Automated foam control in HyPerforma Single-Use Bioreactors using a single-use foam probe

Introduction

The generation of foam is common in aerobic bioreactor systems. Foaming can cause a range of problems, including increased contamination risks through potential exhaust filter fouling, poor mass transfer, product/cell entrainment, increased shearing from bursting bubbles, and cell death [1,2,3]. Proper foam control in bioreactors is essential to ensuring a successful culture campaign. The Thermo Scientific™ single-use foam probe is a composite product-contacting probe that has been designed to detect foam in large-scale Thermo Scientific™ HyPerforma™ Single-Use Bioreactor (S.U.B.) operations. Coupled with proper controller integration, the probe has been shown to effectively control foam, even under high-gassing conditions. This capability allows the end user to shift from a time-depended antifoam delivery protocol developed based on educated guesses to a controlled protocol based on an output signal, resulting in less antifoam consumption and providing traceability of foam generation within the bioprocess.

This application note outlines the cause of foam, use of antifoaming agents to control foam, design and integration of the single-use foam probe (including a mounting system and bioreactor controller integration), and several successful case studies utilizing the foam probe in aggressive bioreactor cultures. This study demonstrates that foam can be controlled across all S.U.B. sizes, 50 to 2,000 L.

Foam production and antifoams

Foam is generated in gassed S.U.B.s when the liquid–air interface of a gas bubble is sufficiently stable, such that the bubble does not easily burst upon reaching the top of the reactor. The surface tension of the bubble, as well as bubble-to-bubble interactions, dictate foam stability. When this foam layer is allowed to build, potentially catastrophic foam-outs can occur in the bioreactor. In both stainless steel and single-use systems, chemical agents are typically used to reduce foam buildup. Chemical antifoams typically work by modifying the surface tension of the liquid-air interface inside the bioreactor.

Antifoams function in one of two ways: they either displace foam-stabilizing agents (surfactants), such as proteins, polysaccharides, or fatty acids [1] from the bubble surface, or they locally burst the bubble [4]. Displacing the foam-stabilizing agents only occurs after a thin layer of antifoam is spread over the bubble. Antifoam success depends on the surface tension of the liquid surrounding the bubble. For the foam to dissipate, the surface tension of the antifoam must be less than that of the foam in the bioreactor.

Antifoams are most effective when located at the liquid–air interface. While the exact compositions of the various commercially available antifoam agents are proprietary, one of the primary differences is the strength or quantity of emulsifiers present in solution. A strong emulsifier will allow antifoam molecules to go into solution more easily, providing a more shelf-stable, homogeneous mixture.

However, because the antifoam is brought more easily into solution upon delivery to the bioreactor, the efficiency of foam degradation is reduced. The opposite is true for antifoams with a weak emulsifier: bolus delivery is less chemically uniform, but more antifoam stays at the liquid–air interface. A need for continual antifoam addition is often observed. Antifoaming agents are composed of monomers with hydrophilic heads and hydrophobic tails, so a monolayer forms on the surface of the fluid upon initial addition to the bioreactor. The monolayer is disrupted by mechanical agitation of the vessel, bringing the antifoam into solution. As the antifoam is brought into solution, the steric nature of the defoaming agent can lead to micelle formation, rendering the molecule ineffective. Other compounds in the vessels can displace antifoam from the liquid–air interface, requiring antifoam addition throughout the duration of the culture [2]. By accurately measuring foam generation and controlling antifoam addition using the foam probe, operators can increase efficiencies in foam management and prevent reactor foam-outs.

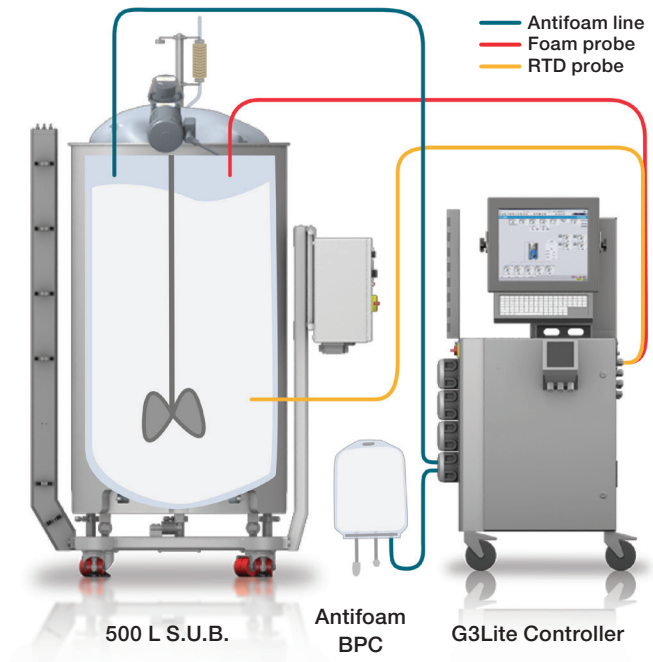


Figure 1. Setup of a foam sensor in a 500 L HyPerforma S.U.B. connected to a Thermo Scientific™ HyPerforma™ G3Lite™ Controller.

