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

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Bioprocessing

ABSTRACT

Purpose: Cell culture and fermentation has the potential to generate foam in great quantity in stirred-tank reactors. To reduce the need for constant supervision, manual intervention, and excessive chemical anti-foam usage, an automated foam probe is proposed.

Methods: A foam detection system was designed to provide accurate and consistent results. It was designed to resist forces applied from manufacturing, shipping, and BPC installation, and to resist fouling in culture. The system was tested to automatically manage foam in several cell culture applications, and was compared to time-based anti-foam addition strategies. Comparisons included viable cell density, and amount of antifoam used.

Results: The design of the foam detection system was proven effective in standard cell culture fed-batch processes as well as ultra-high density perfusion processes. The effectiveness was proven to scale through the full range of HyPerforma™ Single-Use Bioreactors (S.U.B.). It was shown to reduce the amount of chemical anti-foam required, decrease risk, and increase confidence in bioreactor safety and functionality.

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]. The development of proper methods and equipment to control foam is critical. A single-use foam probe would allow the end user to shift from a time-dependent antifoam delivery protocol developed based on educated guesses to a controlled protocol based on an output signal, resulting in greater confidence and control of the system, less antifoam consumption, and traceability of foam generation within the bioprocess.

MATERIALS AND METHODS

Four case studies were performed as outlined in Table 1. Dissolved oxygen (DO) was maintained at 30% with a cascaded mixture of oxygen, air, or nitrogen through a drilled-hole sparger (DHS). Total gassing through DHS and headspace varied by case: Case 1 sparged gas through the DHS as needed, with 3 sLPM through the headspace. Case 2 targeted a total gassing rate of 5 sLPM between the DHS and headspace through the first 6 days, DHS gas flow was ramped to 0.1 VVM in the first 6 days of the culture, and held at 0.1 VVM for the remaining 8 days. Headspace was set to 3 sLPM for that 8 day duration. Additionally, two other cases studies were examined: an ultra-high density perfusion cell culture and a large scale (1000 L) aggressive gassing strategy cell. Also tested was application in HyPerforma™ Single-Use Fermenters (S.U.F.) with very high gassing. Viable cell density, total gas flow rate, and occurrences of and total antifoam additions were monitored through each run [4].

Table 1. Description of case studies for foam probe investigation.

Case Study	Title	Description
1	50L S.U.B. fed-batch side-by-side, standard gassing strategy	S.U.B. A: antifoam controlled by foam probe S.U.B. B: antifoam additions time-based/as needed
2	50 L S.U.B. fed-batch side-by-side, aggressive gassing strategy	S.U.B. A: antifoam controlled by foam probe S.U.B. B: antifoam additions time-based/as needed

DESIGN

The foam sensor uses a conductive loop to detect the presence of foam. One lead is grounded in the bottom of the culture broth through the RTD. The other lead is attached to a specifically designed foam probe shown in Figure 1, inserted into the top of the BPC. The single-use probe 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 foam contacts the probe, the conductivity of the loop changes. A threshold value, pumping speed, and pumping duration can be set in the controller to engage the antifoam pump [6].

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

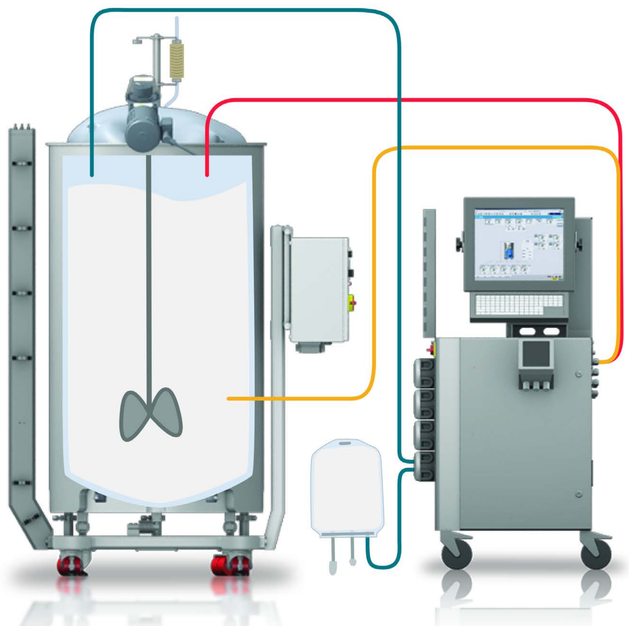
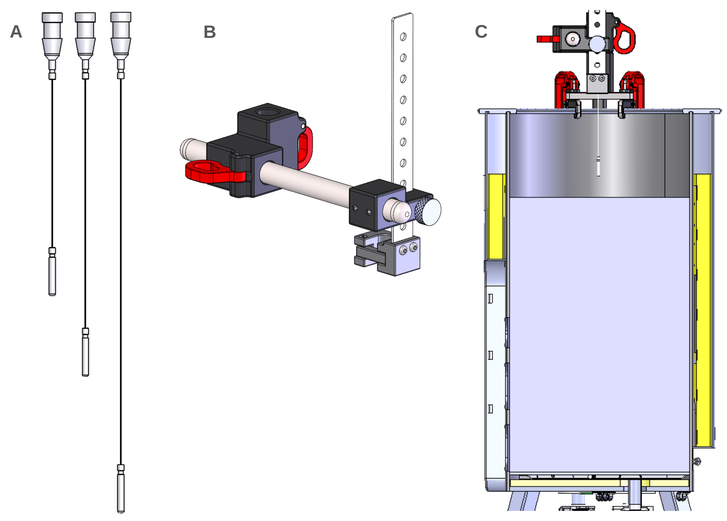


Figure 2. A: Foam probe family – 50L S.U.B. through 2000L S.U.B. B: Foam probe holder. C: 50 L S.U.B. cutout showing foam probe positioning with probe holder.



