Hollow Fiber Bioreactors Technology Introduction


Cell Culture Company (C3) has provided hollow fiber bioreactor systems since the 1980s, and the have been used to produce numerous regulated biologics and other materials. Nevertheless, not everyone is familiar with our cell culture technology.

This bulletin provides an introduction to our hollow fiber bioreactor technology by discussing the topics listed below. For additional technical information, please contact C3’s account services group using the contact information on the back of this brochure.

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Introduction

Achieving high cell densities and reducing the costs to produce biologics are desirable but not always simple or inexpensive goals. C3’s hollow fiber bioreactor systems are a solution to both goals.

High Cell Density

C3’s hollow fiber bioreactor (HFBrx) systems routinely achieve cell densities of 200–400 x 10^6 cells/mL without requiring chemical engineering or bioreactor optimization expertise. Competing bioreactor technologies routinely achieve cell densities of ~2 x 10^6/mL and can achieve 10 x 10^6/mL, but doing so is complicated, expensive, not easily scalable, and requires expertise not available at most organizations.

Because HFBrx technology cultures cells to such high densities, C3’s systems are deceptively small despite their high production capabilities. Two examples:

- **AutovaxID®**: a bench top system that is only 20” H x 20” D, has a production capacity that is equivalent to an 80-L, fed-batch stirred tank bioreactor.
- **ACUSYST-XCELLERATOR™**: pictured at right is a bank of five of its twenty HFBrx cartridges that in total represent the yield capacity that is equivalent to a 1,600-L fed-batch STR, yet the system’s footprint is only 12.5 ft².

Another advantage of HFBrx’s high cell density is cells are cultured at near in vivo-like conditions with direct cell-to-cell contact. An example advantage is for applications using FBS as a growth supplement. Cultures often remain viable when using 1–2% or lower concentrations of FBS, which results in more pure supernatants and less cost. Lower concentrations of FBS often also correspond to increased product yields than when using standard 10% FBS…more product using less supplement.

Production Costs

Starting each new run in any type of bioreactor system includes an amount of risk in addition to turnaround and startup costs that affect the overall cost to produce biologics. HFBrx runs average 60–120 days. Long run times maximize uptime and minimize run turnaround and startup costs and risk. HFBrx runs require minimal support during operation to reduce labor costs. The wetted portion of the system is disposable (see next page) to eliminate turnaround validation and downtime, and its cost is spread out over long run times, making the disposable a very small portion of the run’s total cost. These are just some of the aspects that make HFBrx technology very economical. Competing bioreactor technologies have comparatively short run times, often lasting only two to three weeks. Frequent run starts mean significantly more downtime and incur much more risk and turnaround and startup costs.
Applications

C3’s HFBrx systems successfully culture suspension or adherent cell lines to produce a wide variety of secreted proteins for R&D, diagnostic and therapeutic applications. Cell lines cultured in HFBrx include:

- Hybridoma
- BHK
- MDCK
- Sf9
- CHO
- Vero
- HEK293 and others...

Although monoclonal antibodies are the predominant material produced in HFBrx systems, other secreted proteins are commonly produced. Additionally, HFBrxs are increasingly being used to produce virus and virus-like particles.

Instrumentation and Single-Use Disposables

C3’s HFBrx systems consist of instrumentation and disposables. C3 manufactures these products according to quality system regulations.

Instrumentation

Our two smallest-scale systems—HF Primer™ and Multi-6™—do not require custom equipment from C3. The instrumentation needed to operate these systems is common to most cell culture facilities, so they can be used without capital equipment purchases.

Our larger systems—the AutovaxID® and three AcuSyst® systems—are custom-engineered to automate production processes and increase production yields. These systems allow technicians to support very large cell cultures with minimal support. These four custom instruments include fully integrated hardware and software. Sourcing supplemental third party hardware or control software is unnecessary. No instrumentation is wetted during use. The wetted components are provided as single-use disposables.

Single-Use Disposables

C3 pioneered the use of sterile, single-use disposables in the 1980s to eliminate cleaning validation and expense, minimize downtime, speed run startup, and simplify the manufacturing process. C3’s disposables are provided with a certificate of compliance to document their sterility and specify their manufacturing lot number. The wetted material components of disposables are lot-tracked and manufactured from materials that meet USP Class VI or non-cytotoxicity per MEM-Elution.

