Thermo Scientific HyPerforma Single-Use Bioreactor Bioprocess Containers with Drilled-Hole Sparger

Thermo Scientific™ HyPerforma™ Single-Use Bioreactor (S.U.B.) Bioprocess Containers (also known as Thermo Scientific™ BPC™ systems) are a proven solution for cell culture applications in the biopharmaceutical industry. We have developed a dual-sparger design that consists of a micro- and macro-sparger integrated into the third-generation standard S.U.B. BPC system. The porous-frit micro-sparger (frit) is a sintered PVDF design with pore sizes in the range of 20–40 µm, which creates bubbles with a high ratio of surface area to volume and better oxygen transfer. The drilled-hole macro-sparger (DHS) is a film-based sparger disc with laser-drilled pores that have a specific size and quantity tailored for each S.U.B. BPC system. The production of larger air bubbles by the DHS supports the frit with oxygen transfer, but also improves the removal of carbon dioxide and allows for better scalability throughout the 50 to 2,000 L S.U.B. BPC systems. This dual-sparger configuration is gentle on cell cultures at high gas flow rates, provides sufficient O_2 delivery, and dramatically increases CO_2 stripping capacity. This dual-sparger design sets a new standard for performance, flexibility, and ease of use during scale-up for all cell culture applications.

Specifications

Standard third-generation dual-sparger configuration.

S.U.B. BPC system part numbers	System size	Drilled-hole sparger configuration	Porous-frit sparger configuration
SH30985.01	50 L	1 x DHS with 360 x 178 µm holes	1 x frit with 20–40 µm holes
SH30985.02	100 L	1 x DHS with 570 x 178 µm holes	1 x frit with 20–40 µm holes
SH30985.03	250 L	1 x DHS with 760 x 233 µm holes	1 x frit with 20–40 µm holes
SH30985.04	500 L	1 x DHS with 980 x 368 µm holes	1 x frit with 20–40 µm holes
SH30985.05	1,000 L	1 x DHS with 1180 x 445 µm holes	1 x frit with 20–40 µm holes
SH30985.07*	2,000 L	2 x DHS with 690 x 582 µm holes (1,380 holes)	2 x frit with 20–40 µm holes
SH30985.08**	2,000 L	2 x DHS with 690 x 582 µm holes (1,380 holes)	2 x frit with 20–40 µm holes
SH30994.01	Vent filter assembly		

*With condenser BPC
**Without condenser BPC

Design

The DHS has been carefully designed to produce a consistent bubble size regardless of the gas flow rate delivered to the S.U.B. BPC system within a given agitation range. The parameters of hole size, count, and distribution are critical factors that have been determined for each system based upon the rated working volume. The DHS addresses the needs of cell culture processes for scalable O_2 k_La and CO_2 stripping at dissolved oxygen (DO) set points of 30–50% of air saturation.

Studies have shown that gas entrance velocities near and in excess of 30 m/s are harmful to shear-sensitive cells. The DHS design delivers velocities at less than half of this threshold across all S.U.B. BPC system sizes at 0.1 vessel volume per minute (vvm). In doing so the DHS avoids generating micro-bubbles due to self-cavitation and other phenomena typical of a macro-sparger. The result is more consistent lot-to-lot performance, better process scaling, and reduced foam generation.

Operating guidelines

Operating parameters for cell culture vary greatly between cell lines and media formulations. In developing a gassing strategy for a S.U.B. BPC system using a dual-sparger configuration, it is optional to have a crossover from N_2 to air when progressing from negative to positive DO control. It is optimal to scale the macro-sparger air delivery so that sufficient CO_2 stripping is maintained as the micro-sparger air flow rates increase. As a starting point, scale so that the macro-sparger flow rate is approximately 12.5 times the micro-sparger flow rate, and then adjust for a given cell culture process. When micro-sparger flow rate limits are reached, supplanting the micro-sparger air flow with a steadily increasing ratio of O_2 allows for a higher degree of control while working within a total micro-sparger gas flow rate limit. This approach will provide the best possible utilization of a dual-sparger configuration.

Some users may choose to not use the frit sparger in their current process; in this case it is acceptable to leave it idle and clamped off. The frit works well to complement the DHS with cultures having high demand of oxygen uptake rate (OUR), and the micro-sparger is especially efficient when delivering concentrated gases like CO_2, N_2, or O_2.