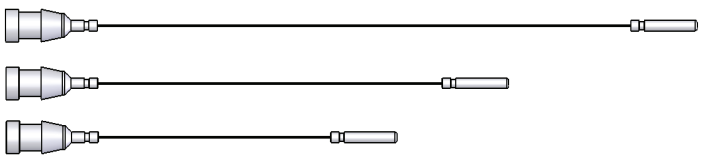


Figure 2. Foam probe family, top: 2,000 L S.U.B. foam probe, middle: 1,000 L and 500 L S.U.B. foam probe, bottom: 250 L, 100 L, and 50 L S.U.B. foam probe.

Foam probe design

Traditionally, foam probes are used in glass benchtop bioreactors to monitor and control process foam levels. Process value readings are conductivity-based; as the amount of liquid and/or air increases and touches the sensor, process values increase and decrease, respectively. Benchtop foam probes are rigid, and are sterilized when the bioreactor is autoclaved. Effectively translating this technology into a single-use design requires low cost, flexibility, and gamma irradiation compatible.

The single-use solution is composed of two 316 L stainless steel components connected by a small-gauge conducting wire. The stainless steel components with hose barb geometry mate to the port of the BPC, and provide an exterior cable connection to the controller. The conducting wire is Nitinol, a shape memory alloy, meaning that despite stressors to the wire (such as fabrication, packaging, shipping, or S.U.B. installation), the wire will return to its original shape when the stressor is removed. The small-gauge wire also aids in preventing sensor fouling, and allows for accurate foam measurement.

As previously mentioned, foam measurement is based on conductivity. Figure 2 shows the family of foam probes for S.U.B.s, each consisting of two conductive electrodes separated by a specific distance. The positive reference electrode is the foam probe in the top of the headspace while the stainless steel casing of the resistance temperature detector (RTD) probe has been wired to serve as the grounding reference (it must be fully seated and directly in contact with stainless steel cap at the end of the BPC thermowell and immersed in the process liquid seen in Figure 3). The controller provides a known AC voltage to one side of the electrode, while monitoring the other side of the circuit for changes in conduction. The conductivity signal will increase if foam or fluid contacts the foam probe. The controller uses this process value as logic for setting antifoam pump output. All physical and wired connections are required to be closed, except the known length between electrodes.

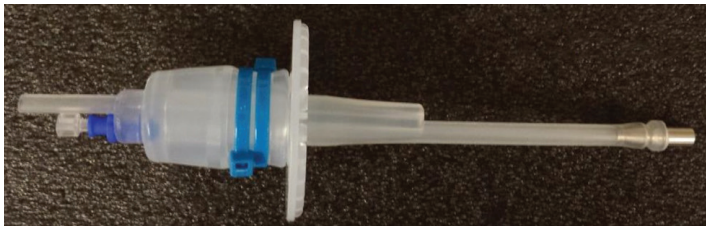


Figure 3. Ground clip connection.

Connecting the foam probe to the controller does not require additional ports on the controller cabinet. The foam probe connection has been engineered to integrate with the RTD connector on the controller side of the cable. It is required to replace the original RTD with an RTD with foam sensor wire. Matching RTD length with BPC temperature probe sheath is essential. Two lengths of RTDs are offered, therefore, two lengths of BPC temperature sheaths are provided. S.U.B.s in the 2:1 and 5:1 configurations typically have the long RTD sheath (Cat. No. SV21487.01), requiring a long RTD (Cat. No. SV50999.05). S.U.B.s configured for 4:1 with upsized impeller operation have the short RTD probe sheath (Cat. No. SV21487.02), requiring a matching short RTD (Cat. No. SV50999.04). RTD with foam sensor, S.U.B. configuration, and standard foam probe recommendations are detailed in Table 1.

The properties of foam determines the process value. Descriptions of the process value output of the foam probe are detailed in Table 2. The foam probe can be used in all HyPerforma S.U.B. sizes from Thermo Fisher Scientific. Sizes available and part numbers are detailed in Table 3.

Best practice is to set the probe height at 2 inches above the liquid surface, which will allow a daily margin for feeds and avoid false-positive readings. The 2-inch tolerance is adjustable by using the foam probe holder illustrated in Figure 4, an appendage to the filter tree bracket that allows standardization of foam probe placement inside of the S.U.B. Figure 5 demonstrates the foam probe holder installed on the 50 L S.U.B. The foam probe holder can be adjusted during cell culture, allowing the ability to maintain a constant distance between changes in reactor volumes (feed addition).

Table 1. RTD with foam sensor cable required for S.U.B. operation.

Size	Configuration	RTD foam probe sensor wire	BPC temperature probe sheath	Standard foam probe
50 L	2:1	SV50999.05	SV21487.01	SV21323.06
	5:1			
	4:1 (with upsized impeller)	SV50999.04	SV21487.02	
100 L	2:1	SV50999.05	SV21487.01	
	5:1			
	4:1 (with upsized impeller)	SV50999.04	SV21487.02	
250 L	2:1	SV50999.05	SV21487.01	
	5:1			
	4:1 (with upsized impeller)	SV50999.04	SV21487.02	
500 L	2:1	SV50999.05	SV21487.01	SV21323.05
	5:1			
	4:1 (with upsized impeller)	SV50999.04	SV21487.02	
1,000 L	2:1	SV50999.05	SV21487.01	
	5:1			
2,000 L	2:1	SV50999.05	SV21487.01	SV21323.07
	5:1			

Table 2. Foam probe process values description.

