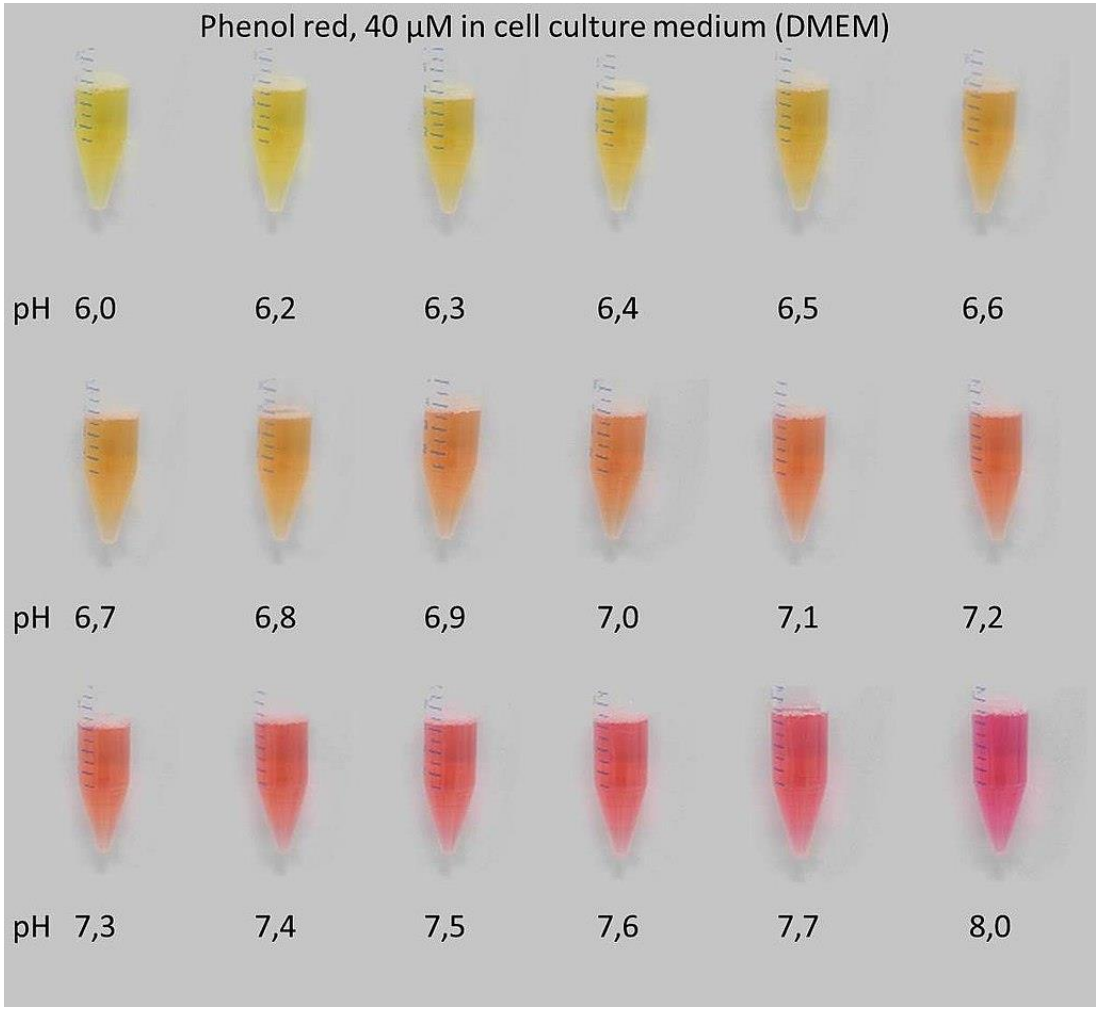
Carbonate-Bicarbonate Buffer



Carbonate-Bicarbonate Buffer



Phenol Red in Medium



Growth Kinetics of Mammalian Cells

- The pattern of the mammalian cell growth is similar to microbial growth.
- Stationary phase is relatively short.
  - The concentration of viable cells drops sharply thereafter, as a result of the accumulation of toxic metabolic products (lactate and ammonia).
- Toxic metabolites
  - Lactate: product of glucose metabolism
  - Ammonia: result of glutamine metabolism

Kinetics of Product Formation

- Most of the products of mammalian cell cultures are mixed-growth associated.
- Product formation both during and after the growth phase
- Luedeking-Piret equation

