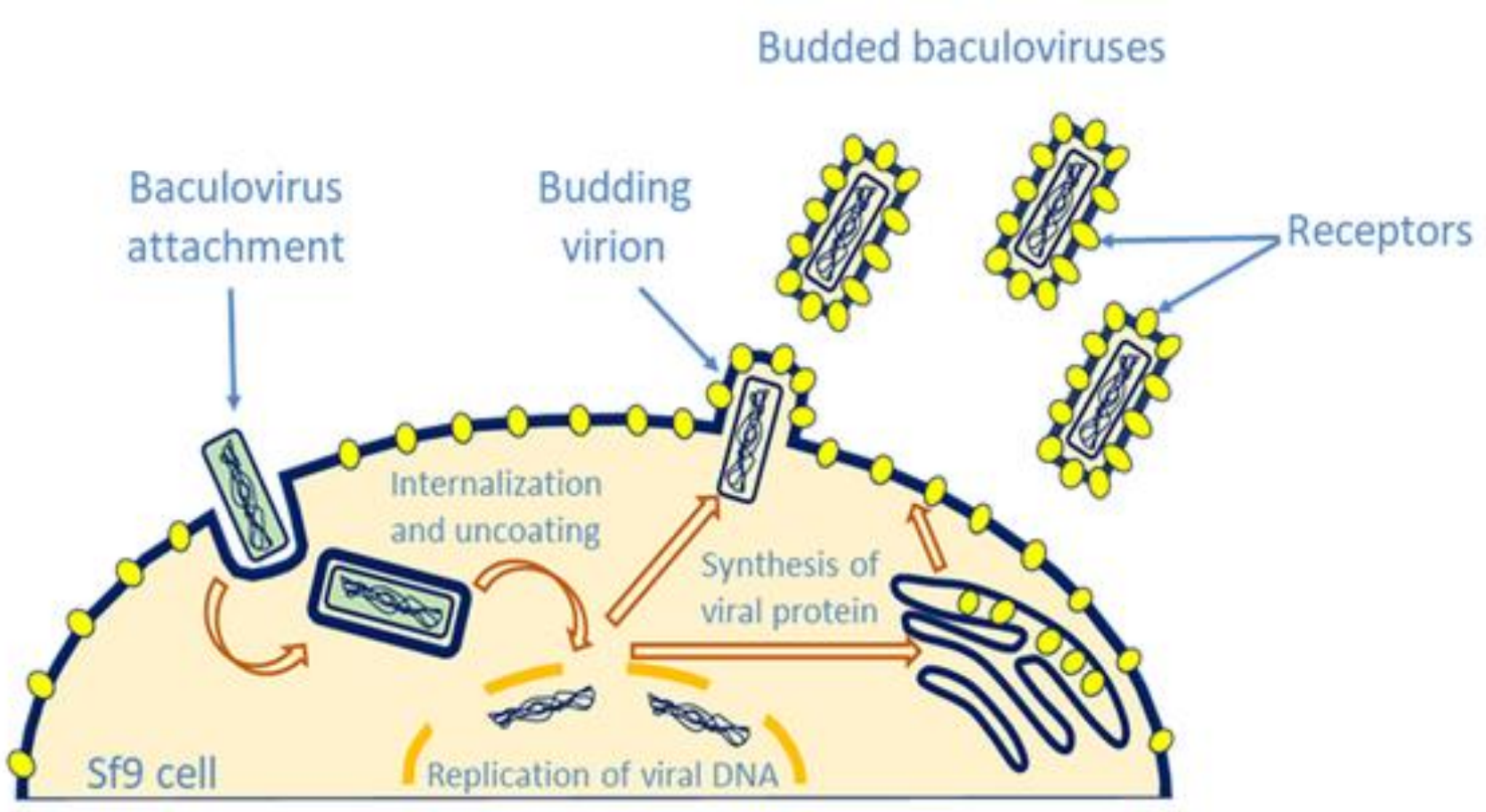
Insect Cell Culture

- Animal cell culture
  - Mammalian, insect, fish, and crustacean cell cultures
- Insect Cells
  - Significant level of glycosylation, but the glycoforms are not authentic with humans However, the difference can be useful.
    - Sometimes more intense immune response or target specific
  - Insect cell/baculovirus expression system is useful.
    - Commercial level of human and veterinary vaccines
    - Gene therapy agent against familial lipoprotein deficiency