Foam probe process value	Physical condition
0%	Foam probe not connected
~25%	Foam probe exposed to air (no foam), static position
~25% to <100%	Foam probe exposed to liquid/air mixture
100%	Foam probe submerged or bridged connection

Table 3. S.U.B. foam probe specifications.

Size	Functional length	Cat. No.
50 L	134.6 mm (5.3 in.)	SV21323.06
100 L	134.6 mm (5.3 in.)	SV21323.06
250 L	134.6 mm (5.3 in.)	SV21323.06
500 L	198.6 mm (7.82 in.)	SV21323.05
1,000 L	198.6 mm (7.82 in.)	SV21323.05
2,000 L	306.6 mm (12.07 in.)	SV21323.07

Foam control

The correct implementation of foam control requires an output from a pump to cascade based on a user-defined process value output by the foam probe. When the sensor detects foam, a wait period is initiated, called a “splash delay.” The splash delay functions as a user-defined snooze timer, suppressing any output for a defined period. When the splash delay timer has completed and foam is still present in the S.U.B., the foam control loop is triggered [7]. The equation governing the control loop is as follows:

$$When\ PV > [\ ]\ set\ HI\ output\ to\ [\ ]\ for\ [\ ]\ sec\ then\ to\ 0\ for\ [\ ]\ sec$$

To set up foam controls, users can select the “Config” tab on the Thermo Scientific™ TruBio™ software home screen, and then the “Foam” icon on the “Configuration” screen. The foam configuration allows the user to set the following [7]:

- Alarm delay: Sets the time in seconds before an alarm is activated (if the alarm is enabled).
- Splash delay: Sets the time in seconds, during which the alarm is prevented from being triggered and pump output is suppressed, which allows temporary contact with liquid.
- High threshold output control: Allows the selection of an action when the process value exceeds the selected range. The controller output can be set to a specified time, with a specified interval for mixing before the controller is again activated.
- Alarms: Can be enabled or suppressed as the user prefers.

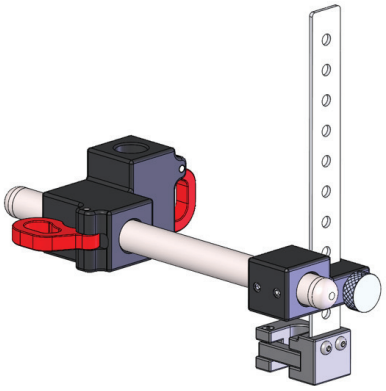


Figure 4. Foam probe holder.

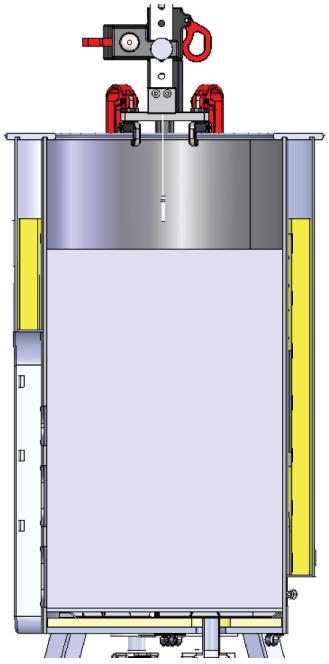


Figure 5. 50 L S.U.B. with foam probe. BPC is hidden in the cross-sectional vessel view.

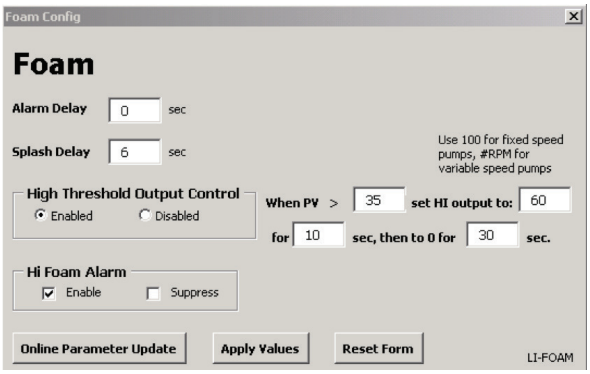


Figure 6. The foam configuration screen of TruBio software.

Cell culture application: materials and methods

Cell culture data was gathered to demonstrate the scalability of the foam probe across multiple S.U.B. sizes, and its application to standard and aggressive cell culture processes. To develop the breadth of data required to show effectiveness of the foam probe in the S.U.B., a phased approach was implemented as a part of a case study. Table 4 provides descriptions of each cell culture case study.

Materials

For each study, similar cell culture protocols were followed as previously described [5,6]. For fed-batch cultures, Gibco™ Freedom™ CHO-S™ Cells were grown in Gibco™ Dynamis™ AGT™ Medium containing 0.1% Gibco™ Anti-Clumping Agent and were fed a combination of Gibco™ EfficientFeed™ C+ Supplement and 45% glucose. For the perfusion culture, DP-12 cells (ATCC, Cat. No. CRL-12445) adapted to LONG™ R3 IGF-1 cells were grown in Gibco™ OptiCHO™ AGT™ Medium in conjunction with a cell separator to maintain ideal operating conditions at high cell density. In all reactors, Sigma-Aldrich™ Antifoam C or Gibco™ FoamAway™ irradiated animal origin–free (AOF) antifoaming agent were used (Table 5).

