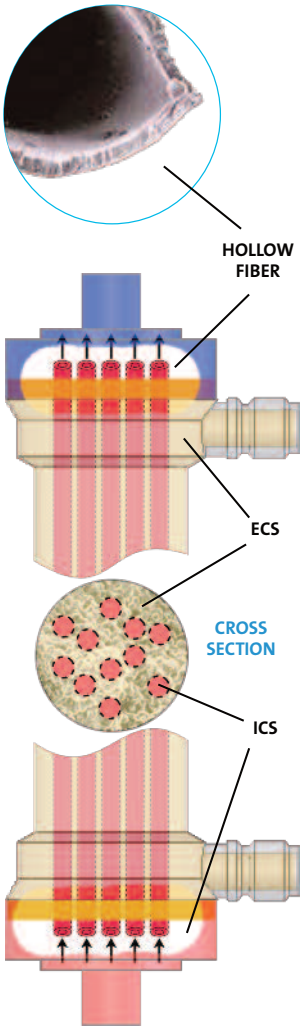
CELL CULTURE COMPANY

Hollow Fiber Bioreactors Comparison to Stirred Tank



Proven Technology

Cost-Efficient Production

Hollow fiber bioreactors are the ultimate solution to achieving high-density, continuous culture.

The upper limit to cell density in most standard stirred tanks is about 2 million per ml, and densities near 10 million per ml are possible with complicated, difficult-to-scale methods that recycle cells.

Hollow fiber systems are inoculated at 5 million cells per mL, and cell densities quickly reach 200-400 million viable cells per mL after 1-2 weeks. Hollow fiber runs average 60-120 days, although much longer runs are not uncommon. As a result, hollow fiber systems provide a compact, efficient, scalable, and economical method for production of biologicals.

Basic HF Bioreactor

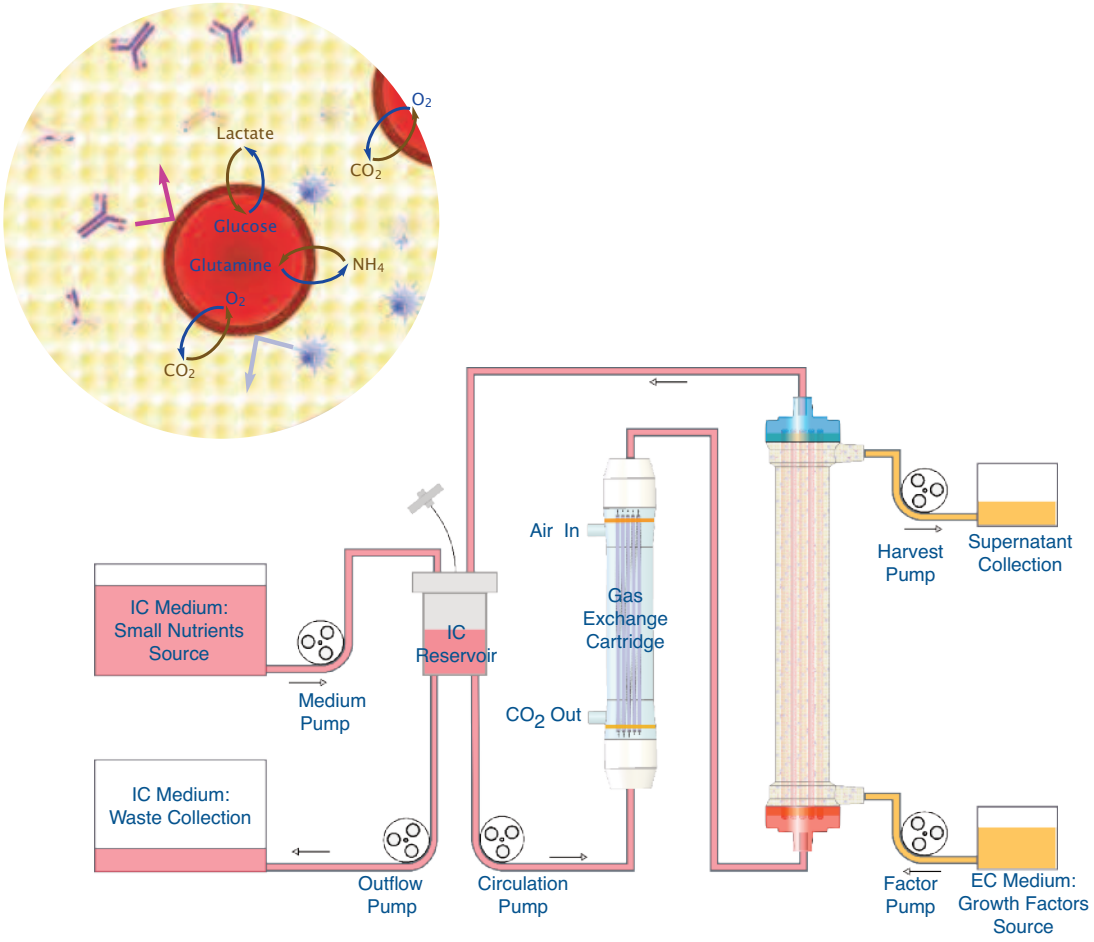
Hollow fiber bioreactors consist of thousands of small hollow fibers encased in a cylindrical housing. The fibers are surrounded at each end by a sealing compound to create two compartments, the intracapillary space (ICS) inside the fibers, and the extracapillary space (ECS) outside the fibers. Communication between the ICS and ECS occurs exclusively through the pores in the fibers, which have a molecular weight cutoff of about 30-60 KDa.

