The Use of Disposable Technologies in Antibody Manufacturing Processes

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Outline

• Rationale for using disposable technologies
• Cell Culture applications (bag focus)
• Purification applications (bag focus)
• Qualification strategy
• Case studies
  – Disposable bioreactors
  – Media / Buffer storage in bags
  – Protein pools in bags
  – Protein bulk drug substance in bags
• Conclusions
Rationale for Using Disposable Systems

• Technologies can offer economic advantages in:
  – Operating expense
  – Capital expenditure
  – CIP / SIP
  – Cleaning validation (between runs, between products)
  – Maintenance (tank inspection, corrosion, etc.)
  – Installation / implementation time

• Improved aseptic / sterile processing and sampling
• Increase plant capacity (turnaround time, space efficiency)
• Consider up-charge on raw materials and disposal costs
Cell Culture Applications

- Disposable Bioreactors up to 500L (or greater) operating volume
- Media & nutrient preparation (dissolution) in disposable containers
- Sterile storage of media and nutrients in disposable containers up to 2500L
- Sterile storage of harvested cell culture fluid in disposable containers up to 2500L
- Sterile sampling
- Sterile / Aseptic connecting devices
- Harvesting using Disposable Systems – disposable depth filters
Purification Applications

- Buffer preparation (dissolution) in disposable containers
- Sterile storage of buffers and protein pools in disposable containers
- Sterile sampling
- Aseptic / sterile connecting devices
Qualification of Disposable Systems (Bags)

- **General Considerations:**
  - Extractables / Leachables
  - Vapor Transmission
  - Integrity / Sterility Validation Testing

- **Application Specific:**
  - Sampling
  - Disposable bioreactors
  - Buffer / media storage
  - Protein pools / Bulk Drug Substance storage

- **Incorporate new technologies early in development:**
  - R&D production / GLP Toxicology
  - Early Stage Clinical Production
  - Late Stage Clinical / Marketed Production
Extractables and Vapor Transmission from Bioprocess Bags using Model Solvents

- Model solvents chosen to mimic aggressive process conditions
- The **complete** bags were tested as one unit (ports, fittings, tubing, & film)
- High storage temp and hold duration
- Small bags used (maximize surface area to volume)
- Measure extractables
  - GC/MS, HNMR, TOC, etc.
- Measure weight loss of liquid (solvent transport through film)
Case Studies Using Disposable Systems

- **Disposable bioreactors**
- **Media / buffer bags & bag containment systems**
- **Protein Pools in bags**
- **Bulk drug substance in bags**
  - Shippable bags
  - Freezable bags

**BAG FEATURES**
- Sterile
- Mixing
- Sensors (pH, DO, Temp)
- Integrated Volume Measurement
- Sampling
- Transportable
Disposable Bioreactor Technology Choices*

- HyNetics
- HyClone
- ATMI
- LevTech
- Xcellerex
- Stedim/Applikon
- Wave Biotech
  - Wave Reactor
  - FlexMixer

*Any of these systems could be suitable for solid-liquid dissolution (media or buffer prep) or protein pools
Inflated plastic bag forms a disposable cultivation chamber.

Rocking Motion

Wave motion sweeps up cells and prevents settling.

Wave action creates large turbulent surface for oxygen transfer.

Cell culture media

Wave action sweeps up cells and prevents settling.
Oxygen Mass Transfer \( (k_{La}) \) in Wave Bags

- Tests performed with water
- \( k_{La} \) increases with angle and rock rate
- Operating at 30-50% volume can still achieve \( k_{La} \)’s over 20 h\(^{-1}\)
Wave System1000

- Evaluated at Genentech for production of monoclonal antibodies in CHO
- 500 liters maximum working volume
- System allows temperature, pH, and dissolved oxygen control
Monoclonal Antibody Production in CHO using Wave System 1000 Bioreactor

- Wave compared side by side with standard 400 liter stirred tank reactor
- High density CHO culture
- Operated under Temp, pH, and DO control.
- Wave system showed excellent cell growth and antibody production characteristics
- Analyses have shown comparable product and impurity profiles
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Media / Buffer bag configuration up to 2500 liters*

*Bags >500L benefit from suspension systems

Vent filter for repeated use

Fill Line

Sterile Inlet Filter

Flush Bag

Sample bags

Drain Line to Chrom Skid; 1.5” Triclamp / Disposable connector
Buffer Bag Holders

- There are several “off the shelf” choices for basic bag holders
- Containment up to 2500 liters
- Smaller systems are stackable
- Bottom feed for easier access when stacked
- Bottom drain for better liquid recovery & priming

200 Liters

500 Liters

Photograph Courtesy of Stedim, LLC website
Bags with In-Line Dilution

- Most buffers can be concentrated >>10-fold
- Buffer concentration allows bags to be used even at very large scale production
- Bags offer corrosion resistance
- Use concentrated buffers in bags then dilute, in-line, to target concentration
- Deliver diluted buffer to unit operations
Inline Dilution of Buffer Concentrates with Bags

1. **Prep Normal-Strength Buffer**
   - Buffer Prep
   - Filter
   - Buffer Hold
   - Filter
   - Process

2. **Prep Concentrated Buffer**
   - Conc. Prep
   - Filter
   - Conc. Hold
   - Water
   - Filter
   - Process

3. **Buy Concentrated Buffer**
   - Conc. Bag
   - Water
   - Filter
   - Process
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Protein Pools in Bags – Recirculation Mixing Example

- Various mixing technologies are available: See Disposable Bioreactor technologies
- Allows bags to be used for intermediate protein pool storage
- Liquid – Liquid mixing strategy using bags
- Not as useful for solid – liquid mixing (dissolution).
- Use of standard bag holder and peristaltic pump
- Slight modification to bag to facilitate sterile recirculation path (Aseptic connecting devices / tubing welders)
- Satisfactory mixing was demonstrated even with high viscosity and density differences
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Bulk Drug Substance in Bags

• Bags can be used for longer-term storage of bulks in bags if product stability permits
• Product can be held as liquid (scales up to ~500 liters) or frozen in bags (at smaller scales: up to about 20 liter per bag currently)
• Great care must be taken to ensure bag integrity is maintained
• Convenient format that allows low-cost storage alternative to stainless steel or higher-alloy tanks
• Useful format for shipping to filling sites
• Product stability testing must be performed on a case-by-case basis
Bags for Liquid Bulk Shipment

• Reduce dependence on stainless or higher alloy tanks for storage of protein solutions
• Heavily used for shipment of WFI, media, buffers, serum.
• 500L is typically maximum shipping volume
• Shipping containers have to be economical but extremely robust
• Bag integrity must be maintained – compare to standard tanks
• Economic evaluation should be performed to determine “acceptable” failure rate 1:1,000? 1:10,000?

200L Commercial System

200L System in Envirotainer at Genentech
Freezable Bags for Bulk Storage

- Bags can be used for frozen storage of protein bulks
- Specialty films available
- Special containment systems to protect bag and tubing must be used
- Freezing and thawing rate can be difficult to control
- More useful with small bulks (<20 liters)
- There are commercially available systems for this application
Conclusions

• Disposable systems offer many advantages in the production of monoclonal antibodies in cell culture, purification, and bulk storage areas.

• Product offerings are becoming more comprehensive and can penetrate many areas of clinical and commercial manufacturing facilities

• Current disposable technologies (bags, fittings, filters, connecting devices) are validate-able for use in cGMP production of parenteral products

• Disposable probes for bioreactors (pH, DO) need improvement to make useful for the rigors of production environments

• Genentech anticipates future production facilities will be designed around the concept of disposable technologies
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