Table 4. Description of case studies for foam probe investigation.

Case study	Title	Description
1	50 L S.U.B. fed-batch side-by-side, standard gassing strategy	Two 50 L S.U.B. fed-batch processes ran side-by-side, each targeting minimal gas input cascading O_2 and/or N_2 as required. S.U.B. A: antifoam additions were controlled by the foam probe; S.U.B. B: antifoam additions were time-based or as needed.
2	50 L S.U.B. high-density perfusion cell culture	50 L S.U.B. perfusion application operated at 40 L volume using alternating tangential flow (ATF) technology. Power input was controlled throughout the culture to observe the relationship between power input by agitation and gassing requirements.
3	50 L S.U.B. fed-batch side-by-side, aggressive gassing strategy	Foam probe performance was evaluated in a 50 L S.U.B. fed-batch process that used an aggressive gassing strategy. S.U.B. A: antifoam additions were controlled by the foam probe; S.U.B. B: antifoam additions were time-based or as needed.
4	1,000 L S.U.B. fed-batch, aggressive gassing strategy	Foam probe performance was evaluated in a 1,000 L S.U.B. fed-batch process that used an aggressive gassing strategy.

Table 5. HyPerforma S.U.B. operating conditions.

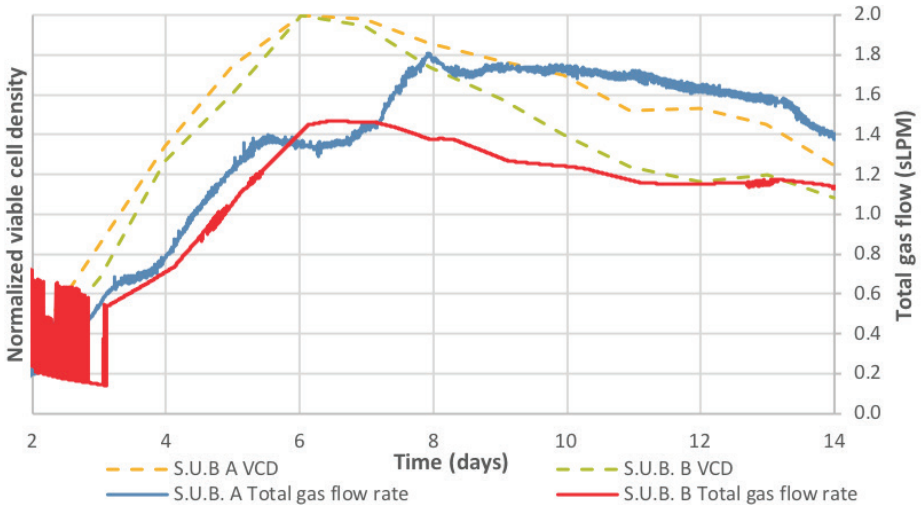
Parameter	Condition					
Case study	**1**		**2**	**3**		**4**
S.U.B.	2 x 50 L S.U.B.		50 L S.U.B.	2x 50 L S.U.B.		1,000 L S.U.B.
Working volume	50 L		40 L	50 L		50 L; 1,000 L
pH	6.8–7.2 (no base)		6.8–7.2 (no base)	6.8–7.2 (no base)		6.8–7.2 (no base)
Agitation	20 W/m^3		Varied (20–100 W/m^3)	20 W/m^3		20 W/m^3
Dissolved oxygen setpoint	30%		30%	30%		30%
Dissolved oxygen cascade	Oxygen and/or nitrogen through drilled hole sparger (DHS) as needed to maintain setpoint		Oxygen and/or nitrogen through DHS as needed to maintain setpoint	D0–D3: Oxygen and/or nitrogen through DHS D3–D6: Linearly increased total gas flow to 0.1 VVM with oxygen, nitrogen, and air D6–D14: Total gas flow rate of 0.1 VVM through DHS using oxygen, nitrogen, and air		D0–D3: Oxygen and/or nitrogen through DHS D3–D6: Linearly increased total gas flow to 0.1 VVM with oxygen, nitrogen, and air D6–D14: Total gas flow rate of 0.1 VVM through DHS using oxygen, nitrogen, and air
Headspace sparge	3 sLPM air		3 sLPM air	D0–D3: Air, (DHS flow rate—0.1 VVM targeting) 5 sLPM total flow rate exiting vent filter D3–D6: Air, linearly decrease head space to allow more gas flow though DHS, targeting 5 sLPM gas flow through DHS D6–D14: 3 sLPM air		D0–D3: Air, (DHS flow rate—0.1 VVM targeting) 100 sLPM total flow rate exiting vent filter D3–D6: Air, linearly decrease head space to allow more gas flow though DHS, targeting 100 sLPM gas flow through DHS D6–D14: 0 sLPM air
Antifoam	S.U.B. A: Antifoam C; automated using foam probe	S.U.B. B: Antifoam C; manually controlled	Antifoam C; automated using foam probe	S.U.B. A: Antifoam C; automated using foam probe	S.U.B. B: Antifoam C; manually controlled	FoamAway antifoaming agent; automated using foam probe
Seeding density	0.46 x 10^6 cells/mL	0.39 x 10^6 cells/mL	0.3 x 10^6 cells/mL	0.3 x 10^6 cells/mL	0.3 x 10^6 cells/mL	0.3 x 10^6 cells/mL

