Breakthroughs in Bioprocessing

Review of Single Use Technologies in BioManufacturing

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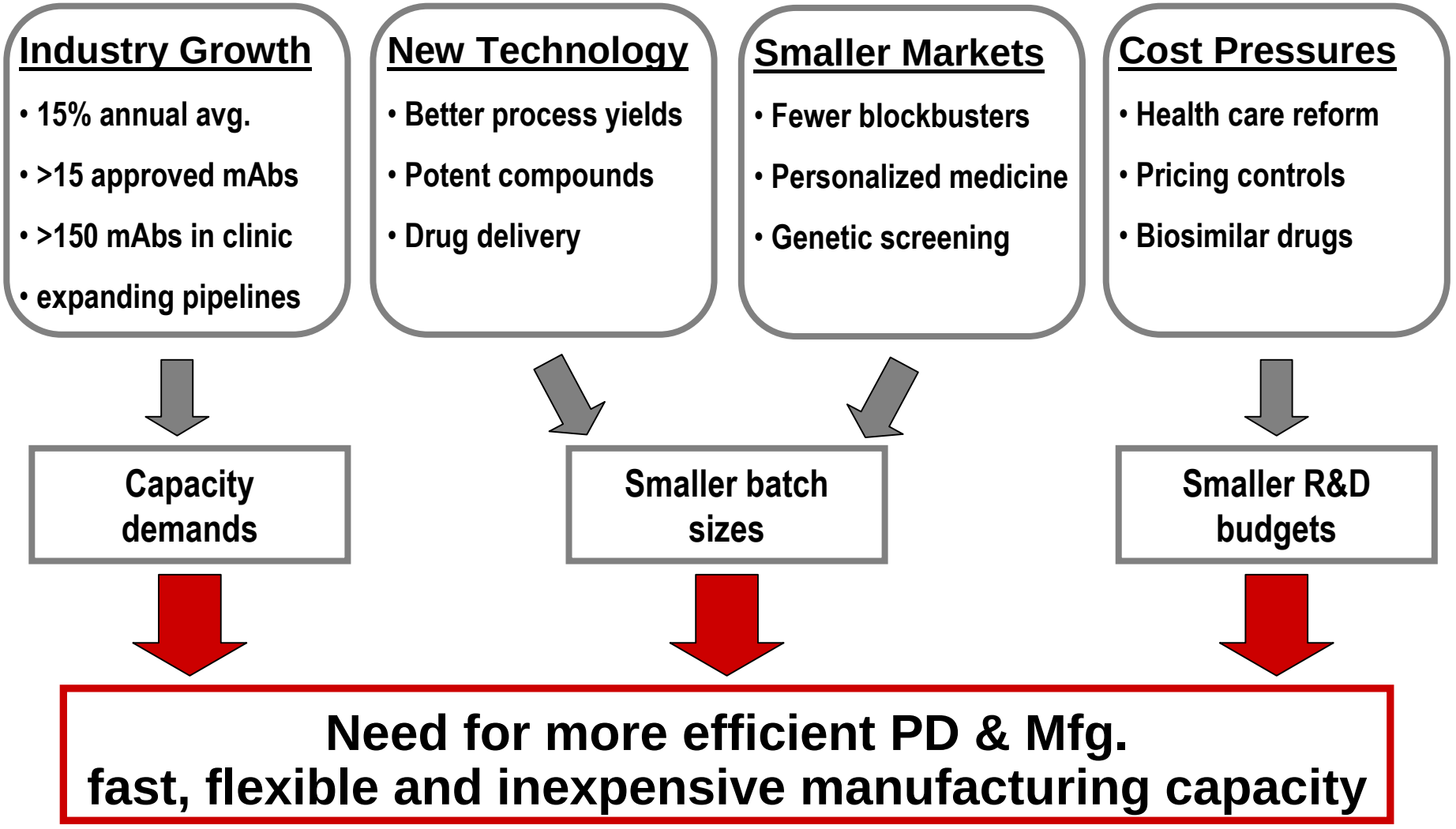
Outline

- Introduction – Strategic Outlook for BioPharma
- Advantages and Limitations of Single Use Systems
- Integration/Scale Up Challenges of Disposables
- Single Use Bioreactor Data
- Conclusions

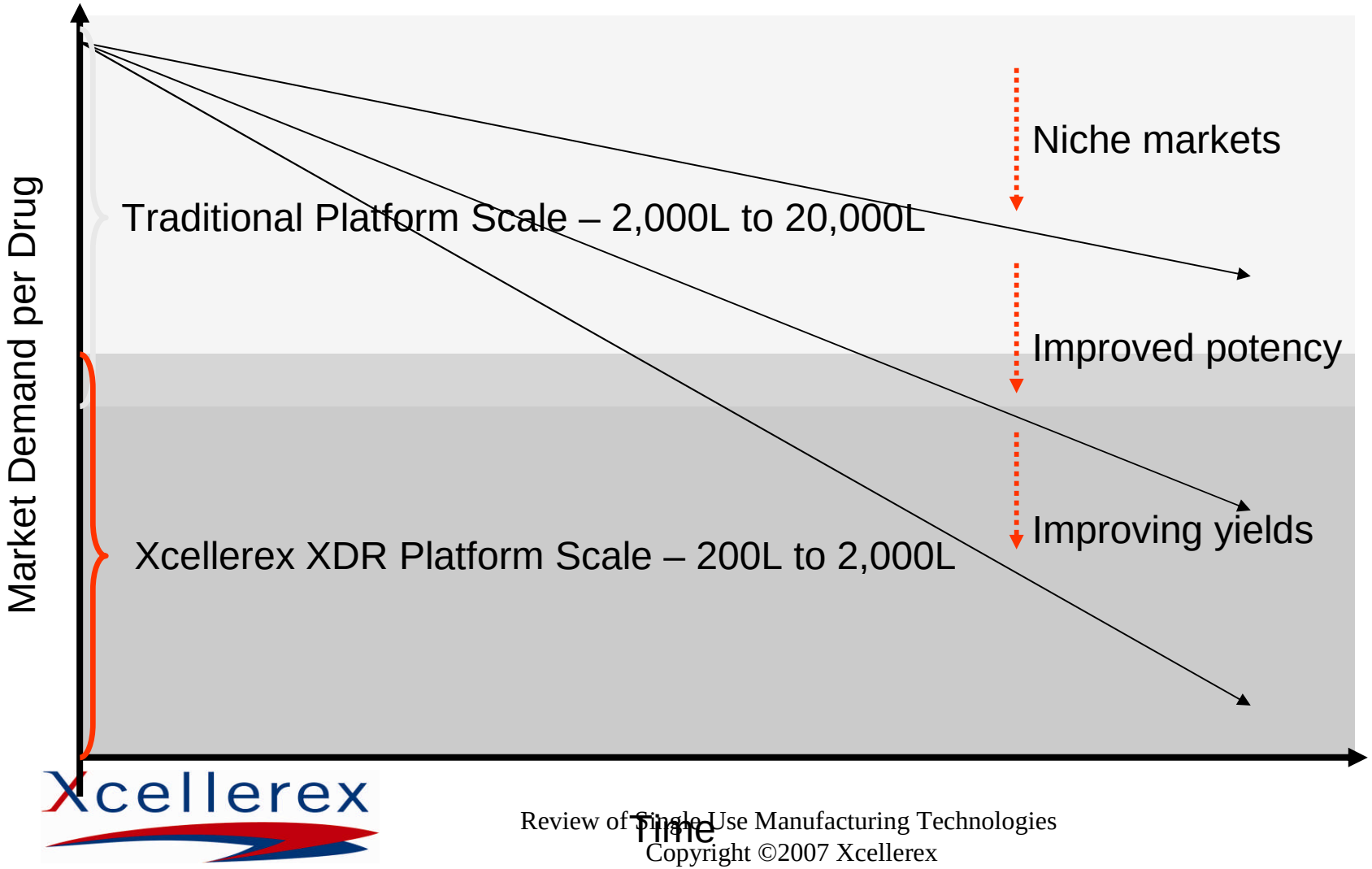
BioPharma Strategy - New Paradigm



Xcellerex

Xcellerex's Technology will be Commercial Scale

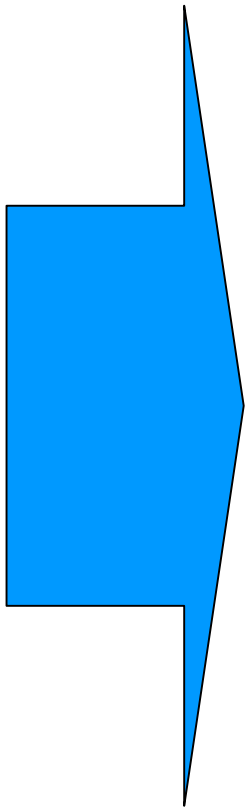
Future Quantity Per Drug Demand for Biologics is decreasing



Advantages of Single Use Systems

Reductions in:

- Cleaning
- Sterilization
- Engineering cost
- Equipment lead time
- Utility requirements
- Validation
- Quality / Regulatory burden
- Space
- Labor
- Waste generation



Improvements in:

- Manufacturing quality
- Capital investment
- Facility buildout time
- Cycle time
- Flexibility
- Environmental impact
- COGS

Limitation of Single Use Systems

- Mixing/Buffer/Media prep – Rate of liquid transfers
- Bioreaction – Lab to commercial scalability within the same reactor design/configuration
- Cell Harvest/TFF - Recirculating processes that require high pressure and high flow rates
- Disposable Chromatography is still TBD
- Non-standard, multiple connection options
- Disposable sensors are limited

Technology Survey

Products and vendors listed in the following slides are provided for reference and do not constitute a complete list or an endorsement of any specific vendor or product

Enabling Single Use Technologies

- **Bioprocess bags**
- **Cell culture bioreactors**
- **Separations (Cfg., TFF, rotary drum, filters)**
  - Harvest
  - Virus removal / sterilization
  - Concentration / buffer exchange
- **Purification – membranes**
- **Tubing welders / connectors / sealers**
- **Integrating stainless and disposables connectors**
- **Sensors**