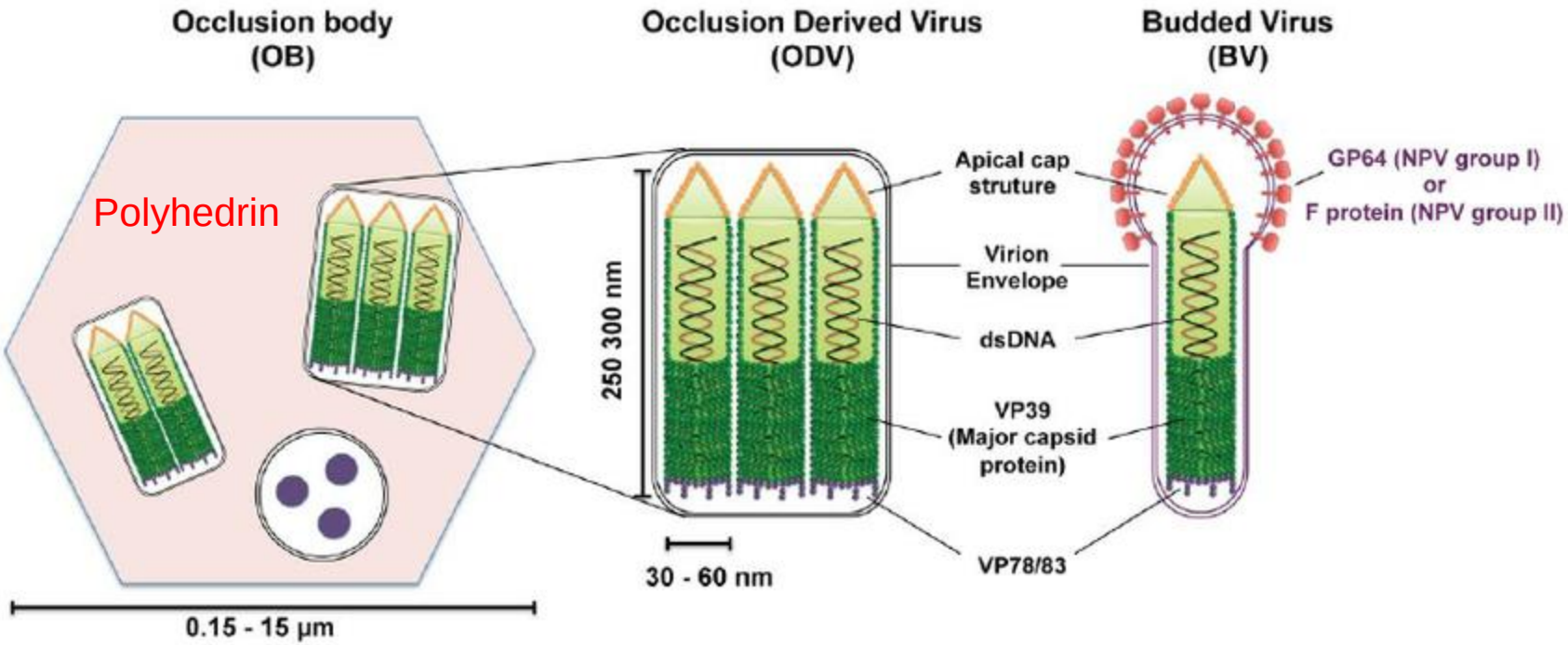
Insect Cell/Baculovirus System

- Baculovirus
  - Baculovirus infects insect cells.
  - Ideal vector for genetic engineering
  - Nonpathogenic to humans
  - Very strong promoter that encodes for a protein that is not essential for virus production
  - High expression level (40% of the total protein as the target protein)