Cell culture application: results

Case study 1 results

Cell culture was performed in 50 L S.U.B.s to test the foam sensor's ability to respond in a standard fed-batch process. Two reactors were operated: in S.U.B. A, foam was controlled by the foam probe on an as-needed basis, while in S.U.B. B, foam was controlled manually using a dosing function based on a time interval between additions. Each reactor was seeded at approximately 0.4 x 10^6 cells/mL. On day 3, feed was initiated in each reactor and ran constantly at 0.65 g/min through day 10. Glucose was supplemented as needed to maintain 3 g/L in culture. Viable cell density and gassing comparisons were measured offline, and are shown in Graph 1.

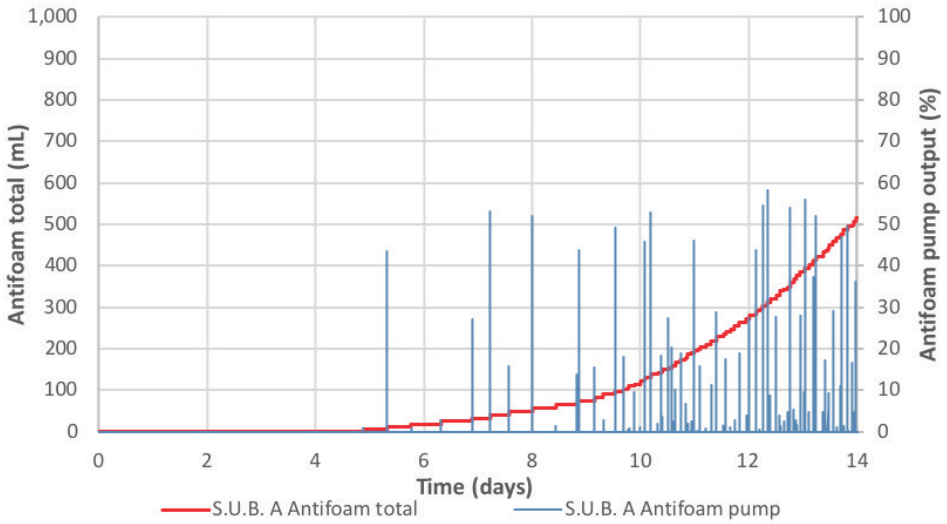
Graphs 2 and 3 detail foam probe performance of S.U.B.s A and B. Similar performance in viable cell densities and gas flow rates were observed in both S.U.B.s. Results from case study 1 demonstrated that when standard operating gassing conditions are employed, the foam probe led to a 47% reduction in the amount of antifoam delivered to the culture. Increased tubing life and less particulate creation is expected by significantly reducing peristaltic pump requirements. Table 6 details pump actions and antifoam control schemes throughout the culture.



Graph 1. Viable cell density and total gas flow rate in 50 L S.U.B. with automated or manual addition of antifoam.

Table 6. Case study 1 antifoam pump output and antifoam control scheme.

Parameter	S.U.B. A: automated	S.U.B. B: manual
Antifoam control profiles	When the foam probe process values reach >35, set pump output to 100 for 10 seconds, then wait for 30 seconds	Time-based dosing function gradually increasing amount of antifoam delivered and decreasing the time interval between doses
Total antifoam delivered (mL)	517	946
Pump cycles	138	510
Average bolus addition (mL)	3.8	1.87

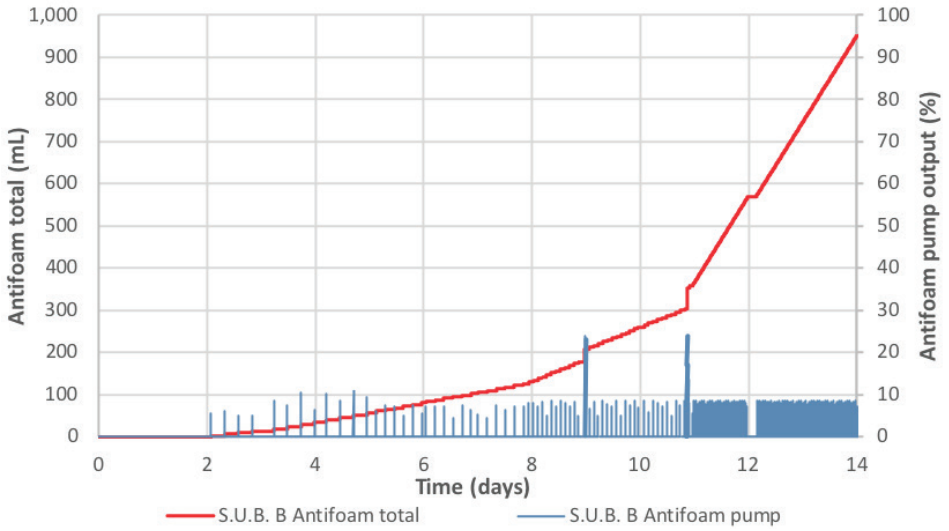


Graph 2. Case study 1 (50 L S.U.B.) total antifoam additions compared to pump output, automated additions.