  (e.g., Mab formation by hybridoma cells)

$$\frac{1}{X}\frac{dP}{dt} = q_p = \alpha\,\mu + \beta$$

Oxygen Requirement of Animal Cell

- Much lower O_2 requirement than microbial cells
  - 0.06 to 0.2 x 10^{-12} mol O_2/h-cell
- Surface aeration from the head space
  - 2 to 5 cm deep for shallow culture
  - Spinner flask with magnetically driven agitator at 10 to 60 rpm

Forced Aeration

- Forced aeration for submerged cultivation of denser cultures
- Shear sensitive issues
  - Special aeration and agitation systems are designed.
  - Rising air bubble and bubble rupture at the medium surface cause the shear damage (cell lysis)
  - Chemicals (e.g., Pluronic F-68) can be added for the shear protection
  - Even the sublytic level of shear can cause the alteration of the product quality
  - Also processes, such as N-linked glycosylation, can be altered by shear.

Bioreactor for Animal Cell Culture

- Gentle aeration and agitation
  - Minimization of shear damage to cells
- Well-controlled homogeneous environmental conditions
  - T, pH, DO, redox potential
  - Supply of CO_2-enriched air
- Large surface-volume ratio of support materials for anchorage-dependent cells
- Removal of toxic metabolic products
  - Lactic acid, ammonium

Lab-Scale Cultivation

- Lab-scale reactors are placed in a CO_2 incubator at 37^{o}C.
- T-flask (25 mL to 100 mL)

- Spinner flask (100 mL to 1 L)

Lab-Scale Cultivation

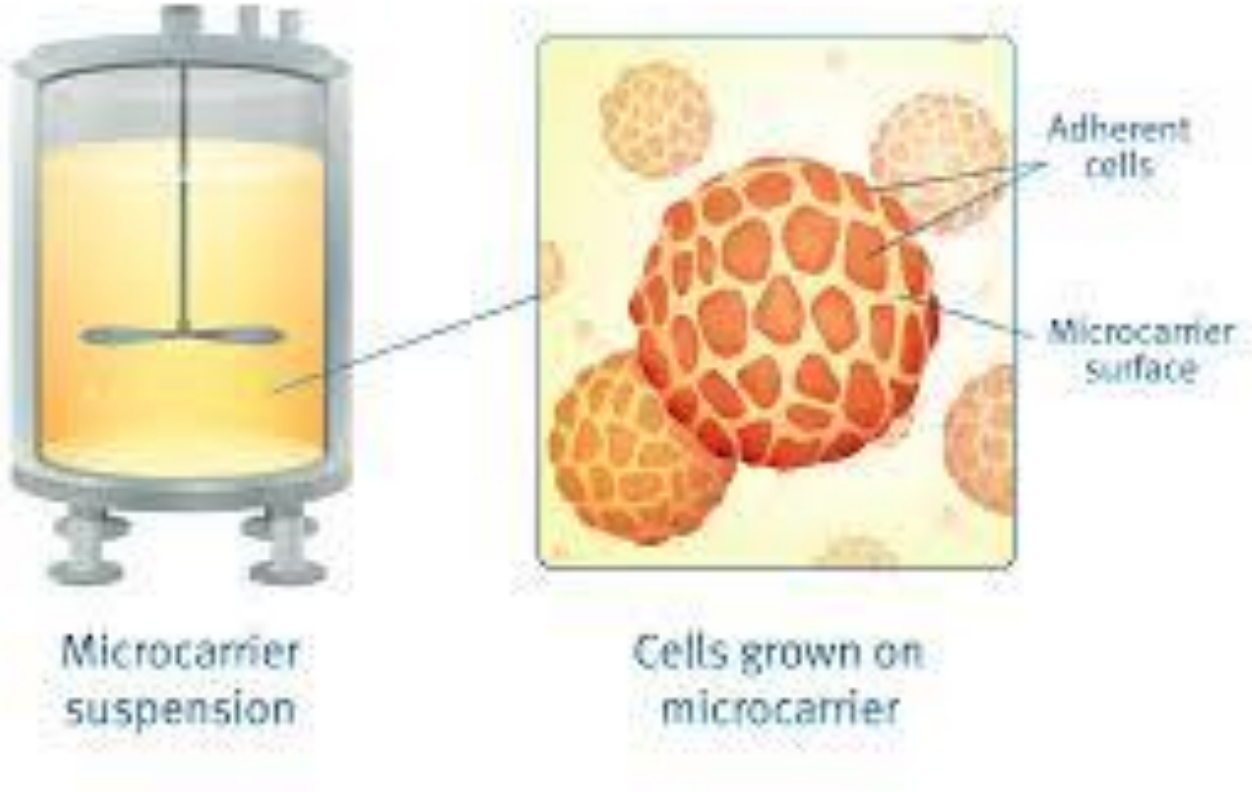
- Roller bottles (50 mL to 5 L) rotating about 1 to 5 rpm