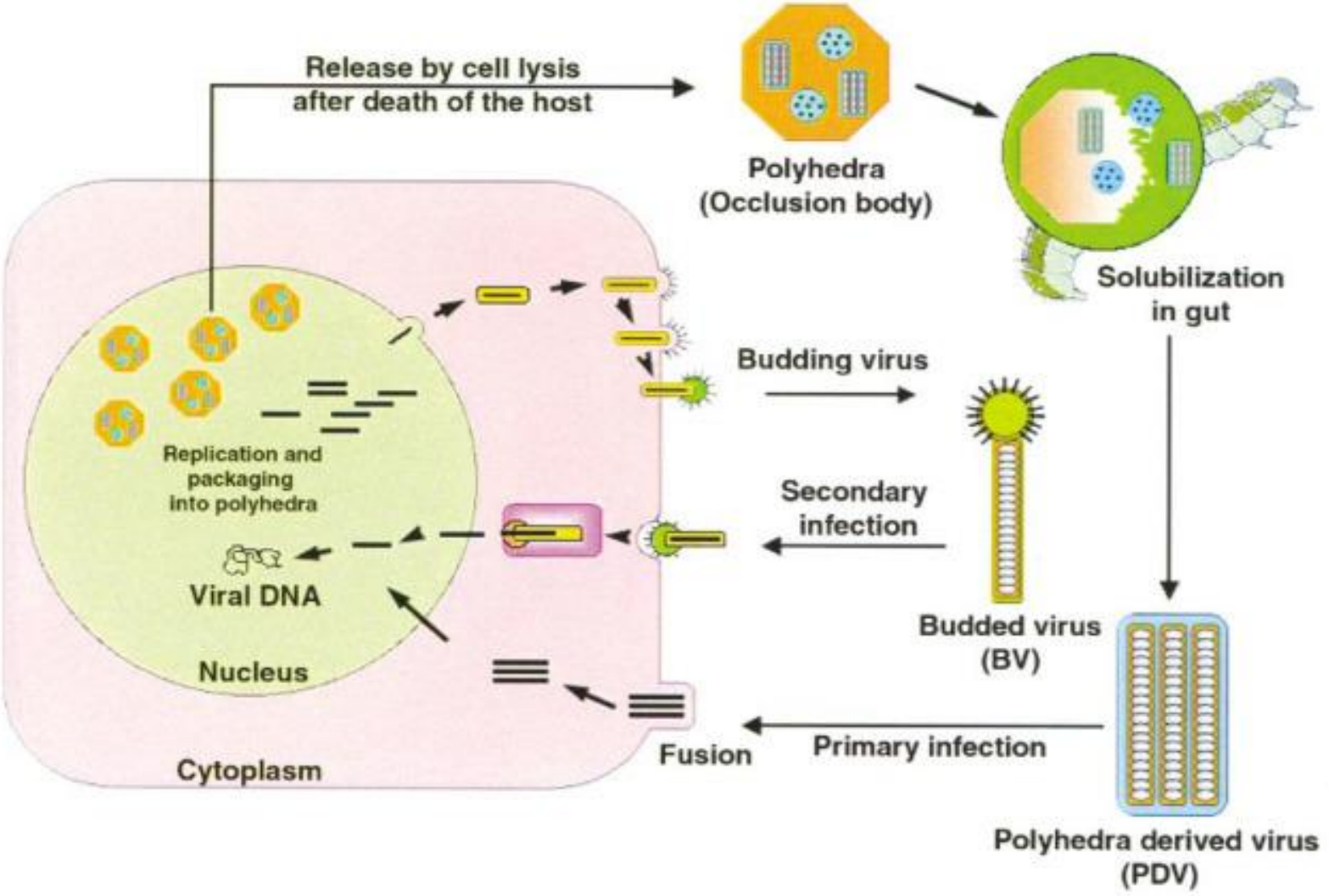
Insect Cell/Baculovirus System



Baculovirus



Insect Cell/Baculovirus System



Process of Viral Replication

1. Early Phase: In this phase, the virus infects the insect cell by attachment, penetration and uncoating. In this phase, the infected cells are prepared for viral DNA replication. Normally, initial viral synthesis occurs 0.5-6h post-infection, along with the shutting down of host gene expression.
2. Late Phase: Genes that code for replication of viral DNA and assembly of virus are expressed during this time. Cells begin to produce extra-cellular virus that contains the plasma membrane envelope and glycoprotein during the time range of 6-12h post-infection. Both are necessary elements for viral infection through the process of endocytosis. The virions are then assembled and budded. Recombinant virions are released 18-36h post-infection.
3. Very Late Phase: Occlusion derived virus particles are produced and cell lysis occurs in this phase.

Polyhedrin

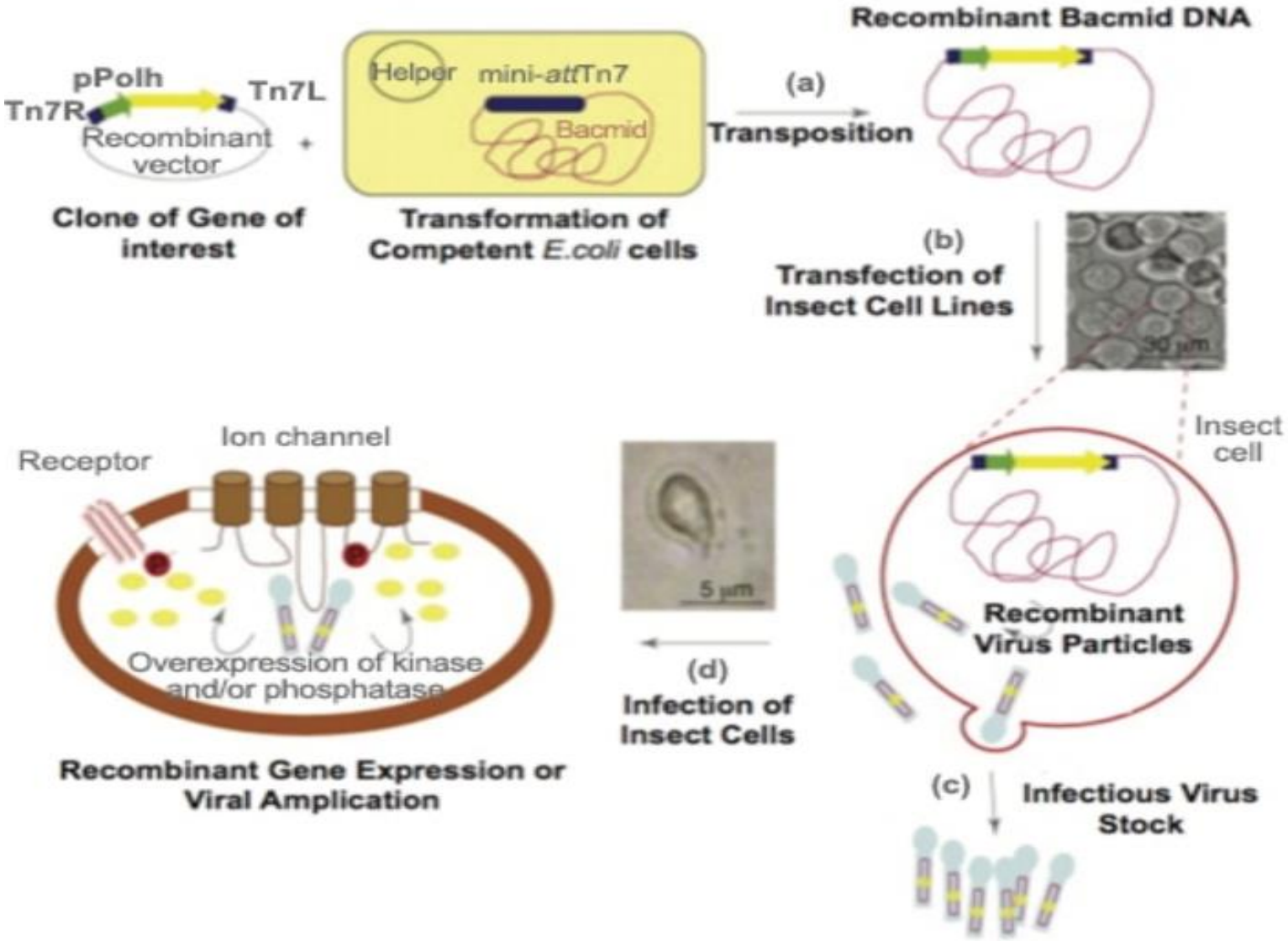
- Polyhedrin has evolved two highly specialized functions.
  - forms a protective crystal around the virus
  - it resists solubilization except under strongly alkaline conditions similar to those found in the insect midgut.
  - Both of these properties allow the virus to remain viable for many years outside the insect host.
- Strong transcriptional promoter.
- Virus-encoded transcriptional complex.
- None-essential for BV replication *in vitro*.
- The crystalline part of baculovirus polyhedra consists of ~29 kDa polyhedrin.