Table 1. Operating conditions for cell culture applications with drilled-hole and porous-frit spargers. Refer to the user manual for more information.

Range of operating parameters for S.U.B. BPC System with dual-sparger design																		
	50 L			100 L			250 L			500 L			1,000 L			2,000 L		
Temperature (°C)	2.0–40.0 ± 0.1																	
Operating volume (L)	25 to 50			50 to 100			125 to 250			250 to 500			500 to 1,000			1,000 to 2,000		
Agitation rate (rpm)	30 to 200			30 to 200			30 to 150			30 to 150			20 to 110			20 to 751		
Recommended max. gas flow rates	DHS	Frit	Overlay	DHS	Frit	Overlay	DHS	Frit	Overlay	DHS	Frit	Overlay	DHS	Frit	Overlay	DHS	Frit	Overlay
Air (slpm)	2.5	1	5	5	2	10	12	4	14	25	6	35	100	8	60	200	16	129
O_2 (slpm)	–	1	–	–	2	–	–	4	–	–	6	–	–	8	–	–	16	–
CO_2 (slpm)	–	0.25	–	–	0.5	–	–	1	–	–	1.5	–	–	2	–	–	4	–
N_2 (slpm)	–	1	–	–	2	–	–	4	–	–	6	–	–	8	–	–	16	–
Total (slpm)	2.5	1.25	5	5	2.5	10	12	5	14	25	7.5	35	100	10	60	200	20	129
Exhaust load (slpm)	20			20			90			90			180			270		

Table 2. Manual drilled-hole sparger flow rate operation recommendation. Due to the higher flow rate capability of the DHS, in most processes, the DHS sparger configurations will require a larger exhaust filter than the current standard S.U.B.

Recommended operation in standard dual-sparger BPC configuration using manual drilled-hole sparger operation (assumes 120-hour log growth phase)						
	50 L	100 L	250 L	500 L	1,000 L	2,000 L
Stage 1: From seed to half-log growth (seed to 60 hr)	0.015 vvm	0.0138 vvm	0.0125 vvm	0.00913 vvm	0.0126vvm	0.0175vvm
Stage 2: Half-log growth to 3rd quarter log growth (from 60 to 96 hr)	0.0288 vvm	0.027 vvm	0.025 vvm	0.0268 vvm	0.0304vvm	0.0375vvm
Stage 3: 3rd quarter log growth through stationary phase (96 hr and beyond)	0.0538 vvm	0.052 vvm	0.05 vvm	0.0539 vvm	0.0618vvm	0.0775vvm

Note: Recommendation based upon cell culture run using CHO cells grown using CHO media with 6 g/L a cell growth media supplement in a 250 L S.U.B. BPC system at 250 L liquid volume, 37°C, 50% DO, pH 7, and 124 rpm agitation. Recommendation also assumes culture is capable of reaching near 10^6 cells/mL density and has a log growth phase of approximately 5 days. Values for 100, 500, and 1,000 L S.U.B. BPC systems with DHS parts are estimates based on parameter scaling. Users should always verify performance in their specific operating environment.

Supporting data

Performance characterization was performed to predict the potential effects of parameter changes under controlled conditions using a buffered medium between the original dual-sparger design (macro: open-pipe sparger; micro: porous-frit sparger) and the next-generation dual-sparger design (macro: DHS; micro: porous-frit sparger).

- The first set of graphs (Figure 1) shows data from the 250 L S.U.B. BPC system and is representative of mass transfer performance for all S.U.B. BPC systems from 50 to 2,000 L. Analysis in this way allows direct and relative comparison of each of the three sparge methodologies.
- The second set of graphs (Figures 2 to 4) shows 3-dimensional representations of the interaction of the sparger channels as tested in the 250 L S.U.B. using regression modeling. The resulting surface plots are helpful in understanding the interaction between different sparge mechanisms. The graphs demonstrate that the key strength of the third-generation sparger platform is its ability to exceed a ratio of 0.33 (k_La for CO_2 stripping to O_2 mass transfer delivery) for nearly the entirety of a specified sparge flow range.