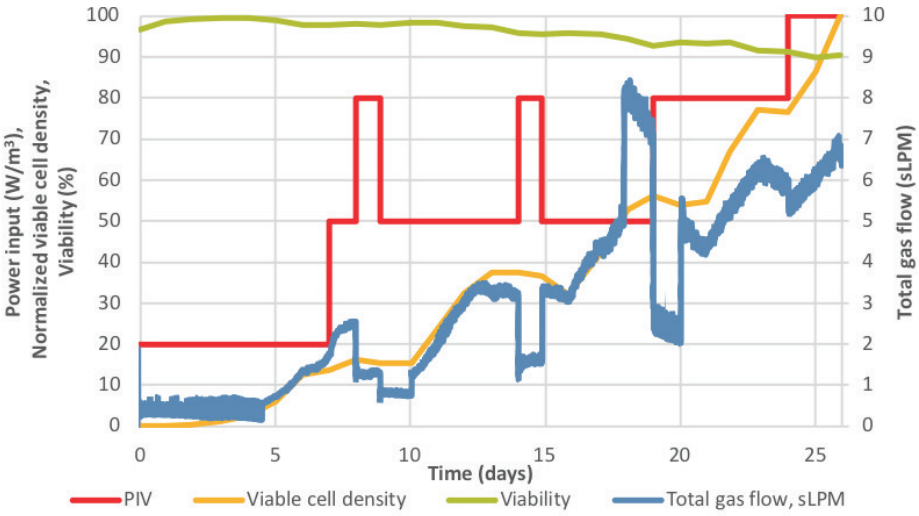
Case study 2 results

A perfusion cell culture was performed in a 50 L S.U.B. to demonstrate the capabilities of the S.U.B., controller, cell retention device, and the foam probe to perform in a high cell density perfusion application. Total gas flow rates throughout the 26-day process varied based on power input by the impeller (Graph 4). As expected, an increase in power input led to a decrease in gassing requirements throughout the process. Viable cell density was controlled throughout the process, and a cell bleed was automated based on feedback provided from a bio-capacitance probe. Graph 5 details the foam probe profile of the high cell density process. On day 26, the ATF filter fouled at a viable cell density of 263 x 10^6 cells/mL, terminating the cell culture. Viable cell densities had been over 200 x 10^6 cells/mL for 4 days prior.

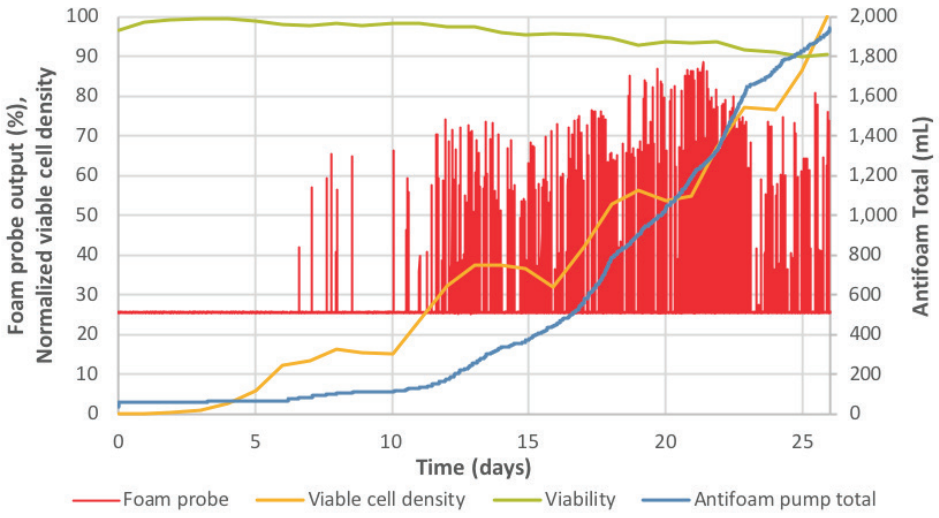
For in-depth technical performance evaluation of this specific culture, refer to: Continuous Processing – Performance Enhancements for Perfusion Applications in 50 L to 500 L Single-Use Bioreactors: A Technical Comparison of Performance Characterization, Cell Culture, and Scale-Up Modeling [5]. Briefly, foam was kept in control through the entire run with no observed anomalies despite the high gas flow rates shown in Graph 4.



Graph 3. Case study 1 (50 L S.U.B.) total antifoam additions compared to pump output, manually controlled additions.



Graph 4. Case study 2 results. Viable cell density and viability compared to total gas flow rate and power input in the 50 L perfusion cell run.



Graph 5. Case study 2 results. Foam probe output, viable cell density, and total antifoam delivered using the 50 L S.U.B.