Insect Cell Lines

- Sf 9 & Sf21 From *Spodoptera frugiperda*
  - Frozen in serum containing medium
  - Can be thawed directly to serum free medium (Ex-Cell 401)
  - From the Moth Army Worm Spodoptera frugipedra ovarian tissue.
  - Reaches 6x10.6/ml in suspension after 6 days with 98% viability
  - Sf9- Faster growth and higher densities than Sf21

- For virus expansion these two cell lines are preferred over High Five
- Common name: Fall Armyworm

Generation of Recombinant Baculovirus & Gene Expression



Expression of Recombinant Protein

1. Early Phase: The virus nucleocapsids pass through the cytoplasm to the nucleus. With the release of the contents of the capsids, the cellular structure changes within the early hours following infection. Normal cellular functions decline sharply.
2. Late Phase: Most of the cellular functions cease within 6-24h post-infection. The infected cells stop dividing; the production of viral genome and budded virus increases. Increased cell diameter and enlarged nuclei can also be observed.
3. Very Late Phase: The infected cells cease production of budded virus and initiate the assembly, production and expression of the recombinant protein within 20-36h post-infection. The density of cell culture decreases dramatically as cells die and lyse. The infected cells continue to increase in diameter and display enlarged nuclei. Vacuoles are visible among the cytoplasm and the nuclei may demonstrate granularity to some extent.

Insect Cell/Baculovirus System

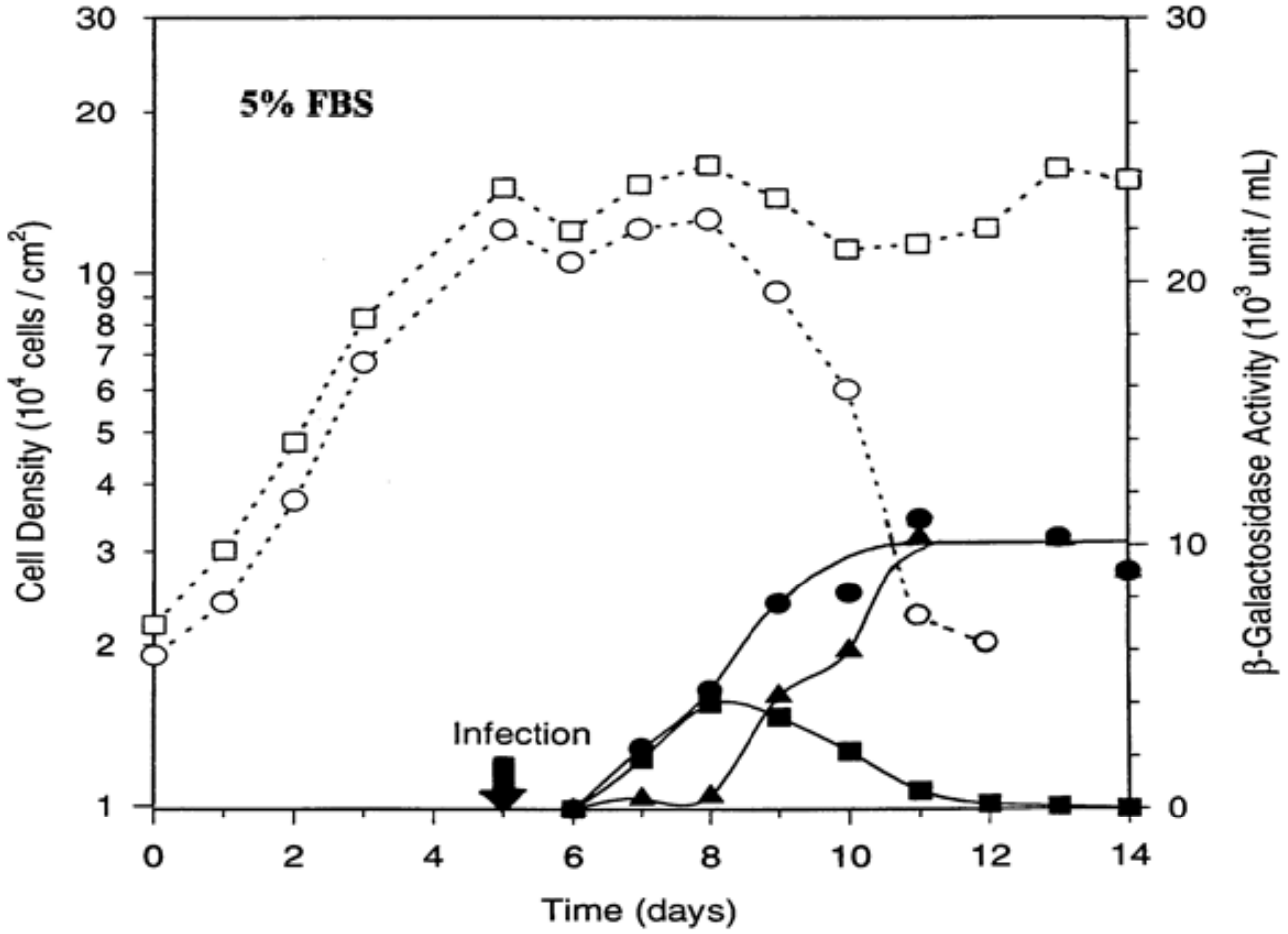
ADVANTAGES

- The polyhedrin gene is **not required** for the continuous production of infectious virus in insect cell culture. Its sequence is replaced with that of the heterologous gene.
- The polyhedrin gene promoter is very **strong.** This determines a very high level of production of recombinant protein.
- This system is capable of post-translational modifications.