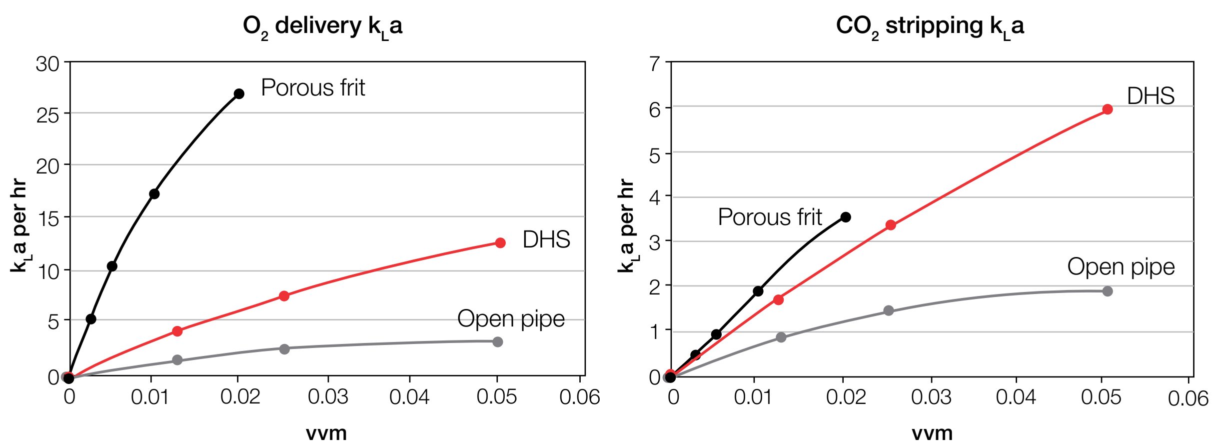

Figure 1. Comparison of performance of DHS, open-pipe, and porous-frit spargers. The DHS provides more linear behavior and 3–4 times increased performance when compared with the open-pipe sparger.

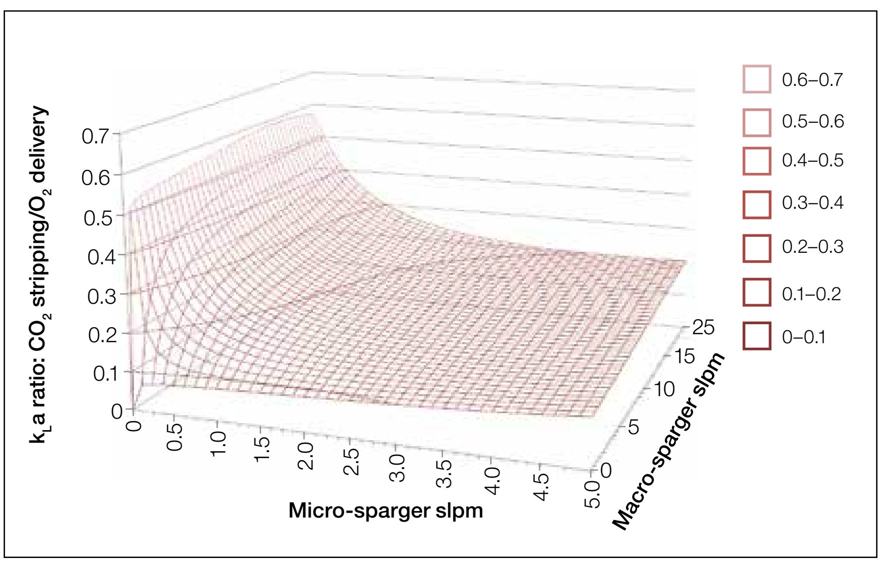

Figure 2. k_La performance ratio for CO_2 stripping/O_2 delivery on a 250 L S.U.B. current standard BPC system with standard design (porous-frit micro sparger plus open-pipe macro-sparger). This 3-dimensional graph shows a reduction in the CO_2 stripping ratio when operating the frit sparger at 1 slpm or higher.

