

Scale-Up to 1000 L Perfusion Cell Culture in a Disposable Stirred-Tank Bioreactor

Application Snapshot

Application Profile

- Cell line: Human cell line
- Culture mode: suspension perfusion cell culture
- Media: animal-product-free media
- Perfusion rate: 1.0 v.v.d.

Process Objectives

Primary objective was to achieve the process advantages offered by a disposables-based process, including:

- Very fast process setup
- No need for CIP/SIP
- Less space requirements
- Short turnaround time between batches
- Easy product changeover
- Facility flexibility

Success Criteria

The criteria established to determine success included:

- Bioreactor working volume: up to 1000 L
- High cell density: be able to reach 10 million cells/ml
- Product comparability
- Reliability: be able to support one month process

Results

The Xcellerex disposables-based process delivered performance comparability at 200L and 1000L, including cell viability, viable cell density, productivity and overall system reliability that exceeded expectations.

Overview

Bioprocess scientists have long sought to expand the application of perfusion cell culture in order to achieve the theoretical productivity gains it promises compared to batch and fed-batch approaches. However, practical challenges often thwart those efforts.

Perfusion operations enable biologics manufacturers to achieve target production volume with a smaller bioreactor than normally required, which results in capital investment savings and reduced footprint. In some cases, smaller seed bioreactors can be entirely eliminated. Perfusion also enables productive processing of unstable products by limiting the exposure of products to damaging proteases. Further, slow-growing or difficult to grow cell lines can often be productively grown only in perfusion operation.

The advent of disposables-based bioprocessing has created a new opportunity to broaden and simplify the use of perfusion, primarily due to the elimination of complex and time-consuming cleaning and sterilization steps, as well as the dramatic reduction in the risk of contamination. Recent advances in the supply of disposable bioprocess systems such as disposable bag assemblies, bioreactors, mixing systems, centrifuges, and filter cartridges make implementation of disposables a real present-day alternative to traditional biomanufacturing for more complex processes.

As demonstrated in this application brief, Xcellerex used its flagship XDR disposable bioreactor and disposable mixing system, and a disposable centrifuge made by Pneumatic Scale/Centritech. The Centritech centrifuge simplified the key cell separation/recycling step that often impedes the success of perfusion processing. The joint operation of these systems permitted successful 1000L/day harvesting.

Xcellerex has developed, scaled-up and operated a perfusion mode operation at the 200L (160L working volume) and 1000L scale using exclusively single-use process equipment. The 1000L process was operated for one month, with 12 harvests over 19 days. The program concluded with a successful demonstration of process viability and reliability that exceeded the initial performance expectations.

System Profile

The Xcellerex XDR single-use bioreactor represented the centerpiece of the disposables-based perfusion train. Key advantages included:

Disposable Assembly

- All product contact surfaces single-use except for sensors
- No assembly required within sterile barrier
- USP Class VI films & components, low endotoxin, low particulate, non-cytotoxic materials

Bioreactor Hardware and Controls

- Turnkey, fully integrated XDR-1000; includes jacketed vessel and process controller
- Stirred tank design — seal-less bottom magnetic drive agitation, integrated sparger with open pipe
- 5:1 turndown ratio (operates at 20% or maximum volume)

The remainder of the perfusion train consisted of:

Media Prep System = 1000L/day: Prototype 1000L media mixing system

Disposable external cell retention device: Pneumatic Scale CentriTech® disposable centrifuge system

Chilled Harvest collection system: Xcellerex XTM chilled mixing/storage system

Process Layout



Harvest hold bag and chilled tank

CentriTech Cell I® disposable continuous centrifuge

Load cell controlling medium feed pump

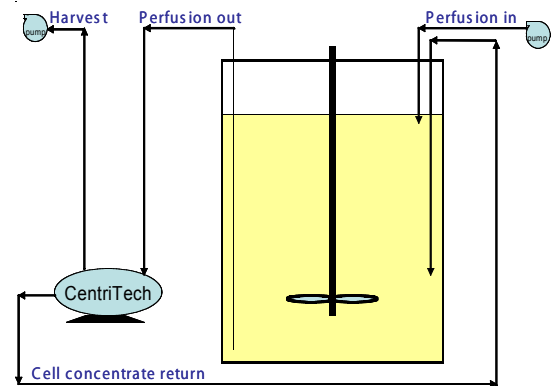
XDR-1000 single-use bioreactor (1000L working volume)