Disadvantages

- Expensive.
- Glycosylation in insect cells is different.
- Discontinuous expression: baculovirus infection of insect cells kills the host and hence the need to reinfect fresh cultures for each round of protein synthesis.
- Inefficient for production on a commercial scale

Insect Cell/Baculovirus System



(❑) total cells, (O) viable cells, (▲) extracellular β-galactosidase, (■) intracellular β-galactosidase, (●) total β-galactosidase

Silkworm Hemolymph (SH)

FBS

- High Cost
- Nonreproducibility
- Contamination Risk
- Complicates Down-Stream Processing

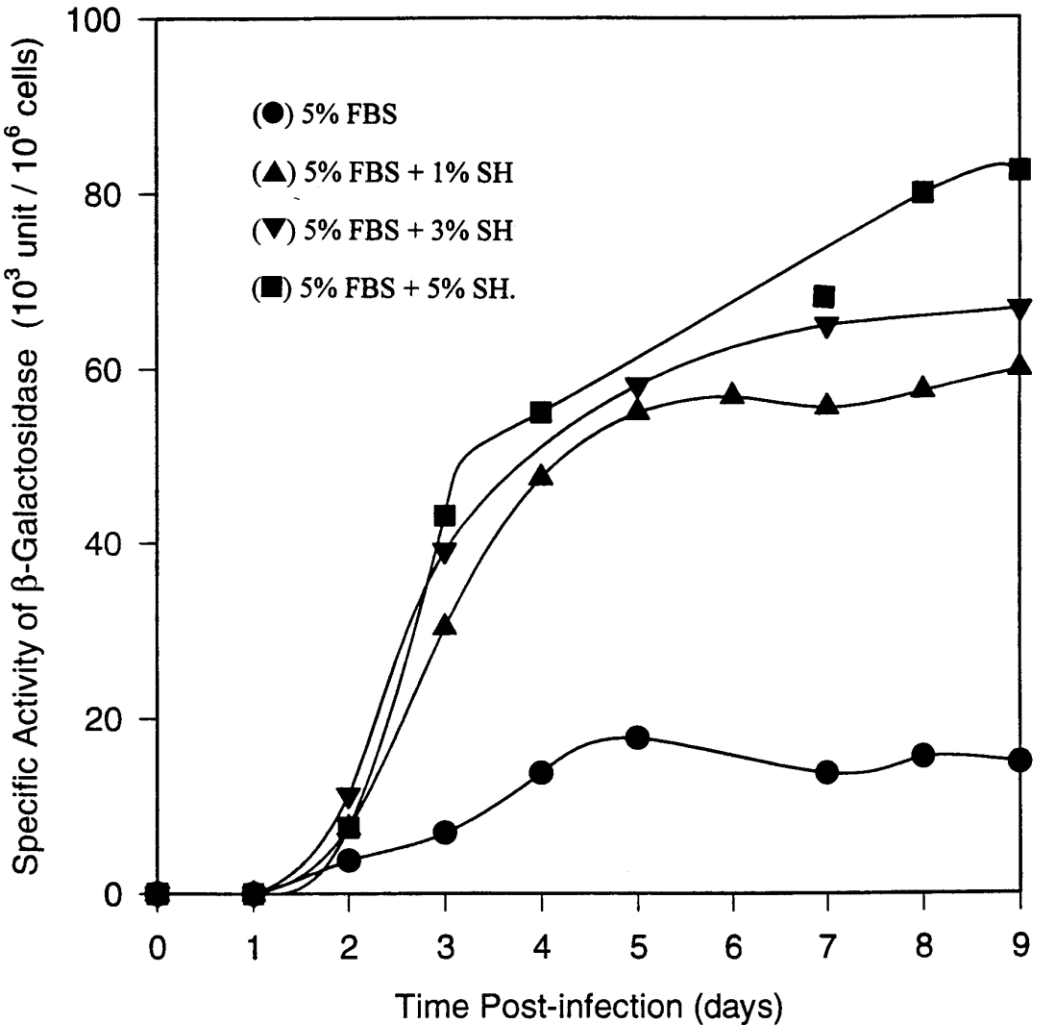
SH

- Insect Serum
- Low Cost
  * $4/100ml SH
  * $56/100ml FBS
- Reproducibility

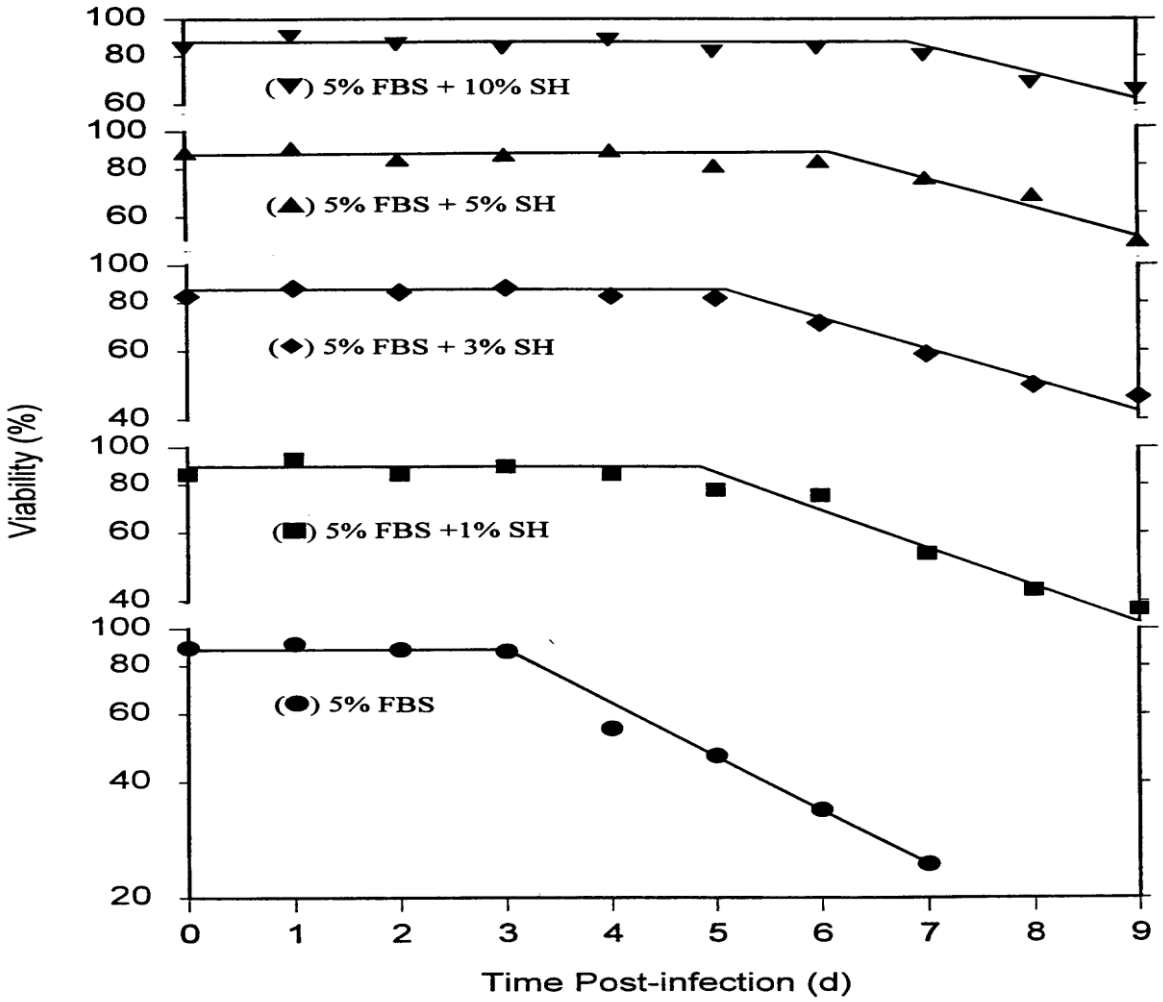
Beneficial Effect of Silkworm Hemolymph on Insect Cell/Baculovirus System