Introduction

Cells are typically cultured in the ECS due to better cell retention. A pump circulates IC medium from the IC reservoir, through a gas exchange cartridge, through the ICS inside the bioreactor, and back to the IC reservoir. Small nutrients and dissolved oxygen diffuse across the fibers from the ICS into the ECS to support cell growth. Small metabolic wastes similarly diffuse in the opposite direction, and are carried away by the IC medium. IC medium is circulated at a high rate (about 0.5 L per minute), primarily to replenish oxygen, which is sparingly soluble in medium. Because this high flow rate occurs in the ICS and cells reside in the ECS, there is no shear force to overcome.

Medium in the IC reservoir is exchanged with fresh medium at a much slower rate (about 0.4 L per hour) for continual replenishment of fresh nutrients and removal of spent medium and metabolic wastes.

EC Medium is added to the ECS at an even lower rate (about 0.15 L per day) to provide high molecular weight growth factors (in serum, serum-free medium, or the large micelles or emulsion sometimes found in protein-free media) and to harvest the product, which is retained and becomes concentrated in the ECS.



Applications of Hollow Fiber Systems

Hollow fiber bioreactors were introduced originally in 1972 as a model system for studying tumors growing at tissue density. Since then, hollow fiber bioreactors have been used in such diverse applications as bioartificial organs, pharmacokinetics, cell therapy, and toxicology. By far, the most widely used application of hollow fiber bioreactors is in the production of proteins by mammalian cells, especially antibodies. Thousands of hollow fiber runs are performed each year for the production of research grade and cGMP in vitro diagnostic applications. Hundreds of cGMP hollow fiber runs are performed each year for the production of clinical grade, injectable proteins. Since 1996, one injectable antibody (ProstaScint®) has been produced under FDA license in C3's AcuSyst-Xcellerator™ bioreactor by Cytogen Corporation.

Illustrative Example:

Comparing Hollow Fiber to Stirred Tank

The following pages discuss results from an experimental production run to assess hollow fiber bioreactors. A cell line was transferred from a 300-L fed-batch stirred tank production method to C3's hollow fiber technology. Typically, such a comparison would be made after optimizing the cell line in the new technology. Optimization was not performed, however, prior to this experiment.

For this experiment, a murine hybridoma secreting an IgG was used. 5x108 cells were scaled up to 1 L in serum-free medium in a spinner flask. The cells were pelleted by centrifugation, resuspended in 50 mL medium, and injected into an AcuSyst-Maximizer® bioreactor containing one 160-mL bioreactor1.

Since the cell line had not been run in a hollow fiber system before, the optimal control strategy was not known. As a result, the control strategy used was that which has worked well in general for other murine hybridomas. The temperature was maintained at 37°C, and the pH setpoint was 7.2. The EC medium, where the cells reside, was the same medium as used for growth in T-flasks (serum-free medium). The IC medium was simple basal medium (no proteins or peptides).