RESULTS

Case study 1, a comparison of automated and timed delivery of antifoam in a standard fed-batch cell culture, resulted in a 47% reduction in amount of antifoam delivered to the culture when using automated dosing as needed (Figure 2). Pump cycles were reduced by 73%, thus increasing tubing life and generating less particulate. Viable cell density and total gas flow are shown in Figure 3.

Case study 2, a comparison of automated and timed delivery of antifoam in an aggressive foaming fed-batch cell culture, also showed flawless performance of the foam detection and antifoam delivery system. Each S.U.B. used similar amounts of antifoam. However, the timed strategy was not aggressive enough resulting in automated antifoam delivery intervening on multiple occasions, as shown in Figure 4.

The other case studies involving ultra-high density perfusion and aggressive gassing at large S.U.B. scale showed excellent performance and scalability of the foam probe with no observed probe fouling. Use of the foam probe and holder provided consistent results at all S.U.B. sizes. Foam probe use in the S.U.F. showed it is also very effective at automatically controlling foam in aerobic fermentation cultures requiring very high gas flow rates (2 VVM).

Figure 3. Case 1, viable cell density and total gas flow rate in 50L S.U.B. with automated or scheduled addition of antifoam.

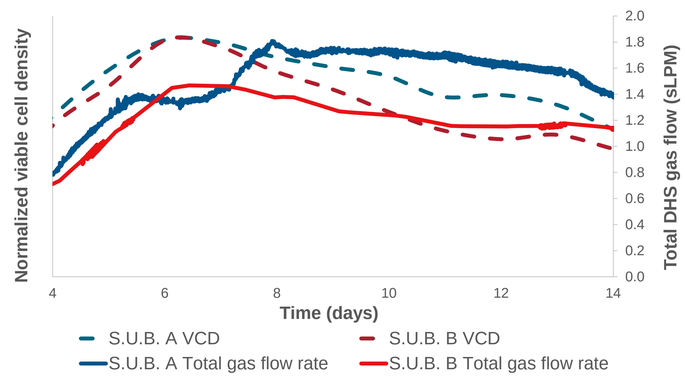


Figure 4. Case 1, total Antifoam additions, comparing automated to scheduled chemical antifoam delivery.

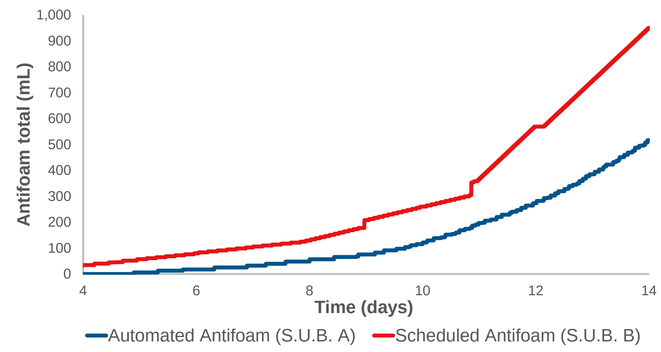
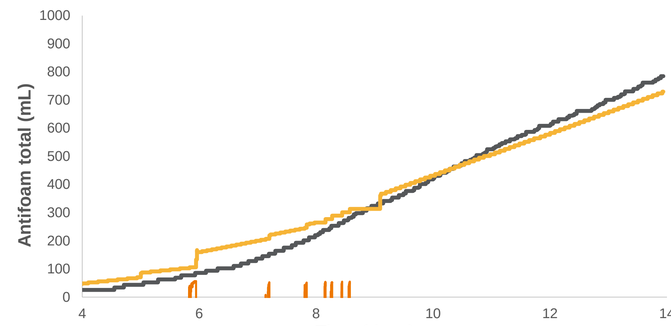


Figure 5. Case 2, total Antifoam additions. Also showing automated additions of anti-foam for vessel B (based on foam probe feedback).



CONCLUSIONS

- The foam probe for S.U.B. systems provides a robust solution for foam management.
- Near 50% reduction in antifoam use in standard fed-batch 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, as well as fermentation 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. Bioreactor operators noted greater confidence in leaving the bioreactor unattended, such as overnight, when using the foam probe.

TRADEMARKS/LICENSING

IN-DEPTH COVERAGE

REFERENCES

1. Doran, Pauline M. “Mass Transfer.” In Bioprocess Engineering Principles, 2nd ed., 405. Waltham, MA: Elsevier, 2013.
2. McClure, Dale D., et al. “An Experimental Investigation into the Behavior of Antifoaming Agents.” Chemical Engineering Science, vol. 160, 2017, pp. 269–274., doi:10.1016/j. ces.2016.11.033.
3. Routledge, Sarah J. “Beyond De-Foaming: The Effects of Antifoams on Bioprocess Productivity.” Computational and Structural Biotechnology Journal, vol. 3, no. 4, 2012, doi:10.5936/csbj.201210014.
4. Automated foam control in HyPerforma Single-Use Bioreactors using a single-use foam probe, 2019. Application Note.
5. S.U.B. enhancements for high-density perfusion cultures, 2018. Application Note.
6. TruBio DV 5.0 User Manual, 2019.