- Replacement of FBS with SH
- Production of Recombinant Protein
- Delay of Virus-Infected Host Cell Death
- Inhibition of Apoptosis

Effect of Silkworm Hemolymph on Recombinant Protein Expression



Effect of Silkworm Hemolymph on Host Cell Viability

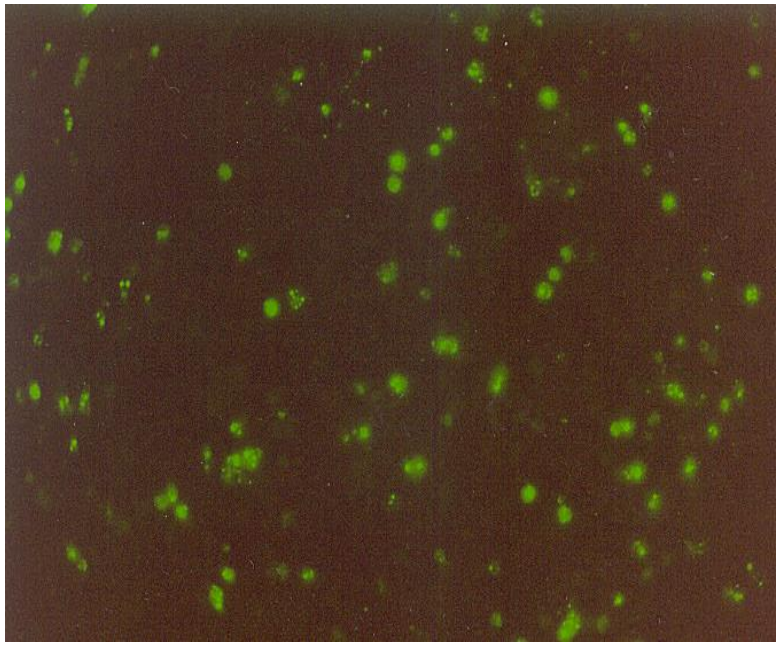


Biotechnol. Prog. 15, 1028 (1999)

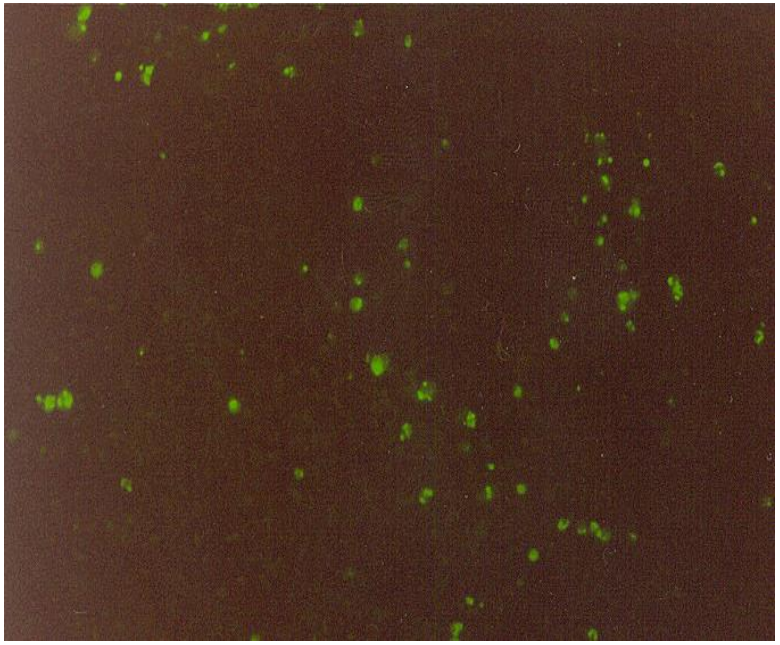
Anti-Apoptotic Effect of Silkworm Hemolymph

(TUNEL Assay)

10% FBS



10% FBS
(5% SH added in the infection step)