The primary strategy was to increase the IC basal medium feed rate, as necessary, to maintain a glucose setpoint of about 2 g/L to drive cell division during the growth phase. Growth is intentionally slowed as the cells fill the bioreactor by limiting the IC feed to a maximum of 400 mL/hr. Glucose and lactate concentrations and pH then slowly shift to conditions that limit cell division. This strategy provides reproducible results from run to run. Some minor modifications were made during the run as described below.

Hollow Fiber Bioreactors - Comparison to Stirred Tank

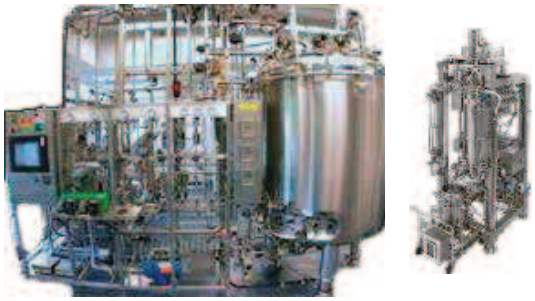
HF productions typically last 60-120 days (during which minimal support is necessary). The 300-L tank requires cleaning every 10 days. Validation for product changeover is critical, making tanks less desirable for multi-product purposes.

Facility Support

Because the hollow fiber's wetted disposable is ready-for-use and is single-use, hollow fiber instruments require no hard plumbing. Their only requirements are a 100% CO2 supply and few standard electrical outlets. As a result, hollow fiber instruments can be set up and taken down in matter of hours in virtually any type of cleanroom space (class 8 is often used). Stirred tanks, however, require hard plumbing for water and steam to facilitate CIP and SIP requirements. This results in extensive, dedicated facility space to support the tank, leading to high overhead costs. The complexity of stirred tanks means they generally are large and immobile. Conversely, even the largest hollow fiber system (ACUSYST-XCELLERATOR™) has casters and rolls through standard doorways, making cleaning the cleanroom simple even for a single technician.

Scale Up

Scaling up a tank is not straightforward. The surface area and paddle speed scale on the tank diameter squared, whereas the volume scales on the tank diameter cubed. These differences result in non-linear scale up problems associated with oxygenation and process control. Hollow fiber systems are scaled from about 10 to 160 mL by increasing the size of a single bioreactor. Further scale up is accomplished by running the bioreactors in parallel. Scale up is linear with respect to total EC volume, making scale up calculations simple and reliable. The largest off the shelf system, the ACUSYST-XCELLERATOR™, has a total system ECS volume of about 3.2 L. Based on the ratios determined in the experimental run, this relatively compact (12.5 ft2) instrument has the production capacity of a 1,600-L fed-batch tank. To achieve the capacity of a 16,000-L tank, ten of them are run in parallel.



Production-scale Skid Tank and Clarification Centrifuge

The XCELLERATOR supports two sizes of disposables. These options represent a 6x to 20x scale-up of the 160-mL bioreactor. The ten-bioreactor (ten 160-mL bioreactors) disposable is pictured above.

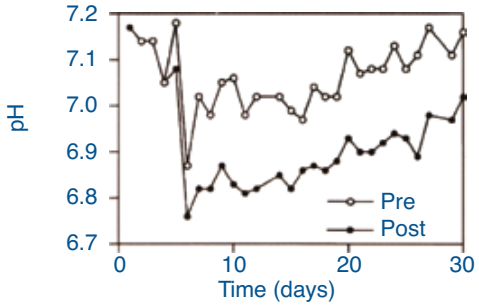
pH

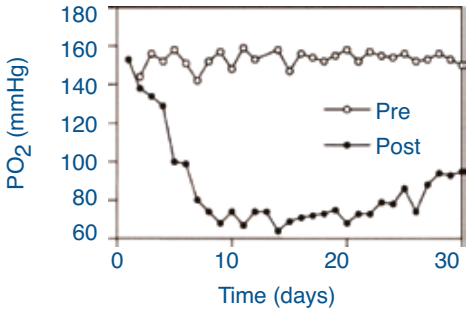
The pH setpoint (pre-bioreactor) of 7.2 was approximately maintained for the first two days of culture (Fig. 2). As the medium reached the initial maximum value of 400 mL/hr, the lactate concentration increased to the point that the gas exchange cartridge was no longer adequate for maintaining pH. The instrument has additional pH control capability, but this feature was not used; according to the protocol, the reduced pH is desired, as it typically slows down growth.