C3’s HFBrx Product Line

Details of C3’s six HFBrx systems are presented in other documentation available from the Account Services group. Please see our contact information on the last page. C3’s HFBrx product line, in order of increasing production scale:

- HF Primer™
- Multi-6™
- AcuSyst-miniMax™
- AutovaxID®
- AcuSyst-MAXIMIZER®
- AcuSyst-XCELLERATOR™
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Technology Background

Hollow fiber bioreactor (HFBrx) is a term with two meanings. One meaning refers to C3’s complete cell culture systems. HFBrx also commonly refers to the cartridge which is the heart of hollow fiber bioreactor technology. The HFBrx (cartridge) consists of thousands of small hollow fibers with an approximate diameter of 200µ. They are encased in a cylindrical housing. The fibers are surrounded at both ends by a sealing compound to create two compartments, the intracapillary space (ICS) inside the fibers, and the extracapillary space (ECS) outside the fibers. Cells are inoculated into, and expand throughout, the ECS. Two ports allow cell culture medium to enter and exit the ICS compartment. Another two ports allow cell culture medium to enter and exit the ECS compartment. As ICS cell culture medium enters the lower port, it is distributed to flow through the inside of the many fibers. As this medium exits the fibers, it collects and exits the upper port. As ECS cell culture medium enters the lower port, it flows through the dense cell culture growing in the ECS compartment. As ECS medium exits the upper port, it is supernatant and is carrying with it the product of interest, such as secreted proteins or virus.

Monitor Process

Periodically remove ICS and ECS medium samples at the user-desired frequency. ICS samples are taken to monitor culture metabolism based on changes to pH and glucose concentration and less commonly on lactate, glutamine, ammonia, and dissolved oxygen concentration changes. These assay data are used to adjust the ICS perfusion rate, which maintains an adequate supply of low molecular-weight nutrients. ICS perfusion is increased by increasing MP and OP speeds in Multi-6, AutovaxID, and AUTOSYST systems or by increasing the frequency of manual replenishment of ICS medium in HF Primer.

ECS samples are routinely taken to monitor product concentration. These assay data are used to adjust the ECS perfusion rate. Increasing ECS perfusion typically results in higher product secretion rates, and it increases delivery of growth-supplements, if in use. ECS perfusion is increased by increasing FP and HP speeds in AutovaxID and AUTOSYST systems or by increasing the volume and/or frequency of ECS media exchanges using syringes in HF Primer and Multi-6.

Run Termination

If reusable sensors are being used, remove and save them for a future run. If the disposable pH sensor is being used, it is simply discarded along with the cultureware.

Turnaround Time

Because the cultureware and pH sensor are disposable, a new run can be started immediately after terminating the previous run. If reusable sensors are used, they can be prepared in advance to achieve the same nearly zero-downtime capability.
Filter Membrane Separation

Hollow fibers are manufactured from an ultra-filtrative membrane. As illustrated below, the membrane allows small molecules to freely diffuse through the wall of the hollow fiber. Diffusion and fluid exchange between the ICS and ECS occurs exclusively through the pores in the membrane, which have a molecular weight cutoff of about 30–60 KDa.

These small pores readily allow passage of low molecular-weight nutrients and wastes. Cells, most growth supplements, and products of interest larger than the molecular weight cutoff (MAbs, other proteins, or virus) do not pass through the hollow fiber pores. Diffusion occurs around the fibers’ circumference and along their lengths. The high number of fibers results in the dense tissue throughout the ECS being bathed in nutrients and having their metabolic waste products removed.

Because the fibers’ pores function as a filter, two cell culture media flow through the HFBrx to deliver the needed low and high molecular-weight nutrients. As mentioned on the previous page, the two media are called ICS medium and ECS medium.

- ICS medium is basal medium without growth supplements.
- ECS medium is either ICS medium plus growth supplements (such as FBS) or a chemically-defined serum-free medium or, rarely, a protein-free medium.

One may successfully use a routine basal medium, such as DMEM:F12 for ICS medium and a serum-free medium for ECS medium, to significantly reduce overall media costs, yet gain the advantages of using animal component-free medium in the harvest supernatant.

Membrane-sequestration of large molecules and the use of two media to feed the bioreactor bring several advantages that are exclusive to HFBrx technology. These advantages are further described in the next section, Production Advantages.
Production Advantages

There are several advantages as a result of hollow fiber bioreactors allowing the exchange of small molecules while retaining large molecules.