Roller Bottle for Commercial Production

- Challenge
  - High labor requirements
  - Bottle-to-bottle variability
- Commercial Production of Erythropoietin
  - Highly automated facility using robots
- Production of Some Vaccines

Bioreactors for Anchorage-Dependent Cells

- Microcarrier systems

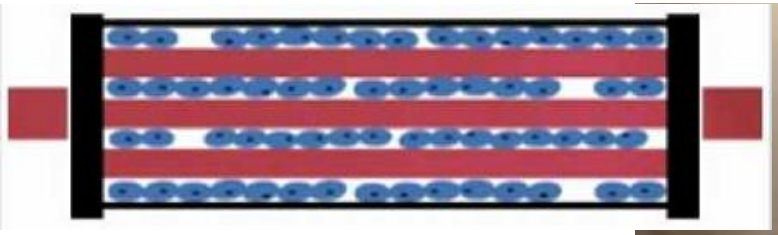


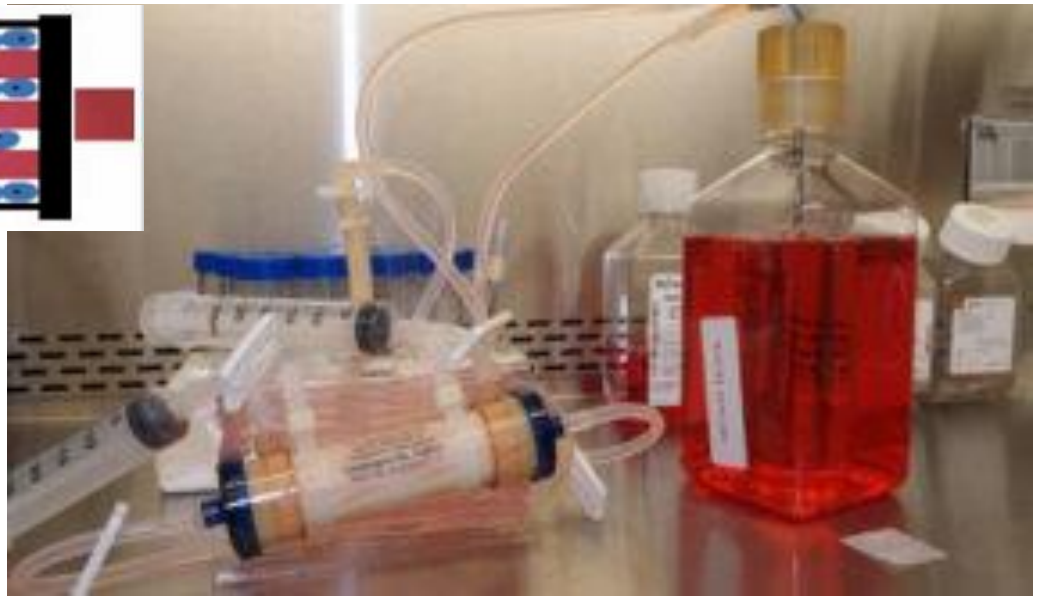
Microcarriers

- Large surface per unit volume of reactor
  - ~70,000 cm^2/L
- High cell concentrations in the medium
  - > 10^7 cells/mL
- Dextran- and DEAE-based microcarriers are commonly used.
  - DEAE-Sephadex, DEAE-polyacrylamide
- The surface can be modified by addition of compound, such as collagen, to promote cell adhesion and enhance cell function.

Bioreactors for Anchorage-Dependent Cells

- Hollow-fiber reactors





- Ceramic matrix systems
- Weighted porous beads

Hollow-fiber Reactors

- The control of environmental conditions especially in the shell side is very difficult.
- Hollow-fiber reactors have been used for the production of extracellular proteins such as Mab's from hybridoma cells.
  - Mab concentration: 50 mg/mL
  - Long-term perfusion operation (e.g., 100 days)
- To overcome some of the difficulties in axial-flow hollow-fiber reactors, radial-flow or cross-flow hollow-fiber units have been developed.

Disposable Systems
Using Multiple Plates