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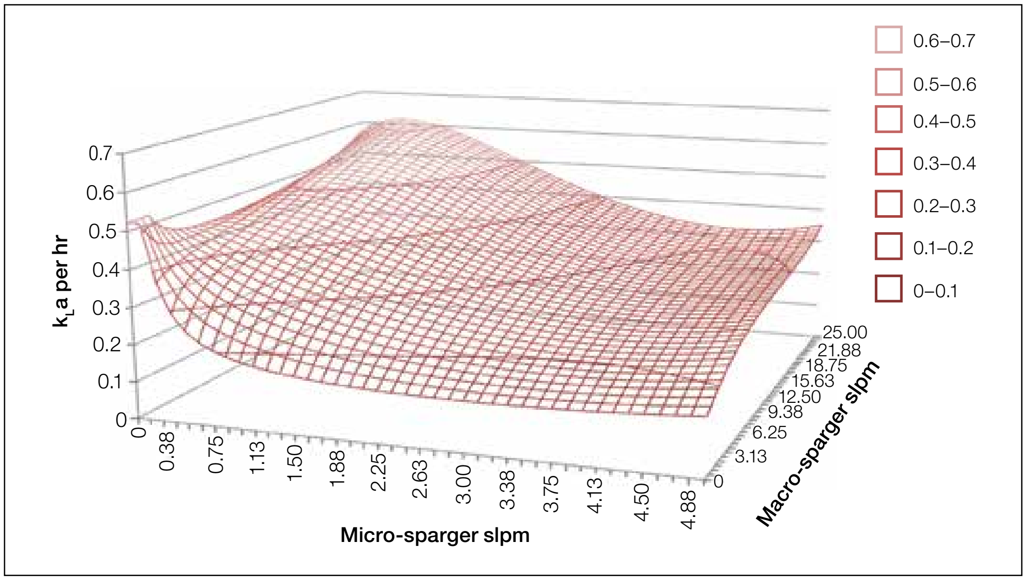

Figure 3. k_La performance ratio for CO_2 stripping/O_2 delivery on a 250 L S.U.B. with porous-frit micro-sparger plus drilled-hole macro-sparger. This 3-dimensional graph demonstrates the increased reach of higher CO_2 stripping into a larger range of operating conditions. Stripping ratio can be maintained even at high flow rates.

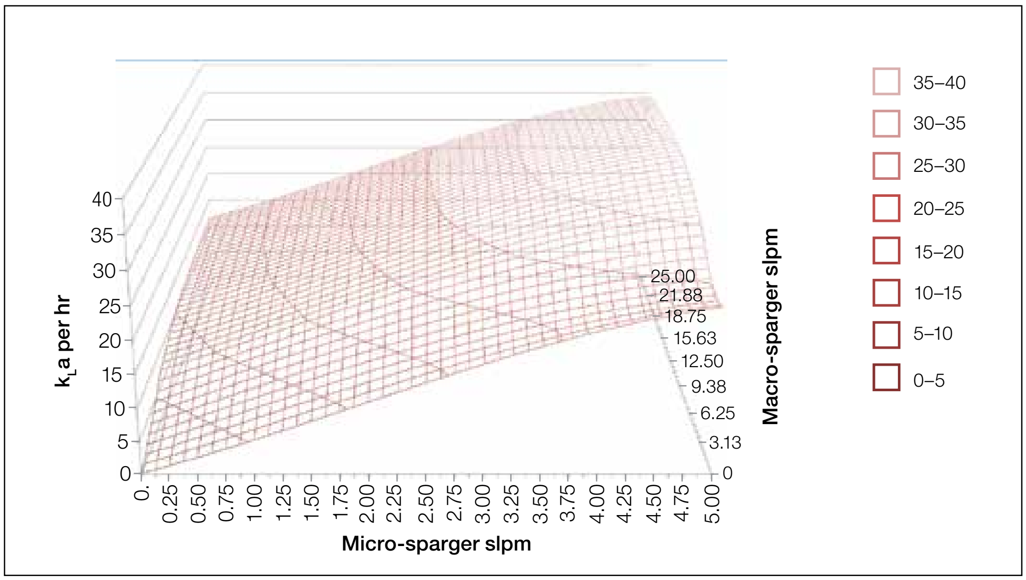

Figure 4. k_La for O_2 delivery at 0.15 HP/1,000 gal agitation on a 250 L S.U.B. with porous-frit micro-sparger plus drilled-hole macro-sparger. This 3-dimensional graph shows a performance map of dissolved O_2 k_La from 0 to 0.02 vvm micro-sparger and 0 to 0.1 vvm macro-sparger flow rates. This demonstrates the performance of the next-generation standard design with good scalability for up to 35 hr [1].

1. Zhu Y, Cuenca JV, Zhou W et al. (2008) NS0 cell damage by high gas velocity sparging in protein-free and cholesterol-fee cultures. *Biotechnol Bioeng* 101(4):751–760.

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