Mixing Systems

Applications: Media, buffer, product processing, formulation

Capacity: 10 L to 10,000 L

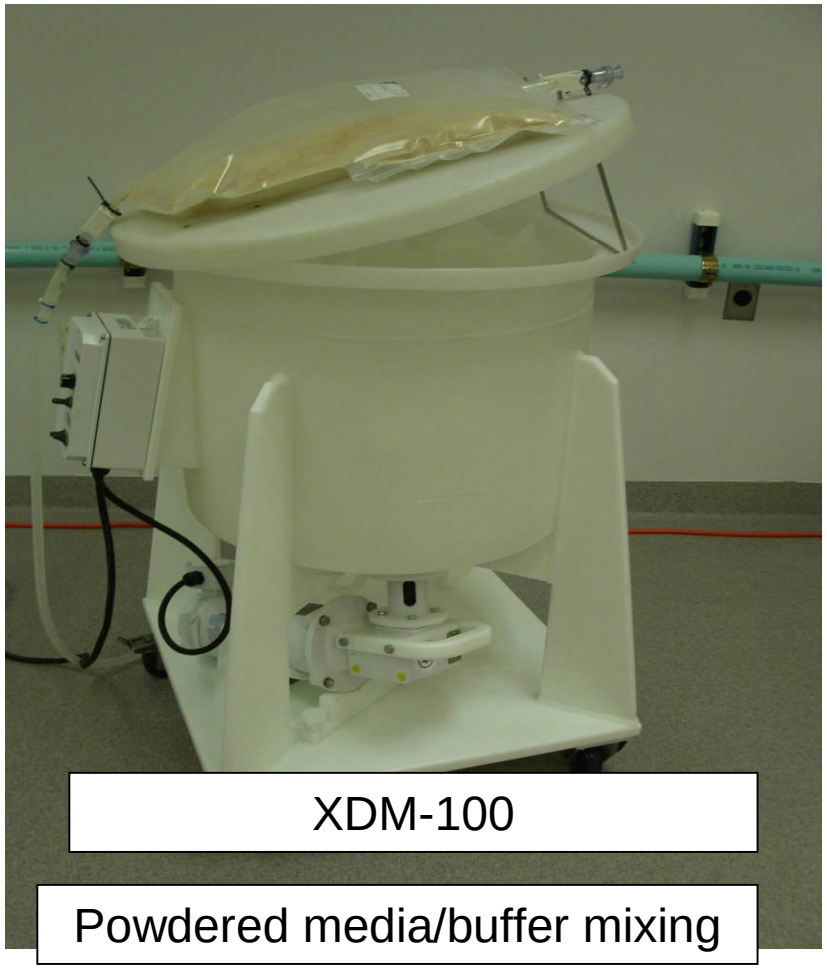
Vendors/Types: Hyclone MixTainer, LevTech/Sartorius levitated prop tank, Wave FlexMixer, Xcellerex XDM stirred tank

Integration Challenges: powder addition, connectors

Scale Up Challenges: powerful mixing, bags that flex to achieve mixing rely heavily on bag seam strength and durability

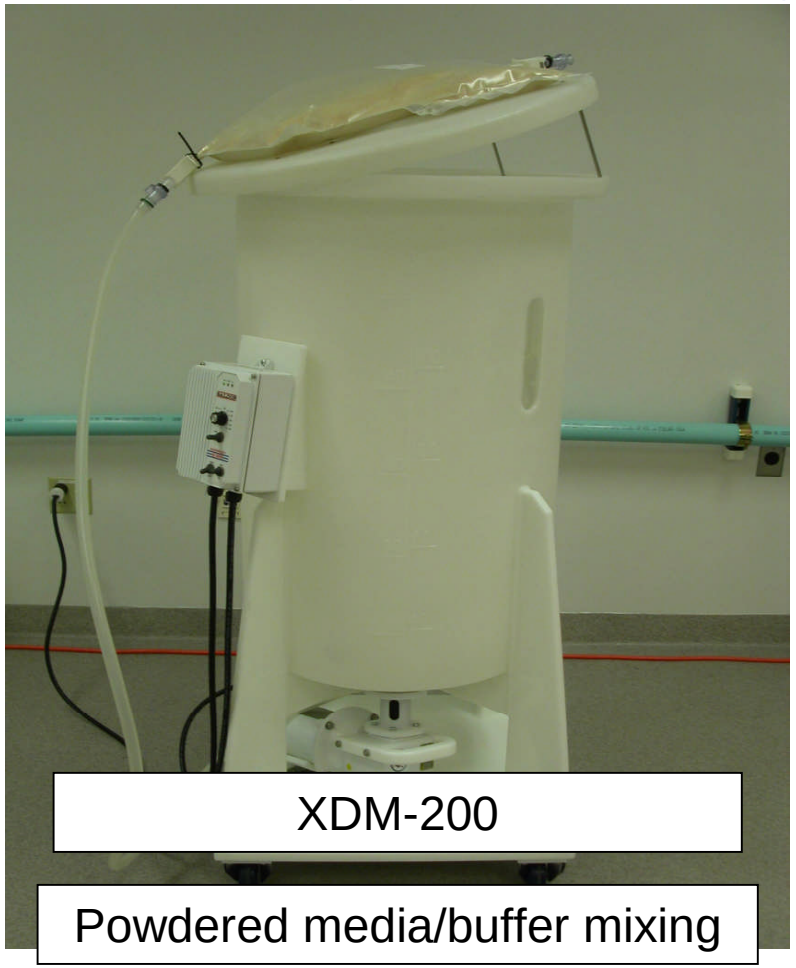
XDM-100 and 200 Disposable Tank Mixer

Invitrogen/Xcellerex XDM Commercial Disposable Mixers for powdered media mixing and delivery



XDM-100

Powdered media/buffer mixing



XDM-200

Powdered media/buffer mixing

BioProcess Bags/Tank Liners

Applications: culture media and buffers, product, samples or waste.

Capacity: 10 mL to 2500 L (up to 10,000 L)

Vendors: HyClone, Stedim, TC Tech, Charter, Newport: USP Class VI tested, gamma irradiated

Types: Monolayer, multilayer, LDPE, EVA, etc.

Integration Challenge: connectors not common

Scale Up Challenge: bag seam weld strength, rate of fluid transfers, robust, cheap tubing >1" diameter

Small Scale Cell Culture Bioreactors

Application: Culture of eukaryotic cells

Capacity: ~10 mL – ~10 L / 25K cm^2

Vendors: Corning, Nunc, Wave, Bellco

Types: TC flasks, rollers, spinners, shake flasks, hollow fibers, expanded T-flasks (Cell Factory, Cell Cube), novel bioreactors (Wave, BelloCell)

Integration challenges: tubing/connectors sizes and compatibility

Scale Up Challenge: model system that scales to 10,000L

Mid Scale Cell Culture Bioreactors

Applications: Culture of mammalian, insect or plant cells in suspension. (Many vendors supply large hollow fiber systems for anchorage dependent cell culture).

Capacity: 1 L – 200 L

Vendors: HyClone SUB stirred tank, Wave Biotech rocking system, Xcellerex XDR stirred tank bioreactor

Integration Challenge: liners, connectors, sensors, filters, controllers

Scale Up Challenge: model system that scales to 10,000L

Xcellerex

XDR-200 working volume disposable stirred tank reactor

Large Scale Cell Culture Bioreactors

Application: Culture of mammalian, bacterial, yeast, insect or plant cells in suspension.

Capacity: 200-1,000L working volume

Vendors: Wave Biotech 500L rocker, HyClone SUB stirred tank, Xcellerex 1,000L stirred tank bioreactor

Integration Challenge: liners, connectors, sensors, filters, controllers

Scale Up Challenge: avoid stressing bag seams, small scale modeling system that scales to 10,000L

Xcellerex 1,000L (wv) Disposable Stirred Tank Reactor - XDR™

Xcellerex

Cell Harvest

Application: Separation of cells from growth medium during perfusion or for terminal cell harvest.

Capacity: Up to 100-200 L/hr

Vendors/Types: Kendro (centrifuge), Steadfast (rotary drum filter), Spectrum and GE (recirc. hollow fiber), Millipore POD system (dead end), Cuno depth filtration. All product contact surfaces disposable

Integration Challenges: connectors

Scale Up Challenges: recirculating systems: disposable tubing not amenable to high flow rates and pressures

Dead End/Depth Filtration

Application: Clarification / sterilization of media, buffers and process intermediates, cell harvest, and removal of particulates.

Capacity: Syringe filters, 30” capsules, flat membrane generally available, (larger by custom order)

Vendors/Types: Millipore POD, Pall, Sartorius, Meissner, Cuno – larger capsules coming available, many available pre-sterilized and integrity tested.

Integration challenges: connector compatibility

Scale Up challenges: >1000L capacity is lacking

Tangential Flow Filtration

Application: Perfusion, cell harvest, purification, concentration, and formulation / buffer exchange.

Capacity: Up to 5.6 m^2

Vendors/Types: Spectrum HF, GE hollow fiber

Integration: disposable pump integration that is durable yet disposable

Scale Up Challenge: recirculating systems: disposable tubing not amenable to high flows/pressures

Virus Reduction

Application: Mechanical reduction of viral load by nanofiltration.

Capacity: 15 - 200 L/hr. (depending on pore size, filter medium & process stream)

Vendors/Types: Millipore dead end, Pall dead end, Asahi-Kasei

Integration Challenges: connectors

Scale Up Challenges: larger scale requires more area

Purification – Membranes

Application: Flow-through removal of contaminants, bind-and-elute purification of small or dilute process streams.

Capacity: 20 L/min., 5g DNA binding capacity

Vendors/Types: Pall, Millipore and Sartorius functionalized filter membranes.

Integration Challenges: connectors, area

Scale Up Challenges: less binding capacity compared to chromatography resins in general

Vial Filling

Applications: Aseptic filling into vials

Capacity: Clinical to commercial {?}

Vendors/Types: Millipore Acerta bag based filling system, MedInstill injection filling/laser sealing

Integration Challenges: connectors

Scale Up Challenges: not clear yet

Sensors

Applications: Process wide

Capacity: N/A

Vendors/Types: Wave Biotech, (pH, DO2), Flourometrix and PreSens optical sensors, microprobes

Integration Challenges: Cytoxicity, irradiatability, fit up into bags, tubing, dead zone elimination, signal response time

Scale Up Challenges: stability, non-fouling, validatable

Sterile Tubing Connectors

Applications: Aseptic / sterile connections

Capacity: 1/4” to 3/4” OD tubing

Vendors/Types : CPT (C-Flex), Pall ACDs, BioQuate, Millipore. CPT connector is similar to a tubing welder. Pall & BioQuate connectors are similar to a quick-connect which can make sterile connections in a non-sterile environment.

Integration Challenges: no one system connects all and they are one time use (versus welders that can reweld)

Scale Up Challenges: connectors >1” diameter

Connectors - Tubing Welders

Application: Aseptic / sterile connections

Capacity: 1/4” to 3/4” OD tubing

Vendors/Types: Terumo, Wave, Sebra. Several devices have been validated by the vendor and/or biopharm manufacturers. Can be used on PVC and EVA (Sebra), or Tygon, C-flex and Pharmed (Terumo, Wave) tubing.

Integration Challenges: no one system welds all

Scale Up Challenges: welding tubing >1” diameter

SIP Tubing Connectors

Application: SIP-able connections between disposable tubing to stainless steel valves.

Capacity: 1/2” ID x 3/4” OD

Vendors: Millipore, Colder, TC Tech

Comments: Can be added as option to tubing on bioprocess bags for sterile transfer to or from stainless steel systems.