Case study 3 results

Cell culture was performed in 50 L S.U.B.s to test the foam sensor's ability to respond in an aggressive foaming application in a fed-batch process. Two reactors were operated: in S.U.B. A, foam was controlled by the foam probe on an as-needed basis, while the foam in S.U.B. B was controlled manually, using a dosing function based on a time interval between additions. Each reactor was seeded at approximately 0.4 x 10^6 cells/mL. On day 3, feed was initiated in each reactor and ran constantly at 0.65 g/min through day 10. Glucose was supplemented as needed to maintain 3 g/L in culture. Viable cell densities and gassing results are detailed in Graph 6. An anomaly in the viable cell density of S.U.B. B was discovered late in the cell run, although this anomaly did not affect the foam probe performance or the culture's ability to produce foam.

As expected, it was demonstrated that the aggressive gassing strategy developed to prove efficacy of the foam probe was detrimental to longevity of the cell line, but demonstrated large amounts of foam production. S.U.B. A required 784 mL antifoam, and S.U.B. B required 729 mL. The difference in antifoam consumption between S.U.B. A and S.U.B. B is considered negligible.

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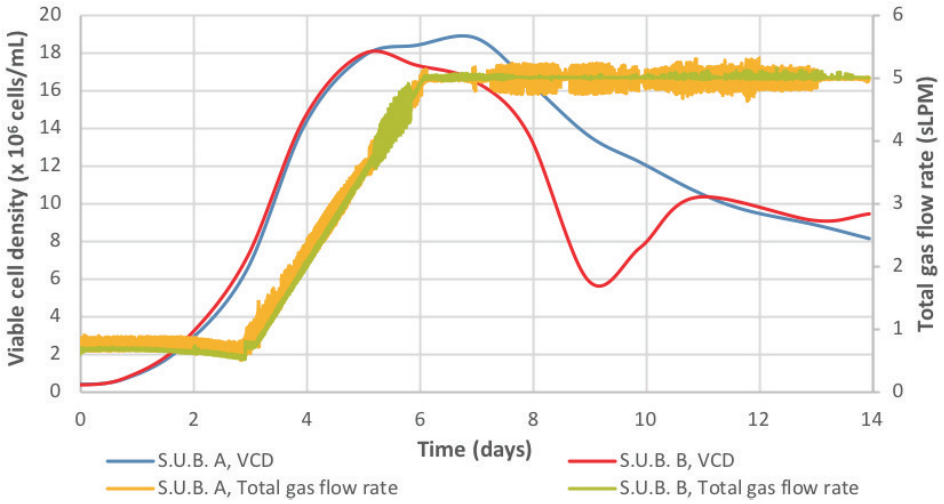
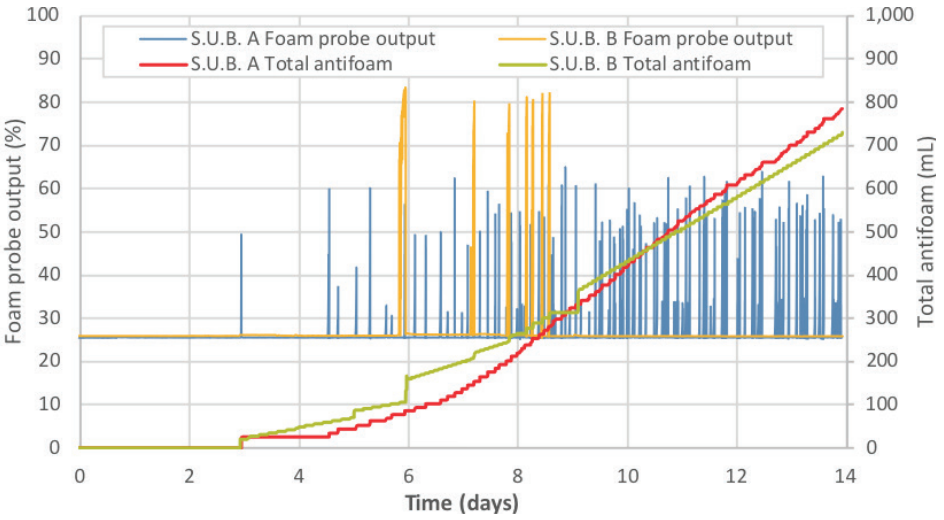


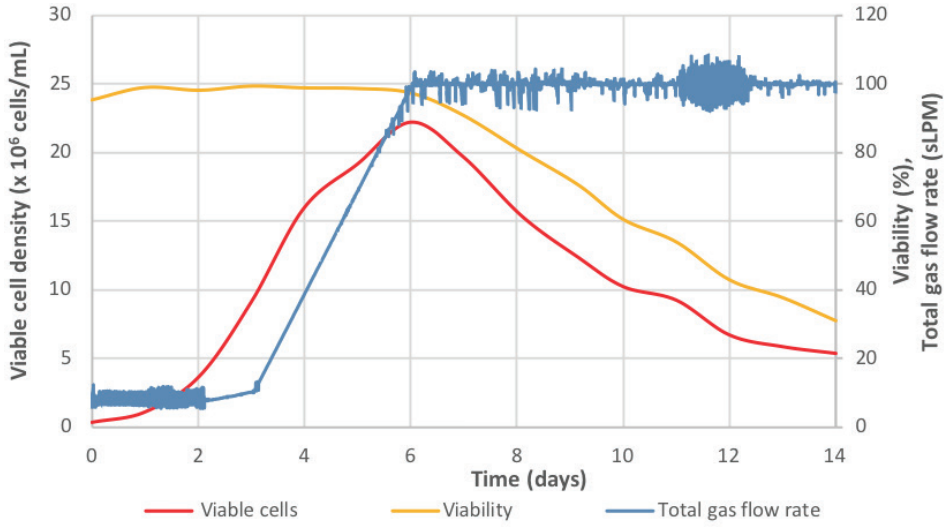
Graph 6. Case study 3. Aggressive gassing in 50 L S.U.B. viable cell density and total gas flow rate.