PO_2 & Circulation Rate

The pH setpoint (pre-bioreactor) of 7.2 was approximately maintained for the first two days of culture (Fig. 2). As the medium reached the initial maximum value of 400 mL/hr, the lactate concentration increased to the point that the gas exchange cartridge was no longer adequate for maintaining pH. The instrument has additional pH control capability, but this feature was not used; according to the protocol, the reduced pH is desired, as it typically slows down growth.

The gas-exchange cartridge typically saturates medium entering the bioreactor's ICS, so the pre-bioreactor PO_2 (dissolved oxygen) stayed near 150 mmHg (Fig. 2). Post-bioreactor PO2 drops as the oxygen demand increases due to cellular expansion. Circulation rate is increased as oxygen demand increases. The circulation rate was increased up to the bioreactor's limit of 500 mL/min on the second day. The post-bioreactor PO_2 dropped to a steady value near 70 mmHg, and remained fairly steady through the run. The oxygen uptake rate averaged about 3 mmol/hr.





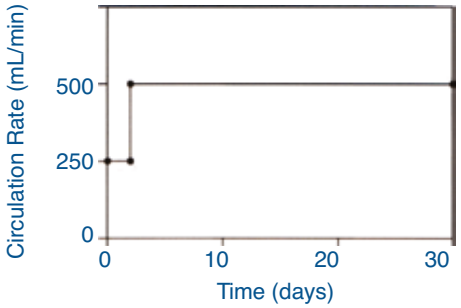


Figure 2: Circulation pump rate, pH and PO_2 from one 160-mL bioreactor.

Pre and Post are defined in the illustration at left.

Antibody Production & Harvest Rate

The cell-side (ECS) feeding rate (serum-free medium) was increased at a 1:50 rate relative to the rate of basal medium addition (Fig. 3). The harvest rate was maintained at the same rate as the cell-side feed rate.

A sample from the instrument on day 10 indicated that the antibody concentration was above 4 mg/mL. In response, the cell-side feed/harvest rate was increased from 10 to 20 mL/hr. In some cases, an increased harvest rate results in increased production; in other cases the antibody just becomes diluted. For this run, the antibody seemed to just be diluted as the concentration fell from above 4 mg/mL to about 2 mg/mL.

The rate of antibody production peaked in the second week, which is typical for some murine hybridomas where the process of slowing growth results in slightly lower productivity. The slow decline in production is also typical of some murine hybridomas. Over the 30-day run, the system produced approximately 25g of antibody in 13L of harvest (1.9 mg/ml).

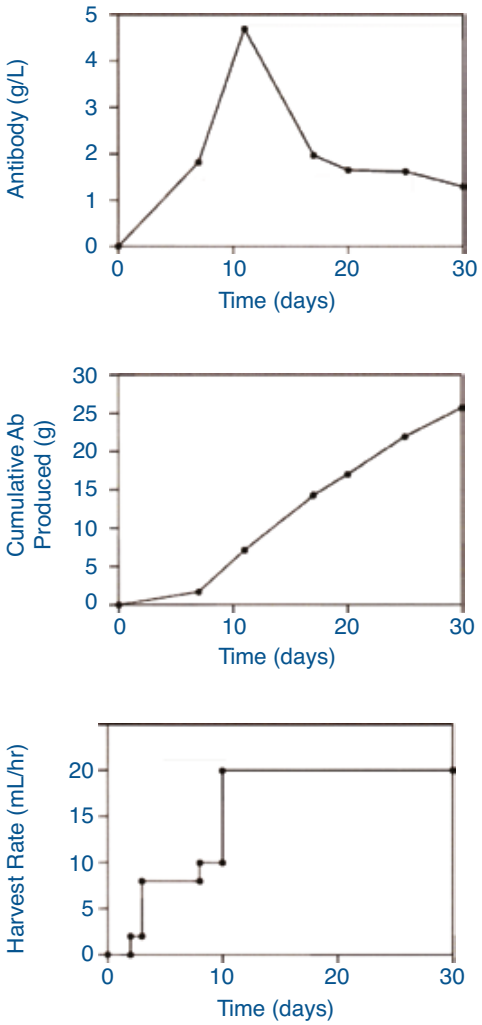


Figure 3: Antibody concentration, cumulative antibody production, and harvest rate from one 160-mL bioreactor

Hollow Fiber vs. Tank

There are numerous advantages to hollow fiber technology when compared to stirred tanks for the production of biologicals. The advantages are perhaps best illustrated using a case study. The cell line described in the previous section was originally produced using a 300-L fed-batch tank. The discussion below is a direct comparison of the results for this cell line in a 300-L tank compared to the 160-mL hollow fiber bioreactor.