Integration Challenges: no one connects all

Scale Up Challenges: none

SIP Filter Capsules

Application: Disposable filter capsule capable of withstanding temperature and pressure required for steam-in-place.

Capacity: 5” housing

Vendors/Types: Pall - Can house vent or liquid sterilizing filters or virus removal filters.

Integration Challenges: connections

Scale Up Challenges: larger size not available

Sampling System

Applications: Used in place of sampling ports on stainless steel tanks.

Capacity: 20 mL - 1 L sample bags

Vendors/Types: NovAseptic - sheathed cannula, tubing set and bag as a pre-sterilized, closed system. Outside of sheath sterilized during vessel SIP. Can load multiple cannulae into single septum sample port.

Integration Challenges: none

Scale Up Challenges: none

Technology Trends

- Standard connectors
- Non-recirculating TFF
- Integration of existing stainless to disposable
- Development of new enabling technology
  - Sensors
  - Chromatography (scaleable and economical)
- Fully disposable biomanufacturing >1,000L
- Yeast, bacteria, fungal systems coming!

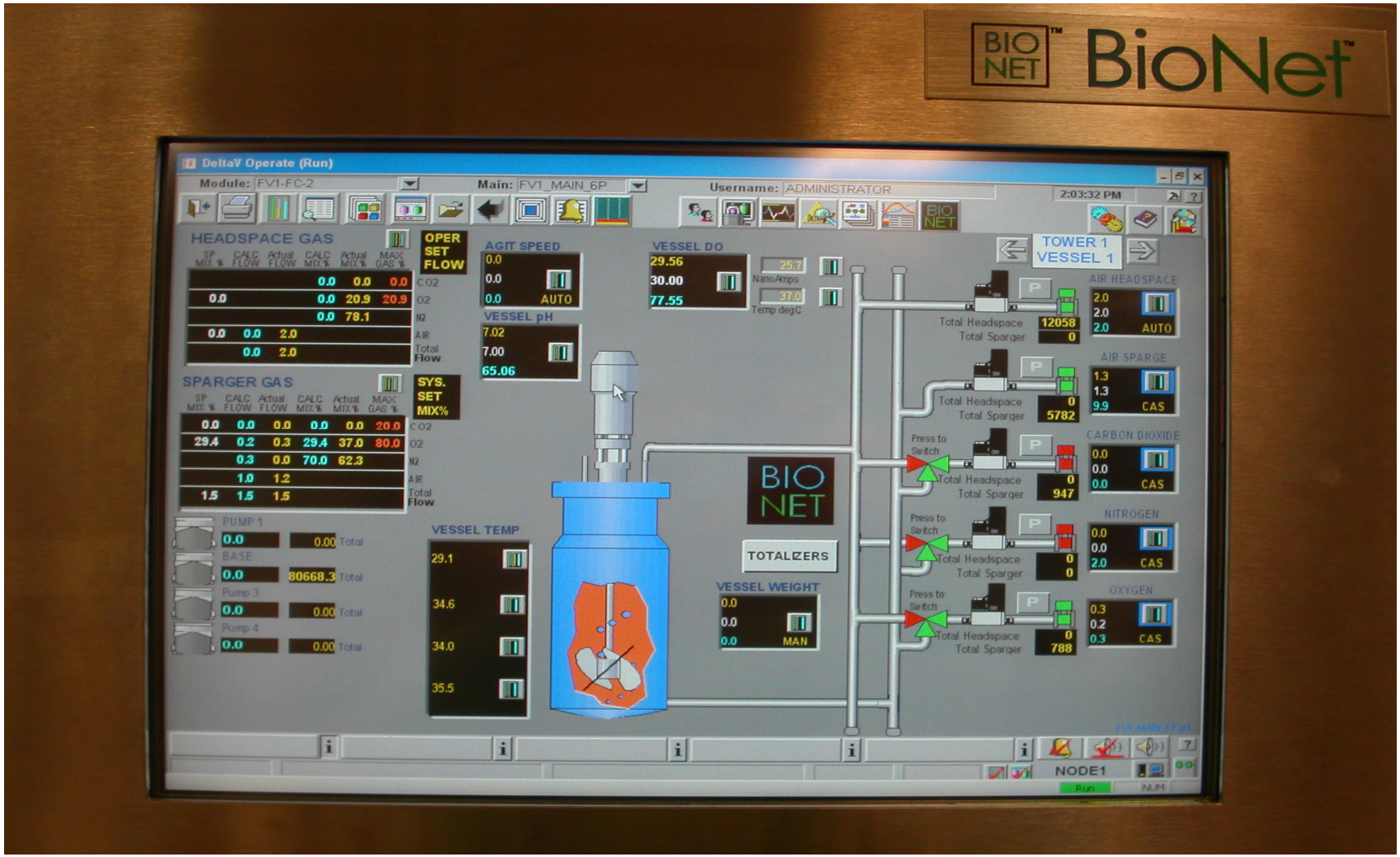
Types of Cells Grown in XDRs

Cell type/product	Mode	scale of runs done L (wv)	2007 planned runs
Hybridoma/mab	fedbatch	2x 200 - GMP	200 - GMP
CHO/mab-fusion	batch	2x200, 2 x 1,000L	1,000L
CHO/fus. protein	fed batch	2x 200, 3 x 500 GMP	200, 500 - GMP
Insect S2/vaccine	fed batch	200	200 - GMP
Insect SF9/vaccine	fed batch		200, 2,000
Human/fus. protein	perfusion	200	1,000
Yeast (Sacc.)	fed-batch	150	1,000
E. Coli	fed-batch		200
Vero/vaccine	batch		200, 1,000