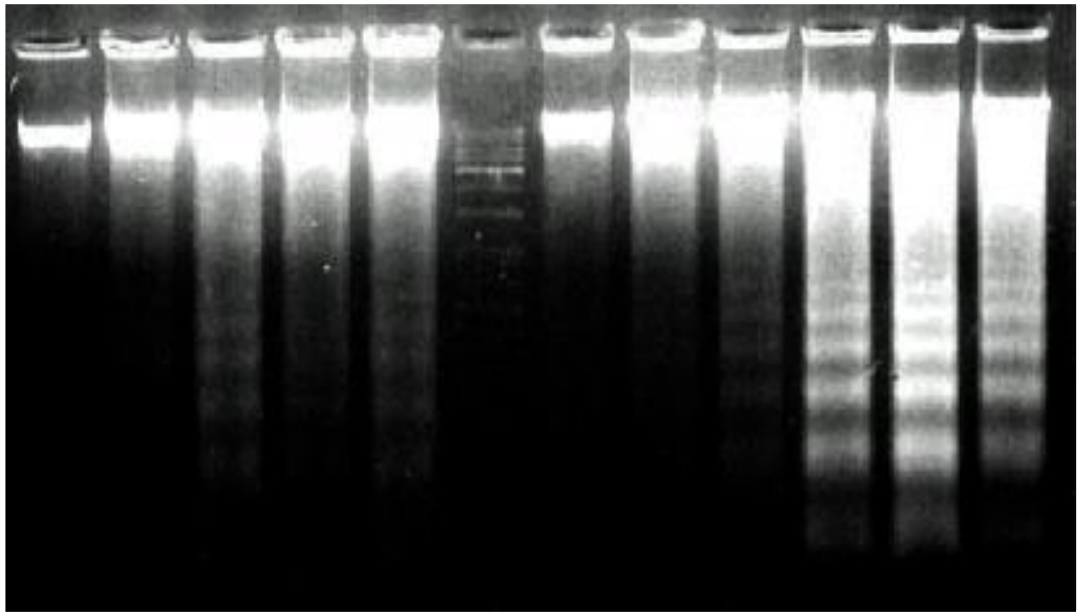


***Biochem. Biophys. Res. Commun. 271*, 186 (2000)**

Virus-Induced Human Cell Apoptosis
(vaccinia virus, HeLa cell)

5% FBS + 5% SH **10% FBS**

Time (hpi) 24 36 48 60 72 24 36 48 60 72



Biotechnol. Prog. 18, 874 (2002)

Systems for Entrapped Cells

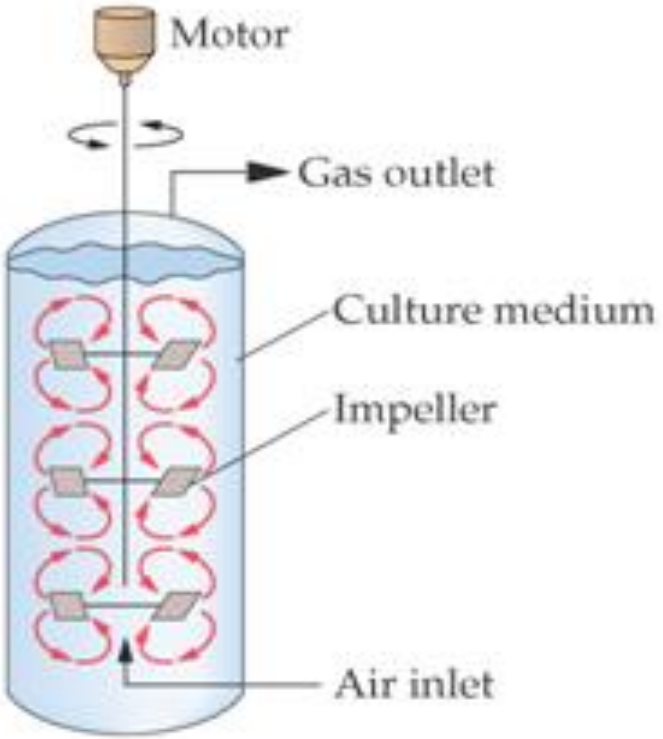
- Immobilization of mammalian cells in gel beads (agar, alginate, collagen, poly-acrylamide)
  - Packed-bed or fluidized-bed reactor
- Microcarriers
  - In a stirred bioreactor
- Microencapsulation
  - Porous membrane (MW cutoff: 60 to 70 kDa)
  - Typical capsule size: 300 to 500 μm
  - High cell concentration: ~10^8 cells/mL
  - Protected from hydrodynamic shear

Bioreactors for Suspension Cultures

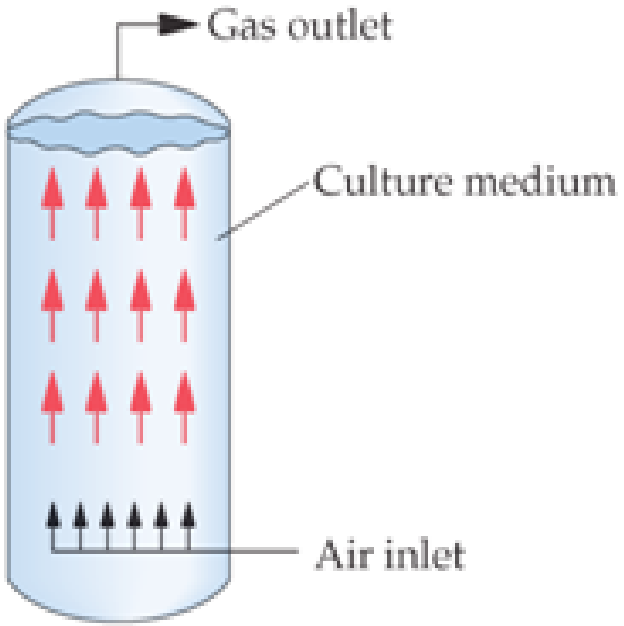
- Stirred-tank reactors
- Modified stirred reactors
  - Sail-type and axial-flow hydrofoil agitators
    (Mild agitation at 10 to 40 rpm)
- Airlift reactors
- Bubble column reactors