Reduced Media Cost
The majority of media that is consumed during the run is unsupplemented ICS medium. ECS medium is consumed at a rate that is only about 1/50th – 1/100th of the ICS consumption rate. Because most of the consumed media is inexpensive and growth supplements are added only to ECS medium, overall media costs are greatly reduced compared to other cell culture technologies.

Concentrated Product
As product is being produced in the bioreactor, it is simultaneously being concentrated. This happens because the product harvest rate from the ECS is 50–100x slower than the ICS medium feed rate (which does not affect product concentration). This inherent concentration allows mgs/mL product concentrations from routine cell lines coming from static culture production methods that have not yet been optimized.

Clarified Product
Because HFBrx productions yield small harvest volumes, harvest fluid is often filtered as it is being collected. Product is now concentrated & clarified!

Simpler, Cheaper & Faster Downstream Processing
1. Very little growth supplement usage
2. High product concentration
3. Small Harvest volumes
4. In-line harvest filtration HFBrx harvest volumes… Skip downstream processing! Go directly to purification!
5. HFBrx harvest volumes mean
6. Skip downstream processing!
7. Go directly to purification!
Operation Overview

The following information describes how our various HFBrx systems work. The focus is on: 1) the various pumps and components and 2) how they affect the cell culture environment and allow a technician to support a very high number of cells in culture with minimal effort. As a visual aide, these three functions are highlighted by different colors.

**pH Control Oxygenation**

**Background**
These critical functions occur in all C3 HFBrx systems.

**Operational Details**
A Circulation Pump (CP) moves ICS medium from the ICS Reservoir, through a gas-exchange cartridge (GEX), through the lumen of the hollow fibers, and back to the ICS Reservoir.

Cell culture medium circulation through the GEX is analogous to the heart and lung functions in mammals: CO\(^2\) exchange and O\(^2\) uptake.

The GEX has a membrane to separate the cell culture medium stream from the Air/CO\(^2\) stream. The exchange of O\(^2\) and CO\(^2\) through the membrane is bubble-free, so there is no foaming potential. CP flow rate is very rapid in order to frequently expose the circulating medium to the GEX to exchange dissolved O\(^2\) and CO\(^2\) in the cell culture medium with the O\(^2\) and CO\(^2\) on the gas-side of the GEX’ membrane.

To decrease pH, air and CO\(^2\) flow through the GEX. Some of the incoming CO\(^2\) dissolves into the cell culture medium, thereby decreasing pH. To increase pH, only air flows through the GEX, which extracts dissolved CO\(^2\) from the cell culture medium, thereby increasing pH. Because the high circulation flow rate occurs in the ICS—and the cells reside in the ECS—the cells do not experience shear force.

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![Diagram of Operation Overview](image-url)
ICS Perfusion.
Deliver Media Nutrients.

Background
Although monoclonal antibodies are the predominant material produced in HFBrx systems, other secreted proteins are commonly produced. Additionally, HFBrxs are increasingly being used to produce virus and virus-like particles.

The delivery of low molecular weight nutrients and removal of metabolic wastes is ICS Perfusion. This critical function occurs in all C3 HFBrx systems.

ICS Perfusion is an automated process when Media and Outflow pumps are used. Automated ICS Perfusion greatly reduces labor time, handling, and contamination risk because it operates as a closed system for long periods of time.

HF Primer, our smallest-scale HFBrx, does not have these pumps in order to keep its cost low. ICS Perfusion is a manual process in HF Primer, which is described on the following page.

The following information applies to Multi-6, AutovaxID, and AcuSyst because they utilize Media and Outflow pumps to automate ICS Perfusion.

Operational Details
A Media Pump (MP) pumps medium from the ICS Medium Supply container and into the ICS Reservoir. The MP speed is much slower than the CP speed! The ICS Reservoir allows the partially spent and rapidly circulating medium to mix with the fresh nutrients being pumped in by the MP.

The MP delivers un-supplemented medium to replenish spent small nutrients, such as glucose, glutamine, etc., to the culture. The concentrations of these nutrients are controlled by adjusting MP speed. Higher speeds are used for aggressive cell lines, while slower pump speeds are used for slower-growing or slower-metabolizing cell lines.

MP flow rate begins slow at the point of inoculation and increases as the culture expands. Technicians periodically remove a sample of the circulating medium, assay its glucose concentration, and adjust MP speed to maintain the desired glucose concentration.

An Outflow Pump (OP) operates automatically to remove spent ICS medium as fast as fresh ICS medium is being pumped in by the MP.
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ECS Perfusion.
Deliver Growth Supplements.