GMP XDR-200 and 1000 Systems

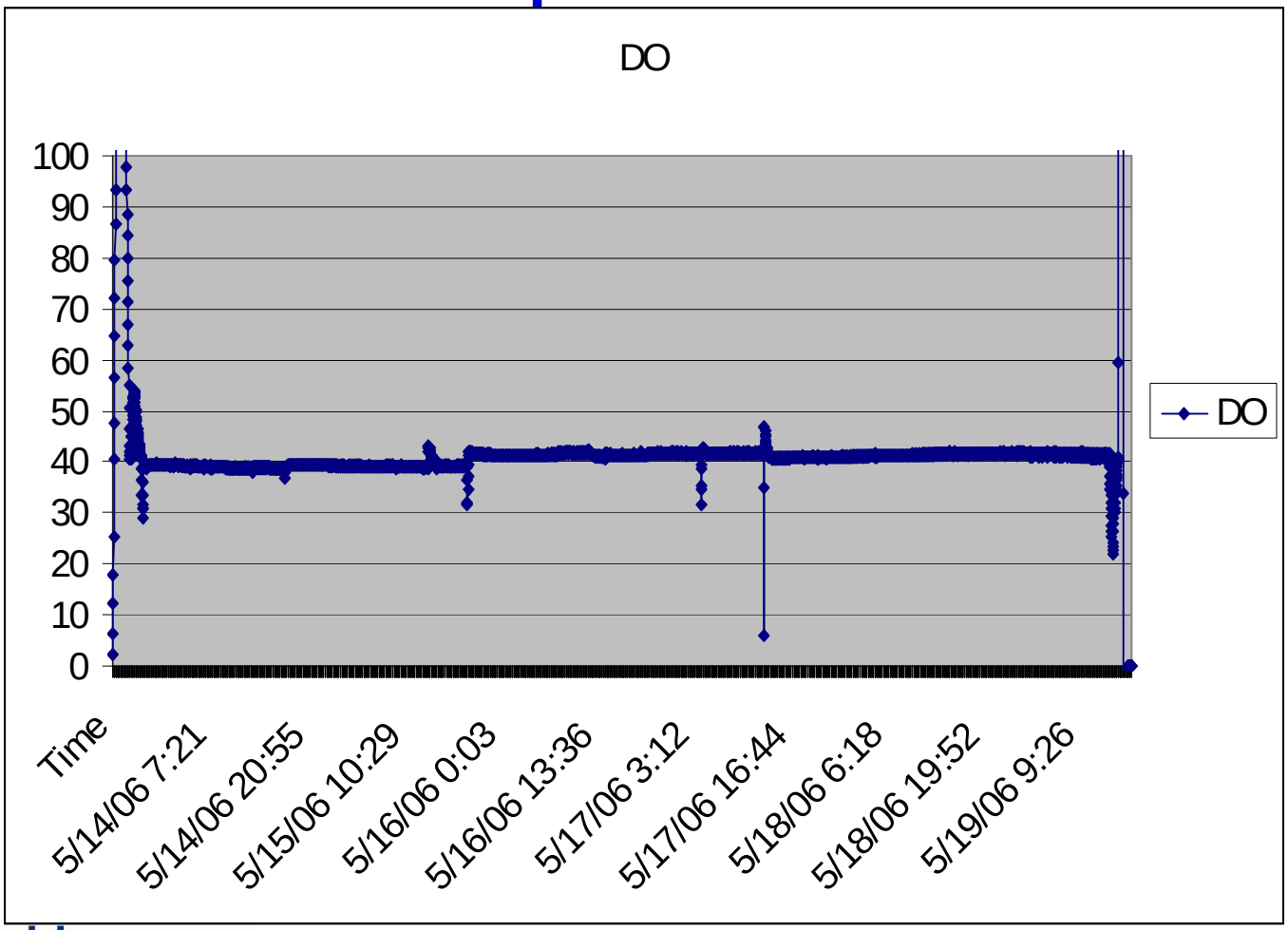
Xcellerex

BioNet DeltaV GMP Process Controller

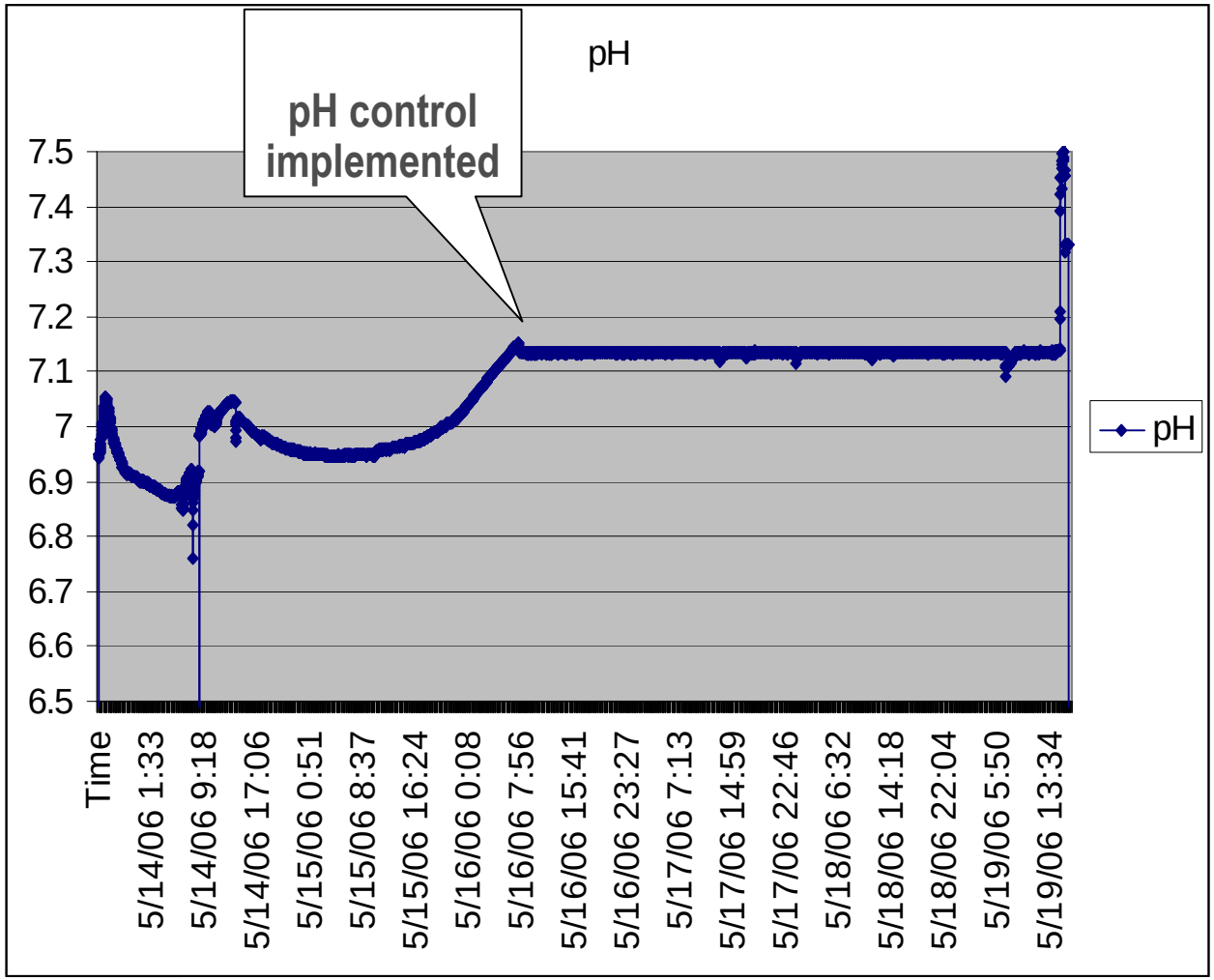


Xcellerex

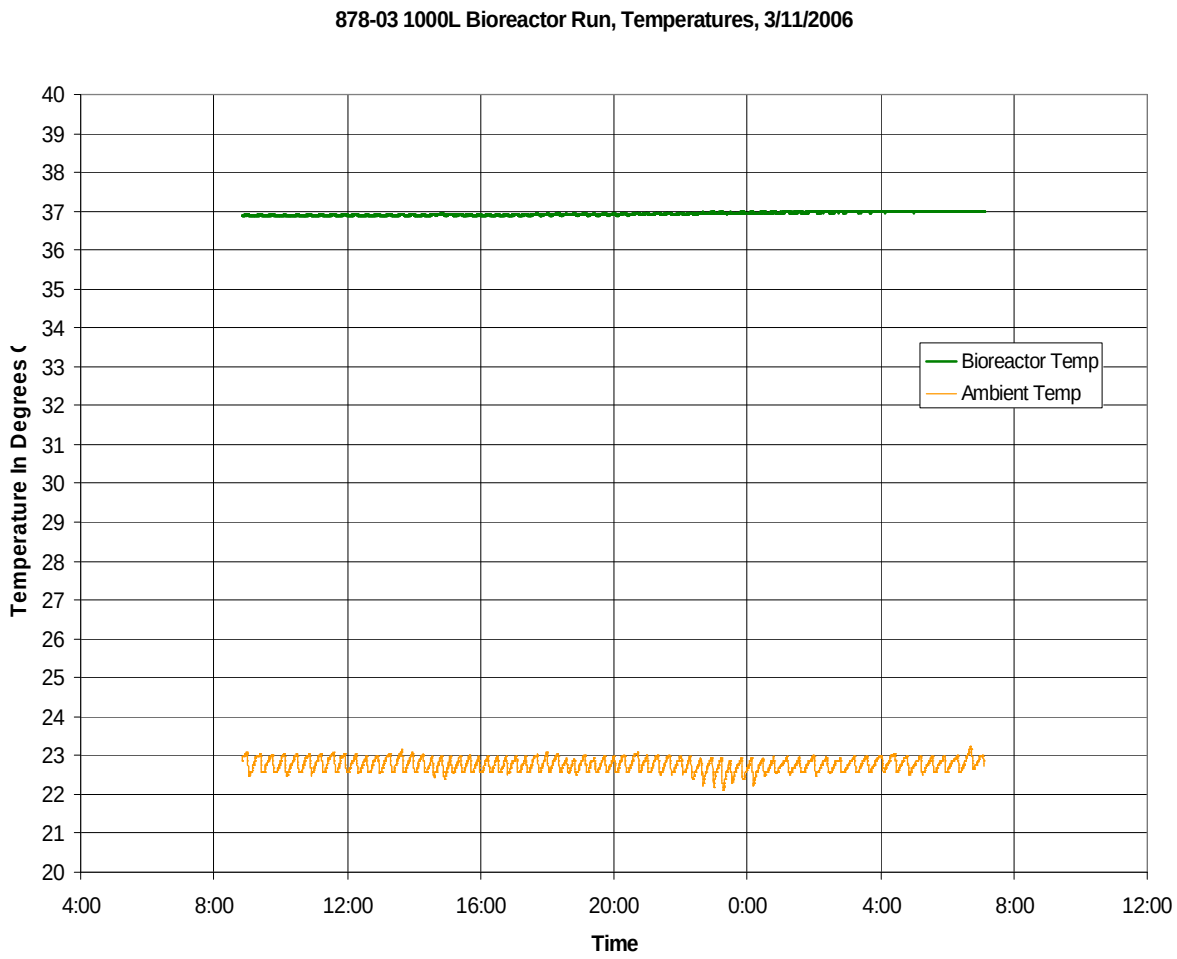
XDR-1000 DO Control, fusion protein



XDR-1000 pH control, fusion protein

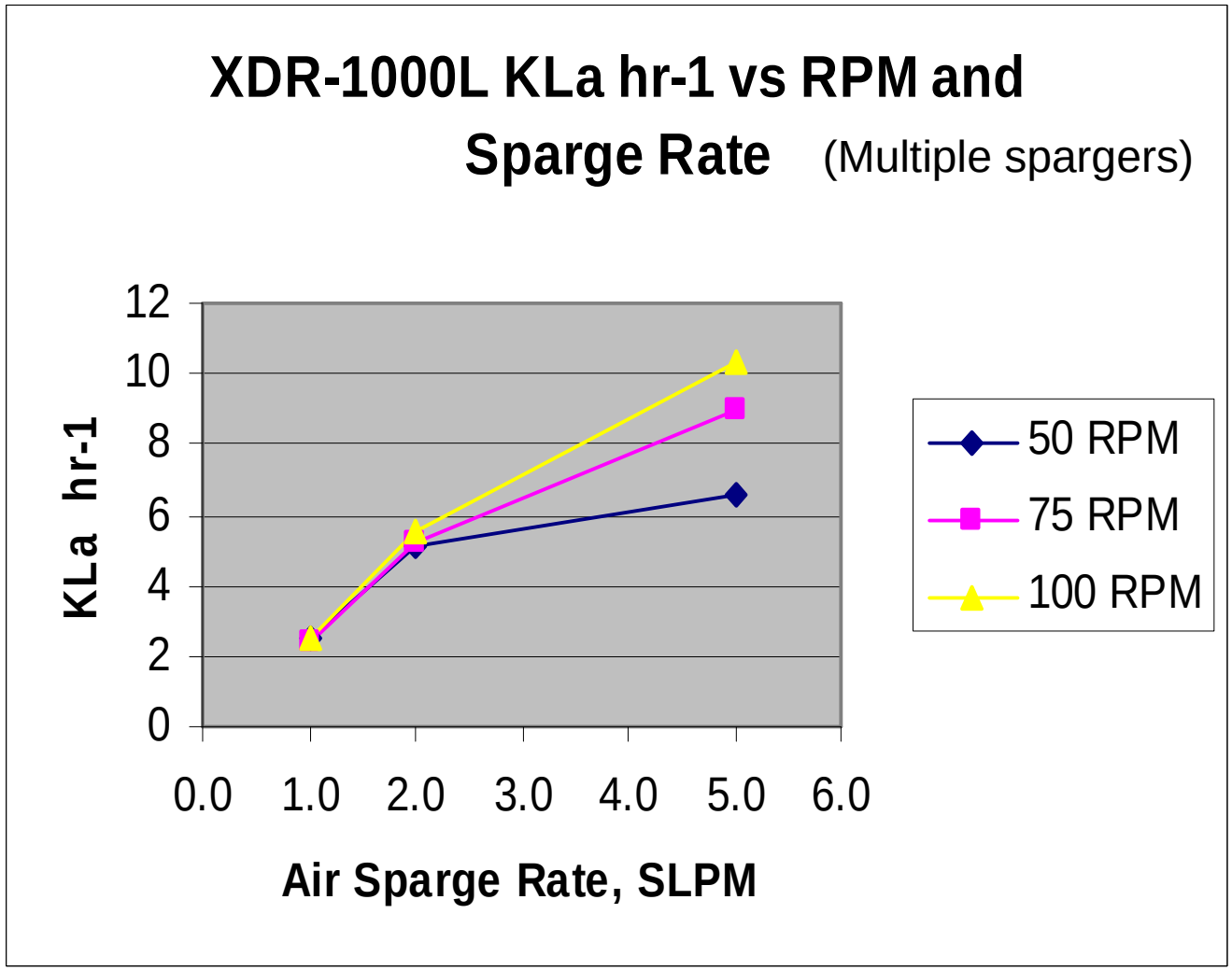


XDR-1000 Temp Control

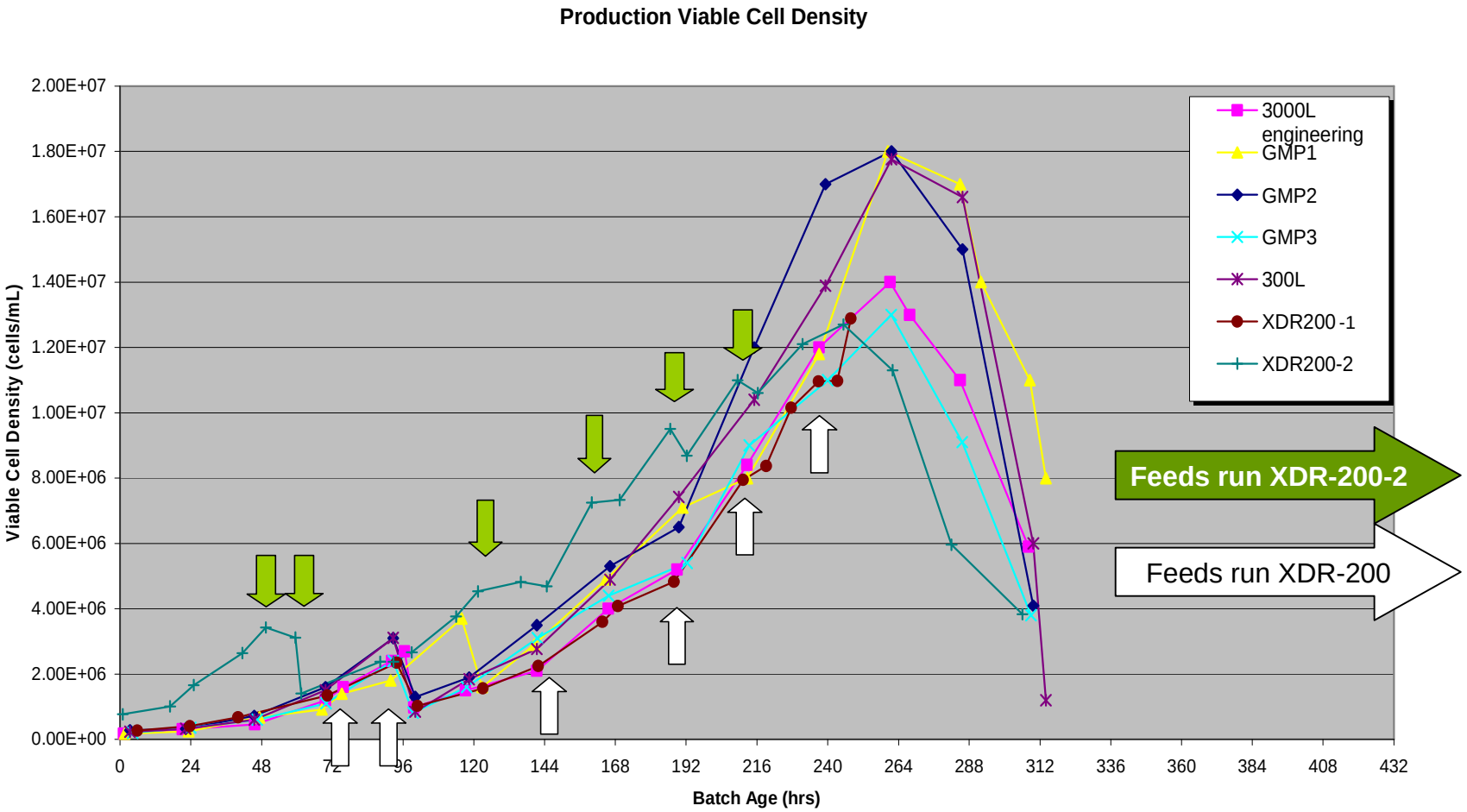


XDR-1000 Mass Transfer