Bioreactors for Suspension Cultures

Stirred-Tank Reactor (STR)

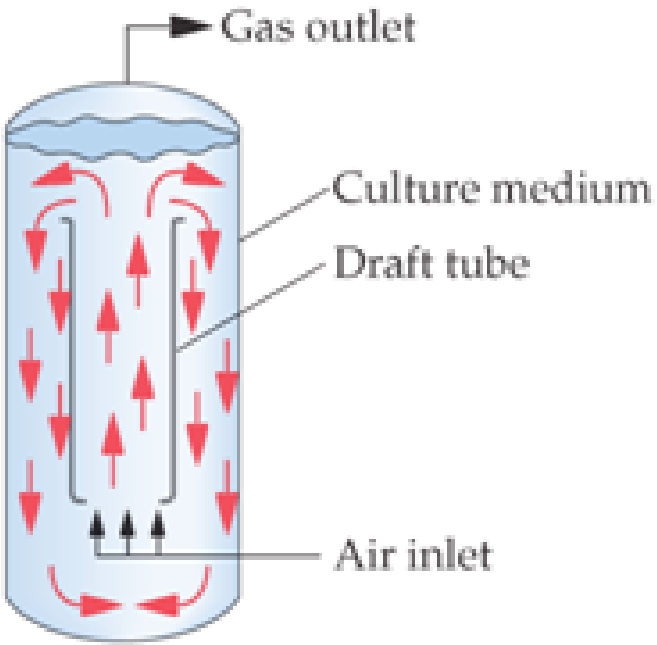


Bubble Column

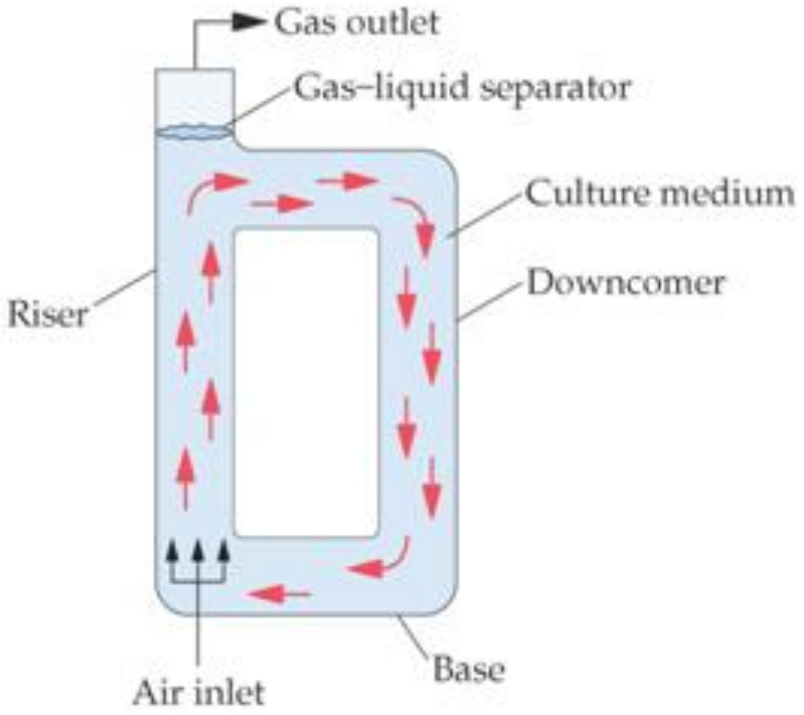


Bioreactors for Suspension Cultures

Airlift Reactor with Internal Loop



Airlift Reactor with External Loop



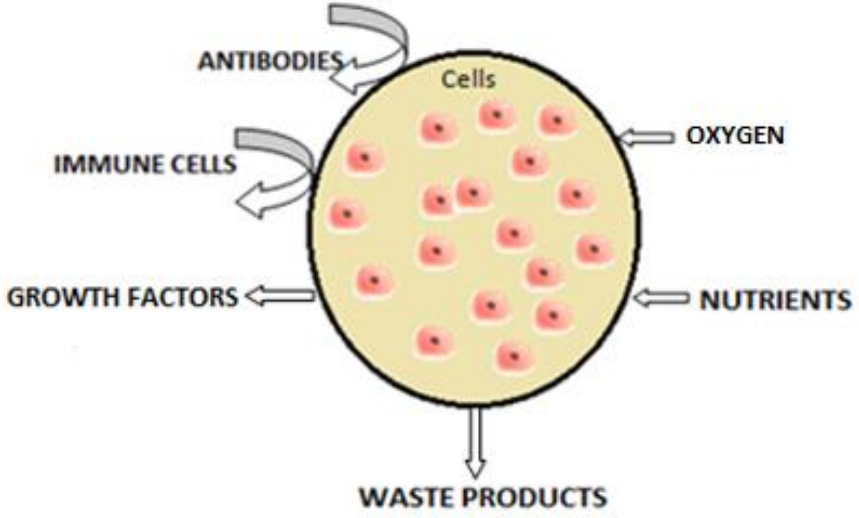
Bioreactors for Suspension Cultures

- STR
  - Traditional and by far the most commonly used
- Pneumatic Reactors

  (Bubble column, Airlift reactors)
  - More energy efficient than STR
  - Elimination of the mixer shaft which is a potential contamination site
  - Lower-shear environment than STR

Bioreactors for Suspension Cultures

- Perfusion culture
  - Cells are retained in the reactor, medium is added continuously, and spent medium is removed.
  - Membrane bioreactors, microencapsulation methods



Products of Animal Cell Cultures

- Biopharmaceuticals
- About 40% of the products are monoclonal therapeutics.
- Generally extracellular proteins
- Require human-like posttranslational modification
- Stem cell technology has great promise.