Background
The delivery of high molecular weight growth supplements and removal of harvest (supernatant) is ECS Perfusion. This critical function occurs in all C3 HFBrx systems.

ECS Perfusion is an automated process when Factor and Harvest pumps are used. Automated ECS Perfusion greatly reduces labor time, handling, and contamination risk because it operates as a closed system for long periods of time!

HF Primer and Multi-6, our two small-scale HFBrxs, do not have these pumps in order to keep their cost low. ECS Perfusion is a manual process in these systems, which is described on the following page.

The following information applies to AutovaxID, and AcuSYS because they utilize Factor and Harvest pumps to automate ECS Perfusion.

Operational Details
A Factor Pump (FP) pumps medium from the ECS Medium Supply container and directly into the ECS of the HFBrx where the cell culture is. ECS medium typically contains growth supplements that provide high molecular weight components such as lipids, autocrine factors, and other components. Because their molecular weights are typically higher than the cutoff of the fiber pores, these components are not dialyzed (lost) to the ICS of the HFBrx.

Cell cultures need very small amounts of growth supplements, so the FP runs very slowly! A Harvest Pump (HP) pumps product-laden supernatant from the HFBrx's ECS. Technicians periodically remove a sample of harvested supernatant, assay product concentration, and adjust HP speed to maintain the desired product concentration. FP speed and HP speed usually begin slowly and at the same rate at the point of inoculation or soon thereafter. Their speeds periodically increase equally as the culture expands.

The HP runs very slowly, which results in high product concentrations and small harvest volumes!
Manual ICS Perfusion / HF Primer™

Although ICS Perfusion is a manual process in HF Primer, the purpose of ICS Perfusion is the same as for our larger systems with automated ICS Perfusion—delivery of small nutrients and removal of metabolic wastes.

HF Primer uses a 2L bottle as its ICS Reservoir, as illustrated below. As described previously, ICS medium circulates through this 2L bottle as part of the pH Control and Oxygenation process.

Instead of using dedicated pumps and medium supply and waste collection containers to automate ICS Perfusion, the 2L bottle also is used to deliver fresh medium to the HFBrx and collect metabolic wastes from the HFBrx.

As medium circulates through the HFBrx, nutrients are consumed and metabolic wastes are secreted. To prevent the circulating medium from becoming too spent, the volume of medium in the bottle is periodically either increased or entirely changed with fresh medium.

At the beginning of the run when the HFBrx contains small numbers of cells and medium consumption is low, less than 2L of medium is maintained in the bottle to circulate medium through the HFBrx. As this medium becomes spent, fresh medium periodically is added to the existing medium in the 2L bottle to increase nutrient concentration and decrease waste concentration. Later in the run when the HFBrx contains large numbers of cells and medium consumption is high, the entire 2L periodically is discarded and replaced with 2L of fresh medium.
Manual ECS Perfusion / HF Primer™ and Multi-6™

Although ECS Perfusion is a manual process in HF Primer and Multi-6, the purpose of ECS Perfusion is the same as for our larger systems with automated ECS Perfusion—delivery of high molecular weight growth supplements and removal of harvest (supernatant).

Instead of using dedicated pumps and factor supply and harvest collection containers to automate ECS Perfusion, these two systems use syringes to inject ECS medium and collect harvest when desired, as illustrated below.

Injecting Factor and removing Harvest often is performed one to three times per week. Injection and removal are done simultaneously. Because Multi-6 has six segregated HFBrxs, injection and removal are done six times, once per HFBrx.

C3 supplies luer-activated valves (LAVs) to which the syringes attach. LAVs keep the luer ports effectively closed during the process of connecting and removing syringes, which helps minimize the risk of contamination!
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Production Run Overview

**Prepare Inoculum**
Common, inexpensive cell culture methods can be used to create the inoculum because C3’s HFBrx systems are inoculated with small numbers of cells, approximately:
- 1 x10^8 cells for HF Primer (our smallest system) to...
- 4-10L of cells for AcuSyst-XCELLERATOR™ (our largest system)

**Prepare pH and Dissolved Oxygen Sensor**
C3’s AcuSyst systems can be used with a disposable pH sensor or reusable pH and DO sensors. When the latter are used, they are autoclave-sterilized. (AutovaxID cultureware have an integral, sterile, disposable pH sensor, so this step is unnecessary. HF Primer and Multi-6 do not use in-line sensors, so this step does not apply to their use.)