Volumetric Throughput

The tank produced 30g of antibody 10 days after inoculation (3g/day). The hollow fiber system produced 25 g of antibody after 30 days of production (0.83g/day). Assuming production is directly scaleable, antibody production from the hollow fiber bioreactor is equivalent to that in an 83-L tank. In other words, the hollow fiber bioreactor produced 500-fold more antibody per ml of cell culture space. The high volumetric throughput of hollow fiber bioreactors results in minimal space requirements, which in turn results in a considerable reduction in overhead costs.

Seed Train

Direct comparison of the seed train for the tank and the hollow fiber system is a bit complicated due to scale differences. For comparison, we will scale the 300-L tank data to that of an 83-L tank, which has the same volumetric throughput as the 160-mL hollow fiber system. An 83-L tank requires inoculation of about 4L of cells. The hollow fiber bioreactor was inoculated with 1L of cells, a four-fold lower amount. The result is that at least one scale up step can usually be skipped for the hollow fiber system. While this may not sound like much gain on the surface, two factors make this difference notable. First, the 4-fold ratio is often the difference between being able to use small, cheap, disposable flasks as opposed to using larger, more expensive flasks that require cleaning and re-use. Second, the 160-mL hollow fiber bioreactor can be inoculated with as little as 0.2L of cells, which would be a 20-fold difference in scale up of the seed train. The 1-L inoculum used for the HF example above was chosen somewhat arbitrarily.

Hollow Fiber vs. Tank Continued....

Medium Use and Cost

30 g of antibody were produced in the tank using 300L of serum-free medium. 25g of antibody were produced in the hollow fiber system using 13L of serum-free medium and 315L of basal medium. With serum free medium at $30/L and basal medium at $4/L, the unit cost of medium was $300/g in the tank. In the hollow fiber system, the unit cost of medium was $66/g, a 4.5-fold reduction in medium cost over tank production.

Downstream Processing

The tank provided 30g of antibody in 300L of cell culture supernatant. The first two steps in downstream processing are cell removal and product concentration. The hollow fiber system provided 25g of antibody in 13L of harvest. This harvest came off the instrument in a concentrated, cell-free format through an in-line filter. As a result, the first two steps in downstream processing are not necessary when using a hollow fiber system.

System Re-Use

Hollow fiber systems consist of a re-usable instrument and its disposable—a fully assembled, sterile and single-use cultureware. Medium is fed to the instrument from a disposable bag (10-500 L size). As a result, all wetted components on the HF system are disposable. Hollow fiber systems require no cleaning or cleaning validation, and there are no issues with product changeover, making hollow fiber systems very versatile, with little labor support.

Glucose and Lactate Metabolism & Basal Medium Feed Rate

The cells rapidly expanded in the bioreactor as indicated by the glucose uptake (GUR) and lactate production rate (LPR) (Fig. 1). The IC feed rate was increased to 400 mL/hr on day 3, which was the maximum rate indicated in the initial protocol. However, it was apparent by day 8 that 400 mL/hr may not be adequate for this cell line since the bioreactor was not yet full of cells. In response, the medium pump was increased to 500 mL/hr on day 8. The GUR leveled off at about 1300 mg/hr with the basal pump rate at 400 mL/hr. Upon increasing the basal medium pump to 500 mL/hr, the GUR increased and leveled off at a proportionally higher value of about 1600 mg/hr. The LPR demonstrated a similar increase. The basal rate was kept at 500 mL/hr for the remainder of the run since the bioreactor was nearly full of cells.