KLa measurements, single sparger

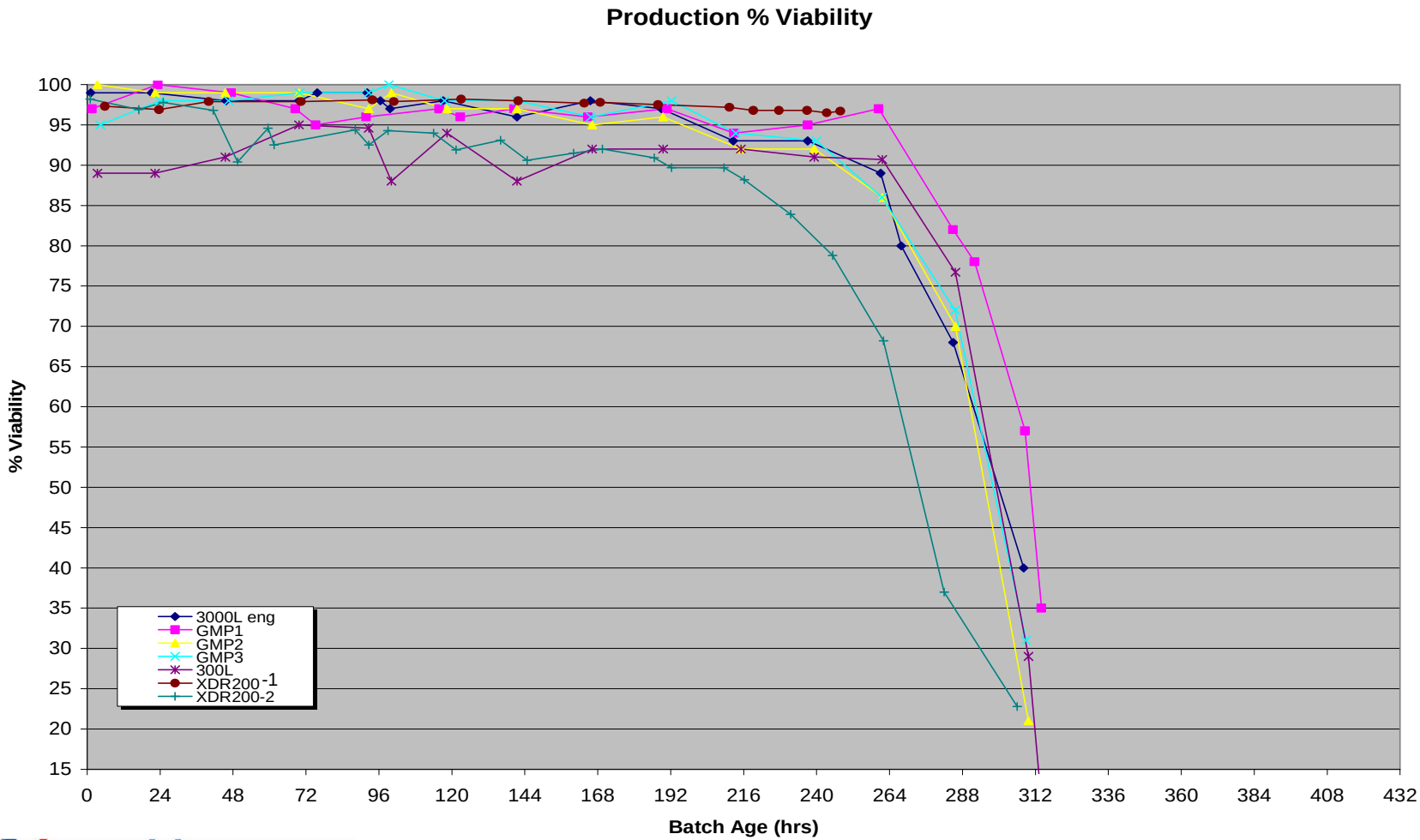
XDR-1000 - F impellers, Air Only, 37C, 6 g/L Salt, F-68 1 g/L, polyol antifoam					
		air flow			
rpm	5 SLPM	10 SLPM	15 SLPM	P/V w/m3	impeller shear sec-1
100	6.84 hr-1	6.61 hr-1	7.09 hr-1	2.5	15
132	7.67 hr-1	9.04 hr-1	9.48 hr-1	5.8	20
167	7.32 hr-1	8.49 hr-1	ND	11.8	25



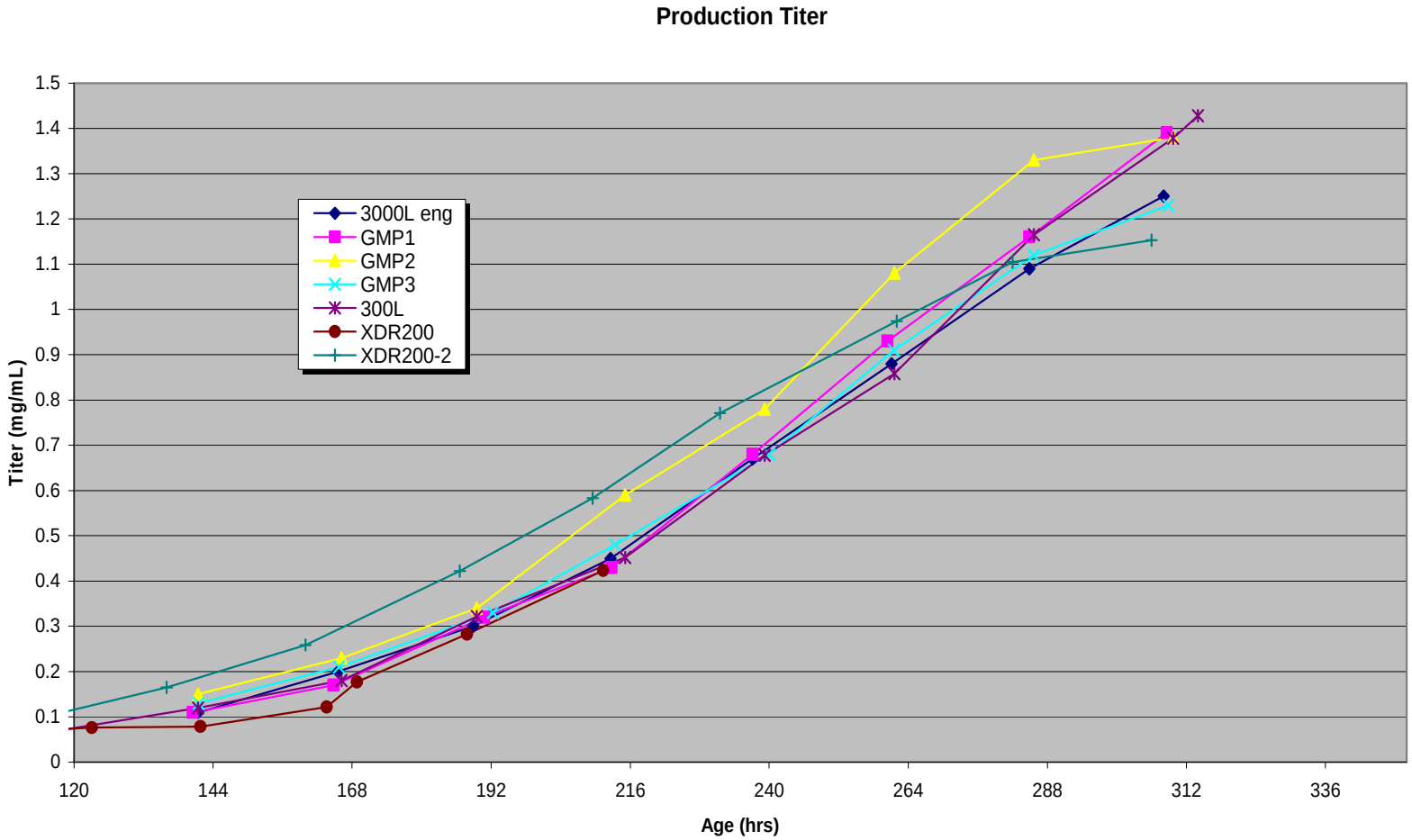
XDR-200 Scalability Comparison Cell Density