Daisy-chained 1000L media hold bags & holders

Prototype 1000L disposable media mixing system

Process Description

Cells were grown in an Xcellerex XDR-1000 single-use bioreactor operating at 1000L working volume. The bioreactor was installed on a load cell to monitor system weight, and was operated at a constant 1000KG. Supernatant was removed at a fixed rate and sent to the CentriTech centrifuge. A concentrated stream of cells was returned directly from the centrifuge to the bioreactor, and the load cell/controller loop governed a pump that would add new media based on the system weight readings in order to maintain constant volume in the bioreactor.



Results

The charts below illustrate the outcomes of the 1000L perfusion run compared with a 200L (at 160L working volume) run and a 10L benchtop run. The results demonstrate successful perfusion process scale-up and continual cell culture at 1000L as measured by cell density, cell viability, protein production/yield, and other criteria.

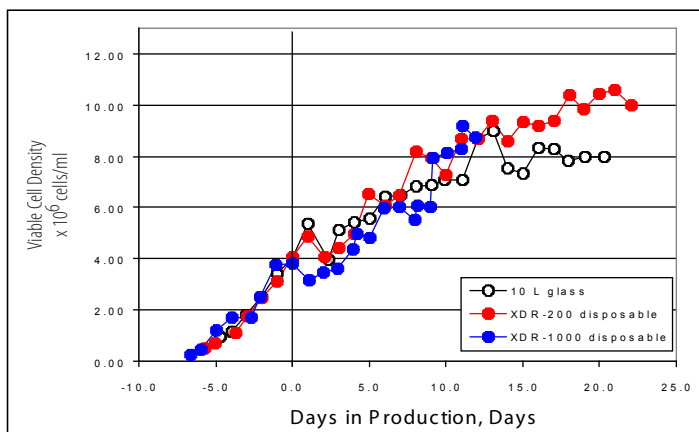
Process Comparisons

- 10 L glass bioreactor run: 27 days with 22 harvests
- 160 L disposable run: 28 days with 22 harvests
- 1000 L disposable run: 19 days with 12 harvests

The process demonstrated equivalent control of:

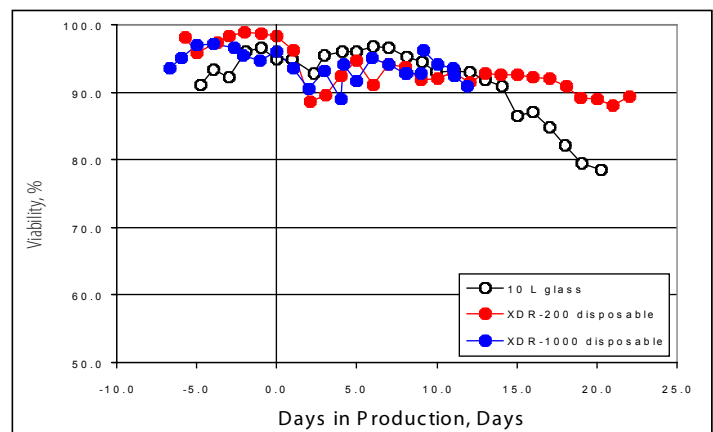
- Bioreactor volume
- Temperature
- pH
- DO

Viable Cell Density



Viable cell density in XDR at 200L and 1000L scale were comparable to 10L benchtop performance

Cell Viability



Cell viability in XDR at 200L and 1000L scale were comparable to 10L benchtop

FOR ADDITIONAL INFORMATION

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