Historically, the rigorous performance demands of industrial scale microbial fermentation processes have required the use of traditional stainless steel cleaning-in-place/sterilization-in-place (CIP/SIP) fermentor vessels. A new commercially available single-use fermentor (SUF) product line delivers scalability and now meets the requirements of dense, rapidly growing microbial cultures used in modern fermentation production facilities. This new technology offers many unique benefits when developing new molecules in microbial systems, revamping and validating older processes, or upgrading aging equipment.

1. Single-Use Platforms Offer Dramatic Advantages

In many areas of biopharmaceutical production, single-use technologies have revolutionized the definition of sterile closed-system processing. The most significant change started over a decade ago as purpose-built polymer bags, commonly referred to as single-use bioprocess containers (BPC), gained broad acceptance. The ability to use a dedicated pre-sterilized container for each batch offers considerably shorter turnaround times between runs compared to stainless steel systems whose processing environments require substantial cleaning and sterilization (SIP/CIP) before they...
can be used again. Avoiding the use of chemicals, water, and steam energy to clean and sterilize leads to direct reduction in capital expenses and the environmental impact of processing.

How single-use systems increase process and system flexibility is another important consideration for biotech companies today. For example, a single-use fermentor can be switched over from one molecule campaign to another in as little as two hours. Compared to stainless steel systems, single-use systems are modular, can be adapted when manufacturers evolve and upgrade processes, and offer significantly lower product quality risks from both regulatory and patient perspectives.

2. Dedicated Technology Built for Pilot and Production Scale Fermentation

Until now, most users have had only one choice to meet their microbial fermentation needs: stainless steel systems. Over the past decade numerous providers of single-use technologies have modified reactors in an attempt to support fermentation, but such attempts quickly reach the performance limits of the hybrid solutions. These hybrid reactors are typically adapted from low shear cell culture applications. As a result they have found moderate success with only slower growing microbes, through the use of higher oxygen supplementation and more taxing agitation rates, all of which push the limits of hybrid systems.

Over the past year, notable successes have been achieved for small-scale microbial fermentation (<3 liters) in the research and academic markets, but for process development activities and pilot scale-up, bioproduction manufacturers have had few choices. The industry now has its first purpose-built SUF designed for the unique requirements of microbial fermentation with the recent release of the Thermo Scientific™ HyPerforma™ Single-Use Fermentor. The SUF is the only single-use fermentation solution created from the bottom up using traditional engineering design principles specific to microbial fermentation. The stirred-tank fermentor system was designed with efficient mass transfer, mixing, and temperature control to meet the broad needs of industrial microbial applications.

3. Optimized for High Oxygen Mass Transfer

Generally, traditional single-use bioreactors (SUBs) deliver oxygen mass transfer rates (kLa) below 20 hr⁻¹, which is inadequate to meet aggressive microbial cultures requirements. Hybrid systems deliver more than this, perhaps in the range of 50-120 per hr. Process limitation for microbes become apparent when fermentation processes demand kLa requirements approach 400 hr⁻¹ during rapid microbial growth (measured without supplementing oxygen). The HyPerforma SUF is optimized to deliver the same oxygen transfer capability as typical stainless steel reactors, producing kLa values exceeding 400 hr⁻¹ (optical sensors, T-95 response time ~ 9 sec.)

![KLA Performance (Air)](chart)

Extensive characterization with the single-use fermentor demonstrates comparable oxygen mass transfer rates to a SIP fermentation system.
and exceeding 600 (as measured using the new DEHEMA industry group protocols).

Achieving high kLa values requires high gas flow rates, typically one to two vessel volumes per minute (vvm). The SUF drilled-hole sparge resists fouling and aids in bubble distribution, Rushton type impellers resist gas flooding and provide two-phase blending performance, and the single-use condenser system, vent heater, and unique high-flow exhaust filter are configured to support airflow of 2 vvm. Together these three exhaust modules are critical in providing the benefits of protecting the filters from fouling and reducing liquid evaporation loss during the process.

4. Powerful Mixing
Delivering high kLa values requires the use of the best mixing and blending performance available. The HyPerforma SUF achieves powerful mixing through direct mechanical agitation using a removable rigid drive shaft inside the bioprocess container. Painstaking effort was taken to insure the physical design of the agitation system matched traditional fermentor design principles. The 30L and 300L SUF vessels are scaled using the same fermentation-specific geometry: Top-driven vertical shaft, three vertically spaced Rushton type impellers, and a 4 blade baffle system which is critical in disrupting radial vortex formation.

This final configuration delivers a new performance benchmark of 11 hp/1,000 gal (2.27 W/L) of scalable mixing power.

Delivering this mixing energy requires sustainable maximum agitation rates of 600 and 375 rpm respectively, which once again is typical of rigid-walled industry CIP/SIP fermentors of this scale. It is important to maintain a delicate balance between aeration and agitation rate in fermentors that operate with a high kLa. Too much aeration increases foaming and hinders process performance and control tuning if the impellers become flooded with gas. Ultimately, at large scale, too much agitation yields excessive power consumption which can produce both unwanted process heat and exorbitant utility bills.

As one might expect based upon the design guidelines, application-based test protocol. Results have shown great success in using traditional dissolved oxygen cascade control methods of progressive agitation, air sparge, and oxygen sparging. As in traditional fermentors, this approach yields a good balance between practical process tuning and meeting desired performance requirements.