Graph 7. Case study 3. S.U.B. A (foam probe controlled) and S.U.B. B (manually controlled) total antifoam delivered with foam probe output. If the manual foam control strategy performed adequately, no foam probe output would be visible. Foam probe output on days 6 through 10 demonstrates the inadequate manual foam control.

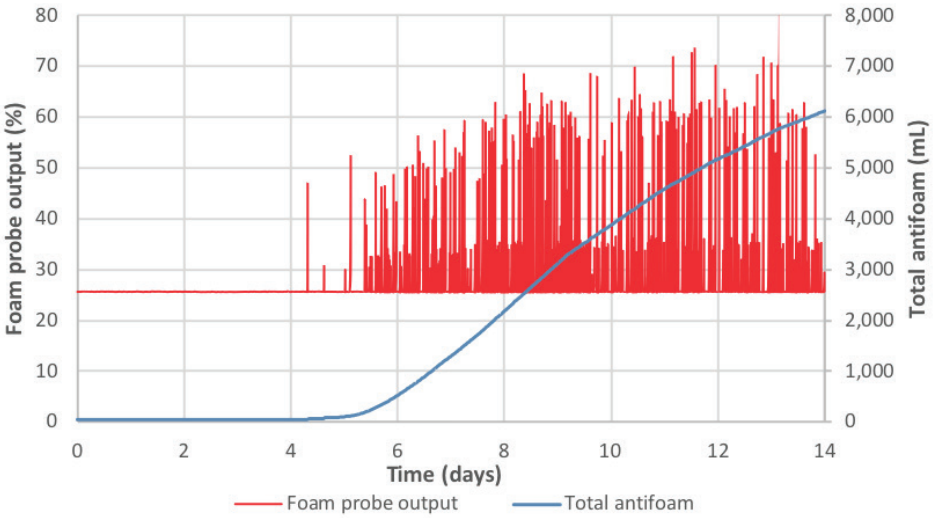
Case study 4 results

A 1,000 L S.U.B. fed-batch process was run implementing the same aggressive gassing strategy of 0.1 VVM detailed in case study 3. The total gas flow rate delivered to the DHS on days 0 to 6 linearly increased from normal gassing conditions to aggressive gassing conditions, totaling 100 sLPM on days 6 through 14. On day 3, feed was started and consistently fed through day 10. Glucose was supplemented as needed to maintain 3 g/L in culture. Viable cell density and viability results are detailed in Graph 8.



Graph 8. Case study 4. 1,000 L S.U.B. viable cell density, viability, and gassing strategy.

Prepackaged and presterilized FoamAway antifoaming agent was used as the antifoaming agent due to the larger storage reservoir available. The foam probe recorded all foaming events and antifoam was dosed accordingly. Foam probe performance is detailed in Graph 9.



Graph 9. Case study 4. 1,000 L S.U.B. foam probe performance.

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Discussion

Results from all experimental case studies demonstrate that the foam probe can respond reliably to both standard foaming and aggressive foaming environments. The foam probe design allows a significant reduction in the amount of antifoam required in traditional fed-batch operations when compared to manual additions. A primary concern of using the foam probe is the reliability of performance in aggressive applications. This study demonstrated robust performance across all applications.

The foam probe consistently responds to foam buildup in ultra-high cell density perfusion applications. The data show foam stability throughout the 26-day cell run, despite minor volume changes due to media exchanges, variable agitation and sparging rates, and high cell density cultures.

Scalability of the foam probe is demonstrated in this study at 50 L and 1,000 L scale, and offers confidence in applying the foam sensor across all S.U.B. sizes. Maintaining a constant allowable threshold of 2 inches of foam is simple using the foam probe holder bracket.

Conclusion

- The foam probe for S.U.B. systems provides a robust solution for customers who seek to improve foam management in their workflow.
- Employing the foam probe in standard S.U.B. gassing conditions led to a reduction of almost 50% of total antifoam delivered throughout the culture.
- Scalability of the foam probe from 50 L to 2,000 L S.U.B.s is straightforward and effective.
- The foam probe demonstrates exceptional performance in aggressive fed-batch and ultra-high cell density perfusion applications.
- Using the foam probe holder in conjunction with the foam probe provides a repeatable target working volume of 90% to 110%.
- The foam probe provides a significant reduction in risk of either exhaust filter foam-out, creating BPC overpressure. Bioreactor operators noted greater confidence in leaving the bioreactor unattended, such as overnight, when using the foam probe.

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