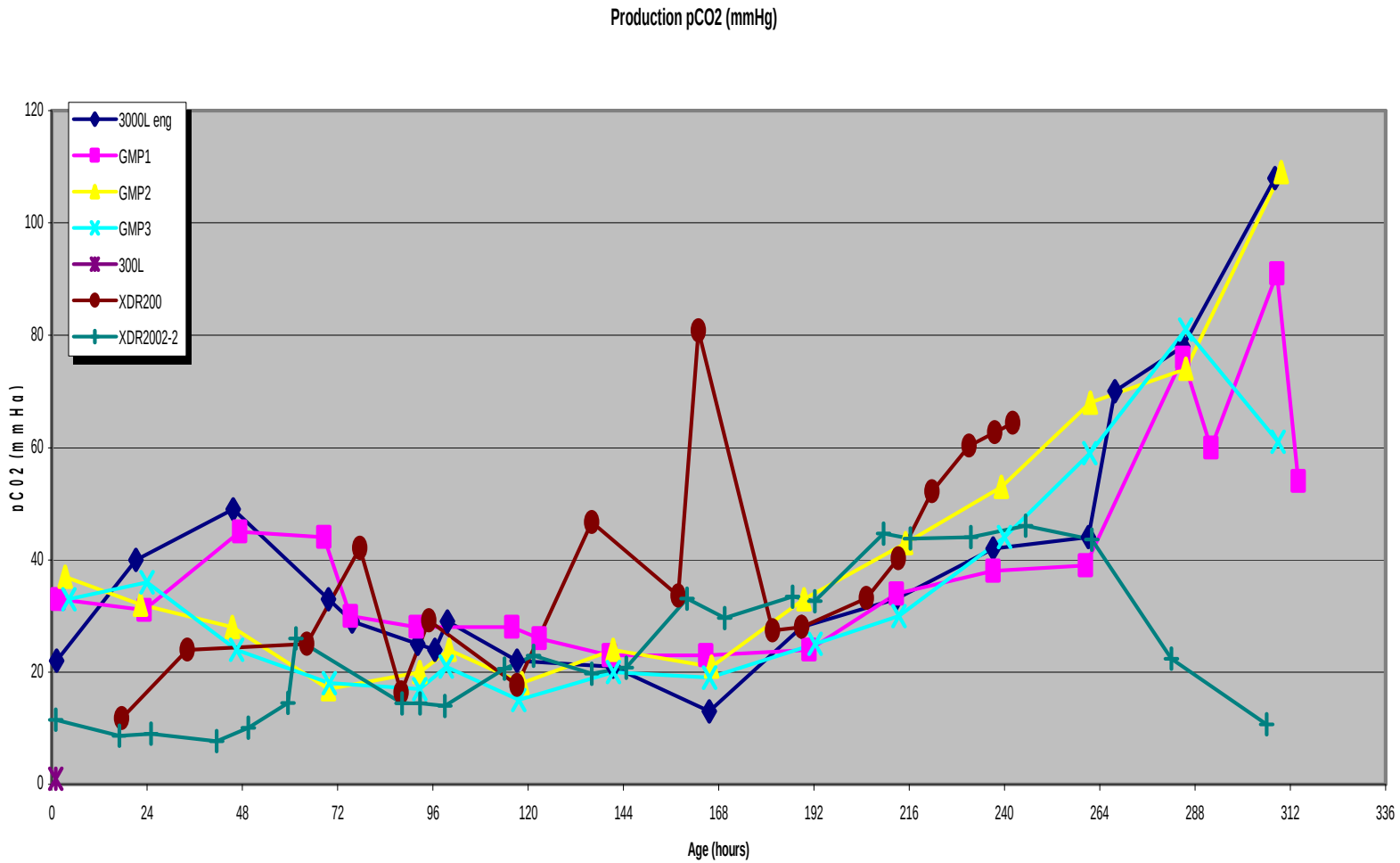
XDR-200 Comparison, Viability



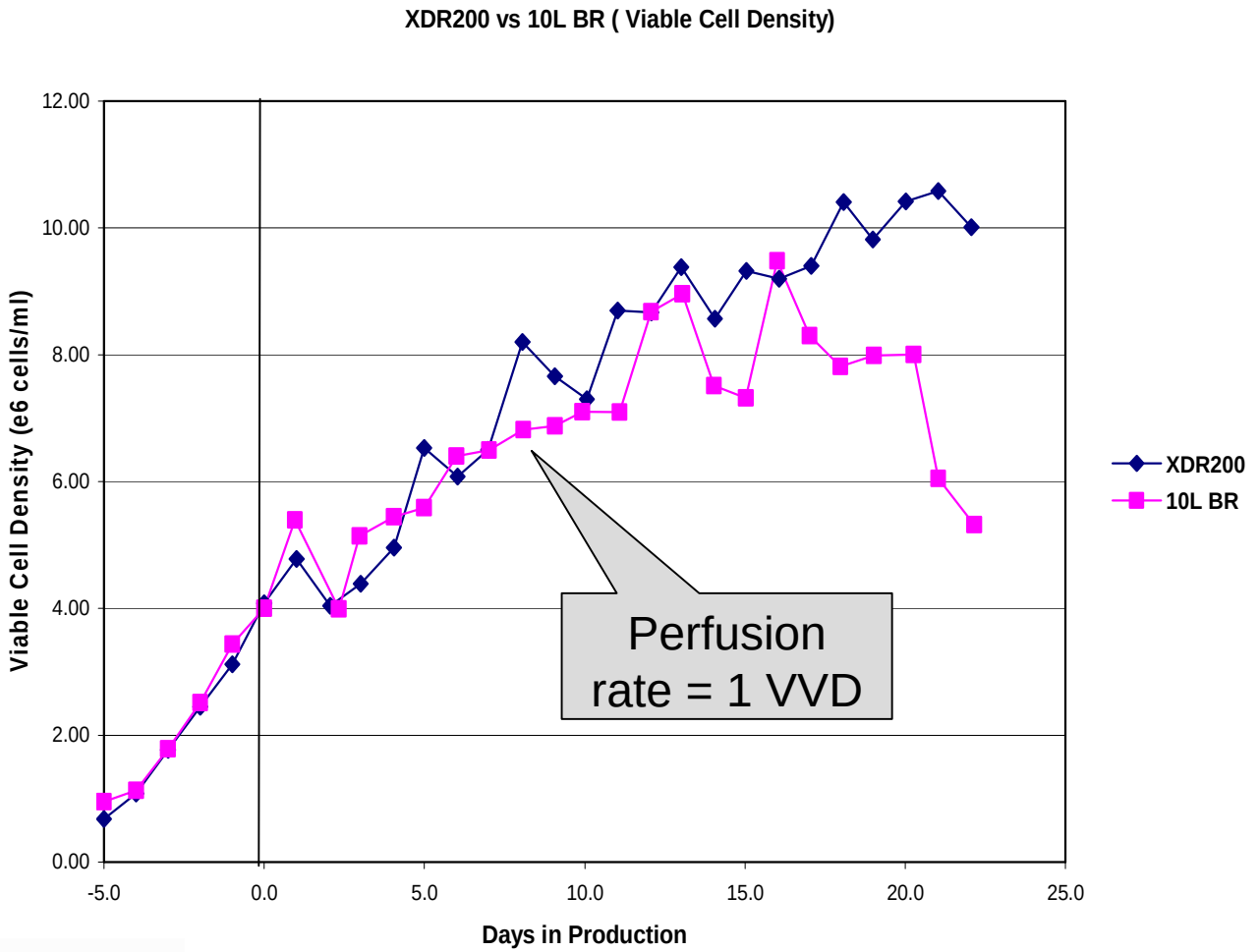
XDR-200 Comparison Titer



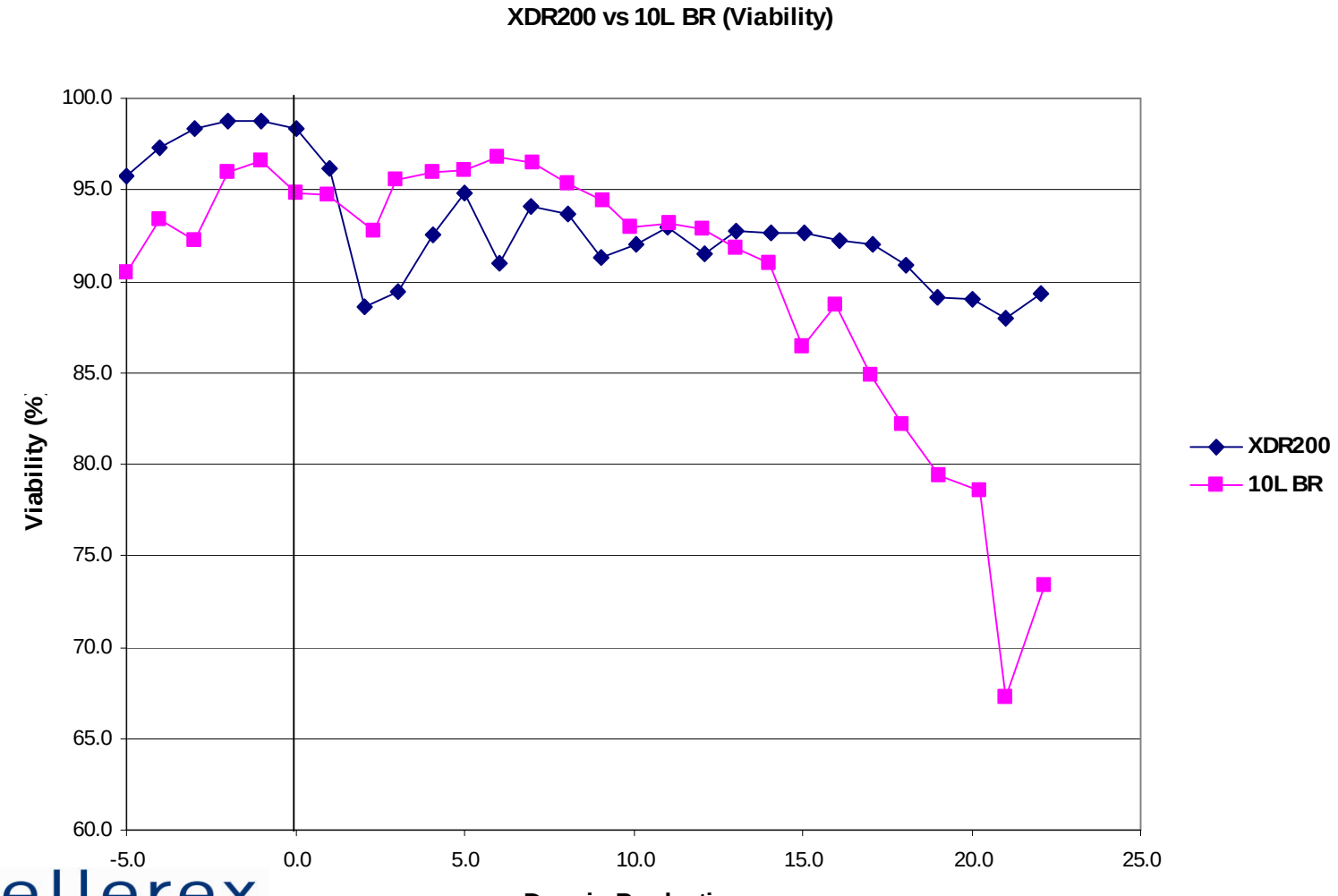
XDR-200 Comparison, pCO2



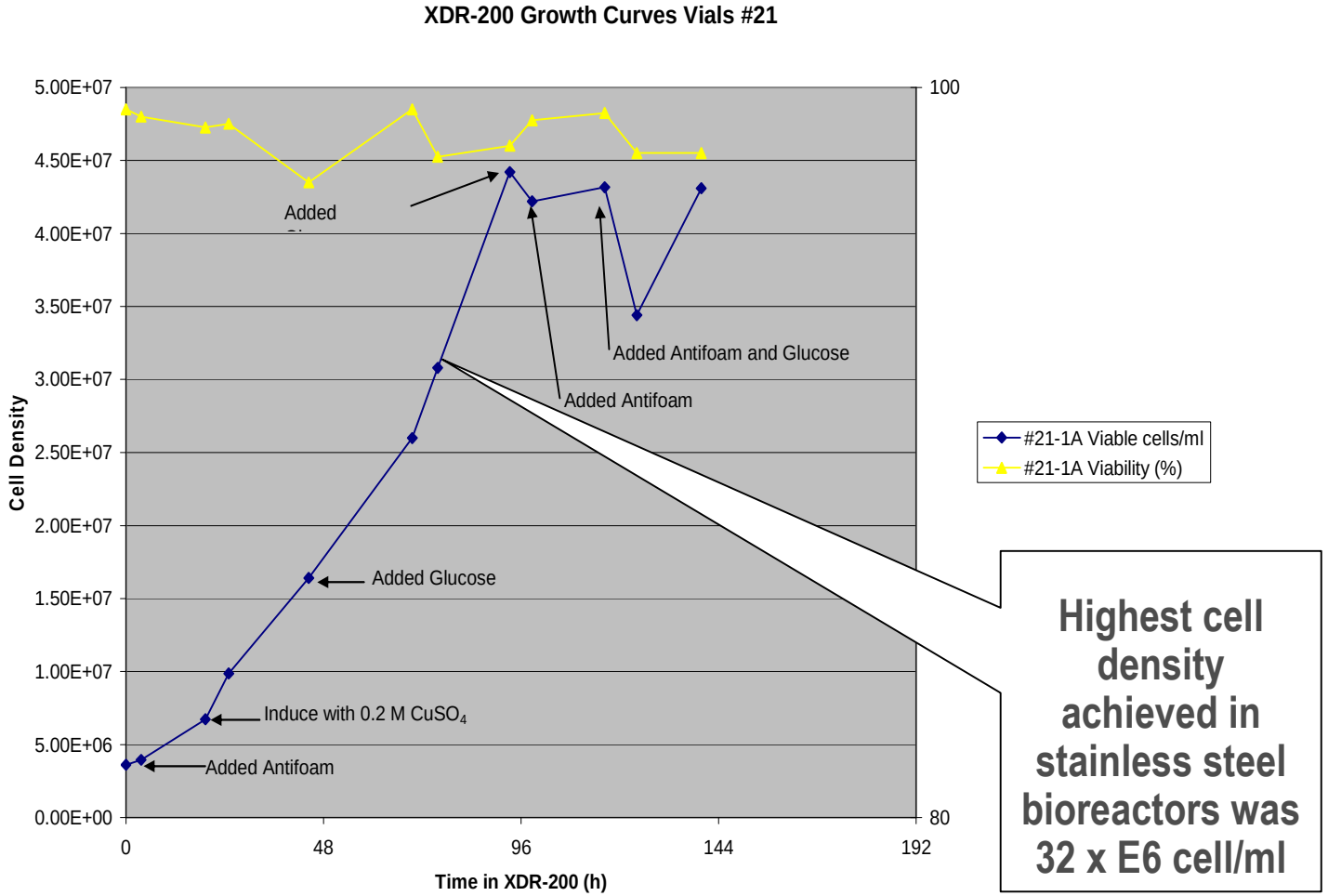
XDR-200 CHO Perfusion Culture - Viable Cell Density