5. Efficient Cooling
The rapid cell growth and metabolism in microbial fermentation generates a great deal of heat that must be controlled in order to maximize productivity. To efficiently remove heat from inside the vessel, the tank has been engineered to provide the maximum possible jacket surface area while eliminating unnecessary liquid flow restriction in the jacket. Maximizing available surface area and fluid flow are required for optimal cooling.

The SUF is a jacketed vessel with a 3:1 height to diameter aspect ratio. It is important to keep in mind that the single-use polymer bag is 0.015” thick and this does provide some undesirable resistance to heat transfer (a notable difference as compared to traditional stainless steel only vessels). These variants serve as an important justification for seeking to optimize cooling efficiency of the SUF and application data confirms that the temperature of the SUF can be cooled sufficiently to regulate temperatures under very aggressive exponential-fed batch E. Coli process conditions.
6. Improved Exhaust Management

Dedicated fermentation systems also offer exhaust improvements over hybrid systems. Single-use systems have constrained gas flow rates due to pressure safety limits of the single use flexible containers. The low operating backpressures decrease the gas flow capacity of exhaust filters. To overcome this, a single-use fermentor effectively condenses exhaust gases and transfers condensate back into the fermentor, preventing potential vent filter blockage and bag pressurization.

The SUF was developed with a proprietary single-use exhaust filter and condenser system that maximizes the filter surface area, reduces the number of filters required, and is scalable depending on the volume required. The exhaust system’s “sweet spot” occurs when it operates below 2 vvm of gas flow in order to balance the kLa, foaming, and agitation. However, it is possible to reach flow rates up to 5 vvm by adding more filters modules.

7. Innovative Process Analytical Technologies

The SUF supports many of the newest Process Analytical Technologies (PAT). Integrated single-use compatible sensors are available for process parameters of pH, dissolved oxygen, temperature, internal pressure, and foam level. The ability to integrate a single-use foam sensor into a traditional fermentor control system is an important way to minimize the risk of foam buildup that can foul or damage exhaust filters. The top-mounted foam sensor combined with the generous headspace height designed into the SUF do serve to actively manage foam and work in combination to eliminate what would otherwise be a potentially high-risk process failure mode. These PAT modules offer much greater system reliability, eliminating a significant source of potential anxiety for the end user, allowing further automation of the processes, and the enhanced sensor response can improve product yield and consistency.

8. Scalability

Scalability should always be considered when investing in single-use technology. Single-use systems tend to be smaller scale than stainless steel tanks. With this in mind, it is not uncommon to find a 5,000 or 6,000L stainless steel bioreactor being replaced with 3 x 2,000 L single-use bioreactors. Benefits of this type of replacement include reduced risk and greater flexibility along with faster implementation times and lower capital investment. The same benefits apply to single-use fermentation.

The SUF is available in 30 L or 300 L sizes (43 L and 435 L total volume respectively). They support a turndown ratio of 5:1, which means it can operate at as low as 20% of its rated working volume. The 30 L size handles working volumes of 6-30 liters, and the 300 L handles 60-300 liter volumes. As process development activities move to production volumes, each can easily be scaled from a benchtop fermentor (or flask) to the SUF. It can also function in a seed train for larger fermentation production runs.

A key attribute of the SUF is that it offers efficient and simple technical transfer, a significant benefit for those desiring to adopt single-use technologies. It has the same fundamental engineering principles of physical geometries,
design criteria, and operating parameters as traditional CIP/SIP vessels. Such as >3:1
turndown ratio, 3:1 height/diameter ratio, mass
transfer capacity, blends time, gas residency
time, impeller diameter, and reactor volumes.
Robust tech transfer and demonstrated process
correlation have been realized because the SUF
utilizes time-proven engineering principles.

9. Performance
A single-use fermentor should be tested to
ensure it meets the performance requirements
and provides optimal conditions for dense,
rapidly growing microbial culture growth equal
to stainless steel fermentors along with quick
process setup, reduced contamination risk, and
high production quality.

Growth tests of an exponential fed-batch
culture of E. coli demonstrated the capabilities
of both the 30L and 300L HyPerforma SUF
compared to a traditional CIP/SIP fermentor
with a 100 L working volume (New Brunswick
BioFlo™ 610, 125 L total volume). While the
traditional 100 L SIP fermentor culture’s optical
density (OD) at 600 nm reached 90 ±10 by 10.5
hours, in the 30 L SUF the cultures reached an
OD600 of 120-140 with a final dry cell weight
(DCW) of 45-47 g/L by 10-14 hours. In the
300 L SUF, the cultures reached an OD600
of 130-140 with a DCW of 36-39 g/L in 14-15
hours.1 Aggressive yeast culture growth for
production of a biocatalyst (P. pastoris via
glycerol and methanol fed-batch culture)
demonstrated the capabilities of the 30 L and
300 L HyPerforma SUF when compared to a 7
L working volume stainless steel SIP fermentor
(SSB BioStat™ DCU, 10 L total volume). This
process demands high gas flow and high kLa
values and produced OD600 of 659, final wet
cell weight (WCW) of 303 g/L, DCW of 95.6 g/L,
and a cutinase yield of 2.1 g/L.2

References:
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transfer optimization, fluid filtration, mechanical drive/sealing systems, biosensors, and downstream purification.