**Prepare Cultureware**
Cultureware are the wetted disposable. For AcuSyst cultureware: the technician installs either disposable or reusable sensor(s) into the cultureware. HF Primer, Multi-6, and AutovaxID cultureware need little to no preparation prior to use.

**Fill & Flush Cultureware**
Cultureware (both its ICS and ECS) are filled and rinsed with a small volume of unsupplemented medium. A very small volume of growth supplements, if needed, are added to the ECS.

**Calibrate Sensors**
Remove a small volume of medium to assay pH, and optionally dissolved oxygen, and use these values to calibrate the instrument’s sensors (AcuSyst and AutovaxID).

**Inoculate System**
For our small systems, pool the scale-up culture, centrifuge it, and resuspend it in complete medium. Use a syringe(s) to inject the culture into the HFBrx’s ECS. For our large systems that require larger inoculum volumes, resuspend the culture in a few 100 mLs, which are transferred to a closed container. Pump the inoculum into the HFBrx’s ECS as a closed system, which minimizes handling and contamination risk.
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Run Termination
If reusable sensors are being used, remove and save them for a future run. If the disposable pH sensor is being used, it is simply discarded along with the cultureware.

Turnaround Time
Because the cultureware and pH sensor are disposable, a new run can be started immediately after terminating the previous run. If reusable sensors are used, they can be prepared in advance to achieve the same nearly zero-downtime capability.
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Facility Requirements

C3’s bioreactors can be operated in ISO 8, or less, cleanroom suites to produce regulated biologics. C3’s bioreactors need a 100% CO₂ supply and a standard electrical receptacle(s). Because our HFBrx systems produce concentrated and optionally clarified supernatant, large-volume, expensive, and time-consuming downstream processing equipment—and the space and staff they require—are unnecessary. The above advantages mean that:

- Supernatant from C3’s bioreactors is ready for purification
- Bioreactor production space is relatively inexpensive
- Minimal facility requirements make overhead costs very low

Competing technologies support comparatively low-density cultures requiring large-volume bioreactors. These big systems are complex and often fixed on large skids. They require clean steam, water for injection, multiple gas supplies, space for all of these supporting utilities, expertise to design, operate, and maintain them… Consequently, they result in high manufacturing overhead costs.

Competing Small-Scale HFBrx Systems

Small-scale should not correspond to inadequate engineering. Using large bioreactor cartridges in small-scale HFBrx systems requires supporting a high respiration rate. Most competing small-scale HFBrx systems do not adequately support the respiration requirements of large numbers of cells in a high-density culture environment. Supporting culture respiration requires adequate gas exchange capability and circulation rate.

Bioreactor Size

C3’s HF Primer is an easy-to-use, inexpensive, small-scale HFBrx system. Despite being our smallest-scale system, HF Primer’s HFBrx cartridge is quite large when compared to our competitors’ products. HF Primer’s large HFBrx cartridge allows it to support a large number of cells that will produce a large amount of material. This productivity is possible because HF Primer includes the necessary gas exchanger and supports the necessary circulation rate and maintain them… Consequently, they result in high manufacturing overhead costs.
**Gas Exchanger Size**
Large cell cultures consume large amounts of oxygen and create large amounts of CO$_2$, and this respiration must be accommodated. Using a large HFBrx cartridge without also using a correspondingly large gas exchange device results in the HFBrx cartridge supporting fewer than its potential number of cells. C3 uses a gas exchange cartridge in HF Primer that adequately supplies the needed oxygen and removes the created CO$_2$. Competing hollow fiber systems do not use a gas exchange cartridge. Rather, they simply use a length of silicone tubing that, while inexpensive, is inadequate for the task and results in insufficient oxygen delivery and CO$_2$ removal. Oxygen limitation greatly limits culture metabolism, and excess CO$_2$ causes acidic pH levels. Both conditions result in inferior production. C3's gas exchanger has roughly seven times more gas transfer capacity than the coiled silicone tubing used by our competitors.

**Circulation Rate**
An adequate gas exchange capability also requires an adequate circulation rate. When cell culture medium flows (circulates) too slowly through the gas exchange device, too little oxygen is added and too little CO$_2$ is removed. HF Primer supports a very high circulation rate to utilize its large gas exchange device. HF Primer is a fully-engineered, small-scale HFBrx system!
Supplying state-of-the-art hollow fiber bioreactors.
Providing custom cell culture services.

Cell Culture Company is very interested in hearing from you, and upon request, it would be our pleasure to email you with news and updates.

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