XDR-200 CHO Perfusion Culture - % Viability

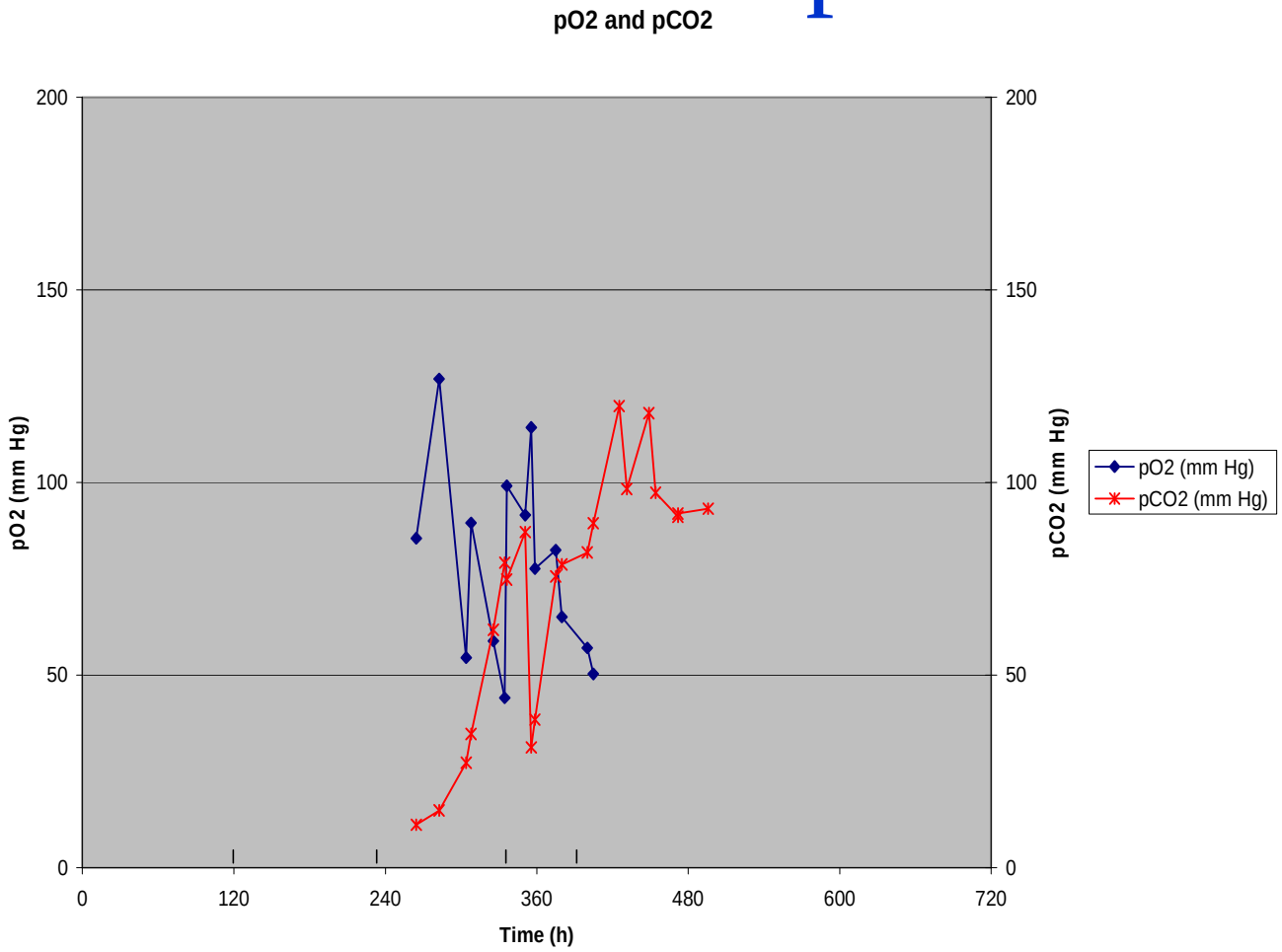


XDR-200 Insect Cells S2 - fed batch



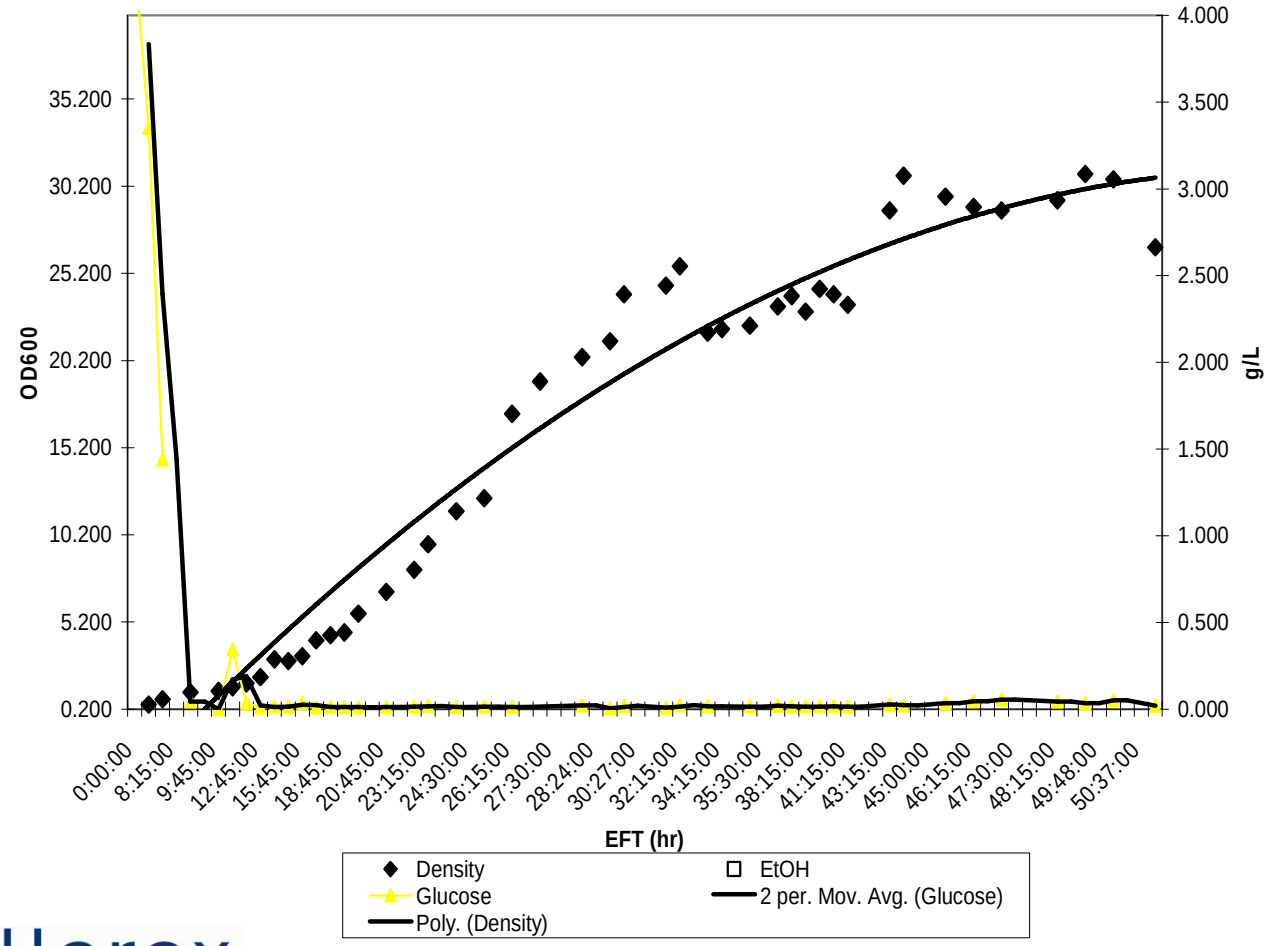
Highest cell density achieved in stainless steel bioreactors was 32 x E6 cell/ml

XDR-200 Insect Cells S2 - fed batch - pCO2

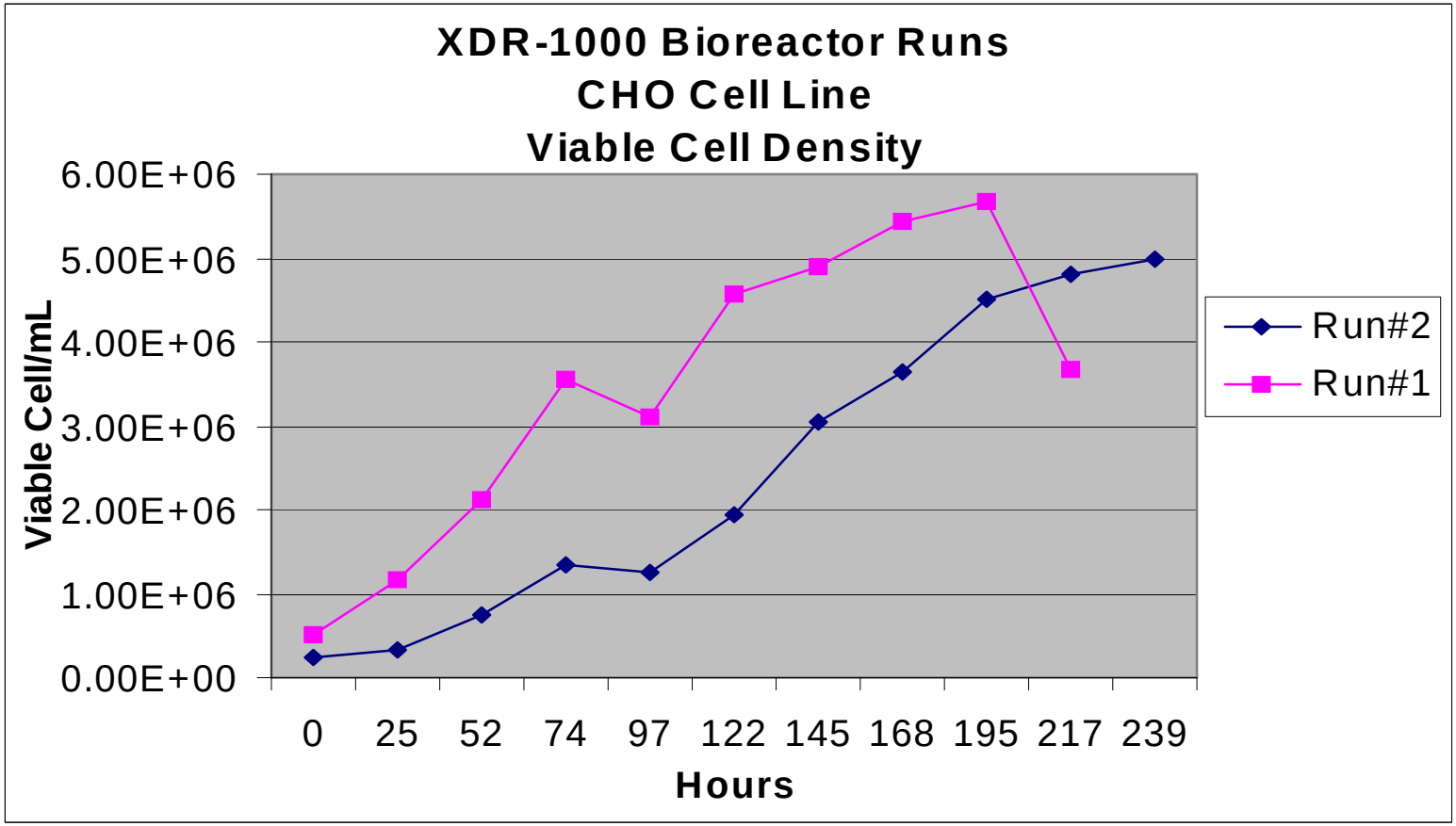


Xcellerex

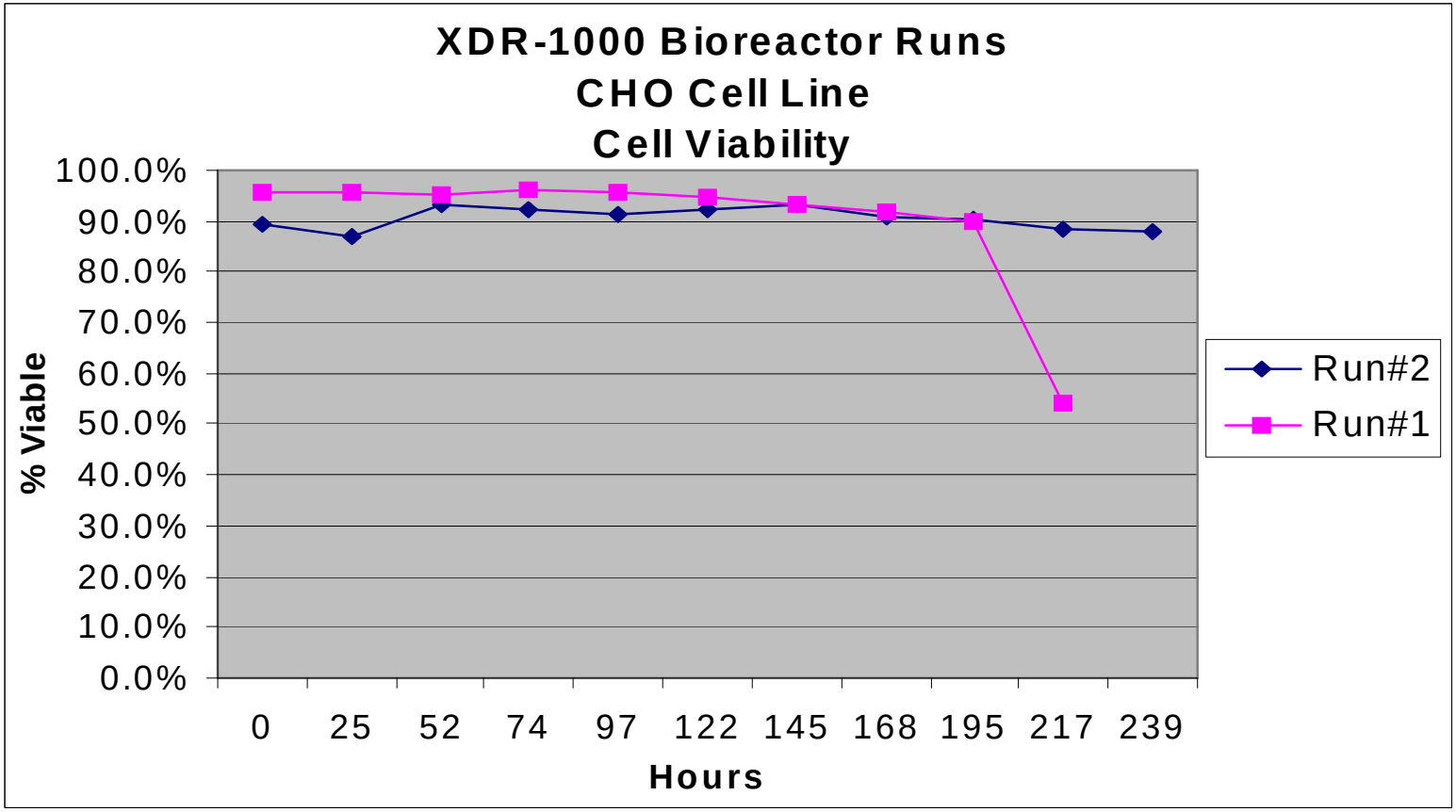
XDR-200 Yeast - S. cerevisiae



XDR-1000, CHO, fusion mAb viable cell density



XDR-1000, CHO, engineered mAb cell viability



Progress for Single Use Systems

- Wide acceptance of bioprocess bags
- Single use bioreactors are scalable and performance is comparable to SS bioreactors
- 1,000L (wv) stirred tank bioreactor breakthrough opens large scale/commercial applications
- Single pass cell clarification/removal (POD) simplifies 1° recovery
- Membranes for purification improving

Summary - Challenges for Disposables

- Mixing/Buffer/Media prep – Rate of liquid transfers
- Bioreaction – Lab to commercial scalability within the same reactor design/configuration
- Cell Harvest/TFF - Recirculating processes that require high pressure and high flow rates
- Membrane Purification – capacity and DNA/virus clearance
- Disposable Chromatography is still TBD
- Non-standard, multiple connection options
- Disposable sensors are limited
- Plastic durability and weld strength needed for scale up

Acknowledgements

- Geoff Hodge
- Dan Mardirosian
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- Keith Kropp
- Pat Puma