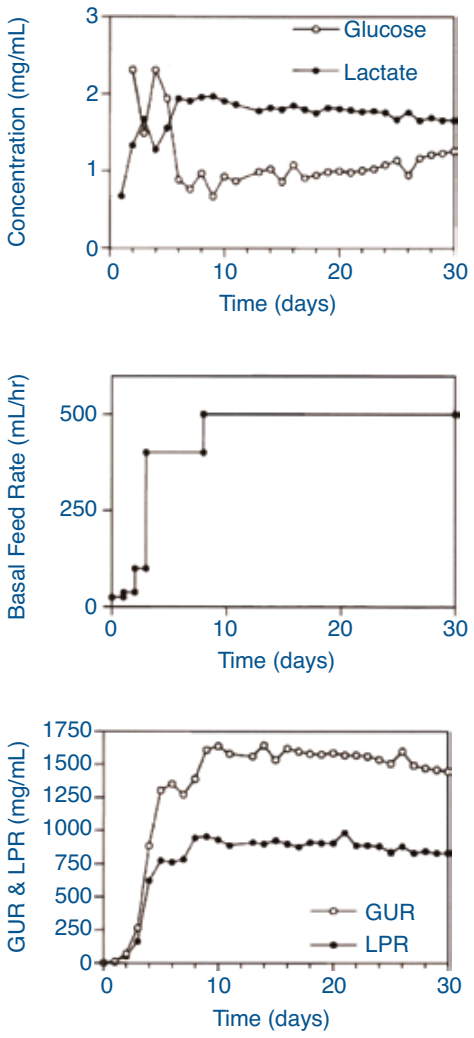


Figure 1: Basal medium feed rate and glucose and lactate metabolism from one 160-mL bioreactor.

GUR = glucose uptake rate
LPR = lactate production rate

What the CFO Would Like About Hollow Fiber Bioreactors

The above comparison of tanks to hollow fiber systems outlines many of the technical and economic advantages that hollow fiber systems have over tanks. However, the advantages are much more far-reaching. Today's biotech executives often struggle with the decision regarding when to invest in commercial production capacity. Heavy investment in a commercial facility before Phase III data are available is a huge risk. On the other hand, hundreds of millions of dollars in revenue could be lost if the facility is not operational upon product approval. Because of the extensive hard plumbing required for tanks, facilities that support tanks take years to design, build, and validate, which further compounds the "Go—No Go" decision. Additionally confusing the situation are uncertainties associated with yearly production requirements, which are only vague estimates at the time of having the make the decision to build. However, since HF systems require minimal support, facility design, construction, and validation are greatly accelerated, substantially reducing the risk of revenue loss associated with waiting for better information as the Phase III study progresses.

A number of cash-strapped biotech companies are also taking great advantage of hollow fiber systems very early in the clinical process. The typical scenario is that the company has a poor producing cell line and has no funds for further process development, yet the desire is to produce enough Phase I clinical material in a very short time at a reasonable price. The Phase I clinical data will be used for another round of financing, which will then involve more substantial process development. In many cases, the needs of this type of company are met with a hollow fiber system, whereas production in a tank would be cost-prohibitive. In a similar scenario, hollow fiber systems are finding a niche as a bridge production technology for transgenic production systems.

Hollow Fiber Bioreactors - Comparison to Stirred Tank

	MAXIMIZER (160 mL bioreactor)	300 L Fed-Batch Reactor
Facility	Simple, bench-top 100% CO_2, Standard electricity.	Large skid, Multiple gases, CIP, SIP, etc.
Seed Train	0.2 - 0.4L Inoculum Simple static culture methods.	14L Inoculum Complex, Expensive.
Media Costs	$1650	$9000
Media Cost per Gram of Antibody Produced	$66	$300
Downstream Process	13L of Supernatant. Supernatant is ready for purification.	300L of Supernatant Concentration, Clarification... Expense & Time.
Turnaround Expense	Runs last months. Maximizes uptime. Disposable = Rapid turnaround.	10-Day Runs. Frequent cleaning and startup costs. Lower productivity.

Summary

The role of hollow fiber systems in the biotechnology industry has expanded rapidly in the last few years. Economic considerations, regulatory acceptance, and scientific advancement have all contributed to this expansion. These factors will continue pushing hollow fiber bioreactors to the forefront of production technology options.

Supplying state-of-the-art hollow fiber bioreactors.
Providing custom cell culture services.
Developing personalized cancer immunotherapies.

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