DiSTEK

Characterizing the Effects of Antifoam C Emulsion on Oxygen Mass Transfer within the BIOne Drilled-Hole Sparger and Microsparger Single-Use Bioreactor Systems

Jake McAndrew, MSc and Greg Kauffman
Distek, Inc. | North Brunswick, NJ
Contact: bione@distekinc.com

BIOPROCESSING

Abstract

Excessive headspace foaming within mammalian bioreactor systems can become highly problematic for upstream bioprocesses. Consequences of foam accumulation can result in decreased cell culture growth, decreased culture productivity, or complete loss of bioreactor system integrity. Due to the severity of these consequences, foam control is often specifically addressed within most biopharmaceutical production manufacturing processes.

Foaming is often controlled through the supplementation of the basal medium with silicone-based antifoam emulsion additions. The administration of such emulsions has been demonstrated to effectively mediate excessive foaming. Despite this advantage, antifoam supplementation has also been shown to unfavorably decrease the rate of oxygen mass transfer within bioreactor systems.

To better understand and characterize the effects of antifoam supplementation on oxygen mass transfer, our team performed a dose-response evaluation of Antifoam C in both drilled-hole sparger and microsparger BIOne Single Use Bioreactors (SUBs). Within the drilled-hole sparger system, results were consistent with previously reported data. A $40 - 50\%$ overall reduction in k_La was observed upon treatment with 30 ppm Antifoam C emulsion. In contrast, no effects from the antifoam treatment were observed within the microsparger bioreactor system. Overall, these results suggest that use of a microsparger may support consistent oxygen mass transfer within high-foaming upstream bioprocesses where antifoam supplementation is required.

Introduction

Several attributes of traditional upstream bioprocessing stir-tank reactors (STRs) can result in excessive headspace foam accumulations. The use of sparged gases to maintain both dissolved oxygen (DO) and pH setpoints provides the aeration necessary to generate foam. The expanding foam mass is then often stabilized due to the presence of cellular proteins which have been released into the extracellular matrix.[1] The resulting foam accumulation has the potential to negatively impact overall cell culture health and productivity within the bioreactor system. In severe cases, foam accumulation can ultimately compromise the overall integrity of the entire system, resulting in the forfeiture of the production batch.

The rupture of foam bubbles has the potential to result in the generation of hydrodynamic shear forces. Depending on the duration of shear force exposure, cells can be irreversibly damaged though the induction of apoptotic pathways.[2] As a result, cells in close proximity to the foam can lyse and become sources of cellular debris.[3] This increased lysis can result in elevated concentrations of undesired host cell proteins (i.e. proteases, sialidases, and glycosidases) within the product pool. The presence of high concentrations of such proteins can potentially jeopardize the integrity of the final product quality.[4]

The presence of foam can decrease the efficiency of bioreactor gas exchange at the gas-liquid interface.[5] Dissolved carbon dioxide (dCO_2) removal from a bioreactor is often dependent on this type of gas exchange, particularly in smaller-scale systems.[6,7] As such, excessive foaming has the potential to result in elevated concentrations of dCO_2 within the system. Increased dCO_2 concentrations have been described to negatively impact both culture growth and productivity.[8] Therefore, by reducing the dCO_2 removal capacity within the system, headspace foam can decrease overall bioreactor production yield.

If let uncontrolled for extended durations, headspace foaming has the potential to foul bioreactor exhaust filters. Such filters are used in a bioreactor system for off-gas venting and to ensure internal pressure remains stable. If filter fouling occurs, the sterility of the system can be compromised.[9] Additionally, such

clogging can result in internal bioreactor pressures reaching unsafe levels. With such an outcome, the production batch may have to be forfeited. Such a result ultimately means the loss of valuable drug substance and wasted manufacturing resources.

The negative consequences of bioreactor foaming have resulted in the regular inclusion of supplemental antifoaming agents within most upstream biopharmaceutical manufacturing processes. These media supplements have been demonstrated to be effective at controlling foam within bioreactor systems through the destabilization of the foam film.[10] Antifoaming supplements are traditionally administered to the bioreactor on strategically defined dosage intervals as bolus additions.

Silicone-based antifoam emulsion supplementation has been accepted by much of the industry as an effective means of foam control within bioreactor systems. However, the addition of such types of antifoaming emulsions has also been reported to significantly decrease the oxygen mass transfer capacity within the culture media. This effect has been described through evaluations of the response of silicone-based emulsions on the volumetric mass transfer coefficient of oxygen ($k_L a$) of bioreactor systems.

The $k_L a$ term can be used to describe the rate of oxygen mass transfer within a bioreactor. The system $k_L a$ is the product of both the liquid phase mass transfer coefficient (k_L) and the gas-liquid interfacial surface area (a).[11] The overall oxygen transfer rate of a bioreactor system (OTR) can be defined as the product of both $k_L a$ and the oxygen concentration gradient $(C^* - C_L)$, as described in **Equation 1**. Due to its direct relationship with bioreactor OTR, $k_L a$ is a highly suitable metric for describing the oxygen mass transfer within a such a system.

$$OTR = (C^* - C_L) \times (k_L a)$$

(Eq. 1)

When administered to a bioreactor at low concentrations (10 – 100 ppm), silicone-based antifoaming emulsions have been shown to decrease system $k_L a$ by approximately 40 – 70%.[12,13] Suitable oxygen mass transfer is essential to satisfy the metabolic demands of aerobic mammalian cell cultures.[14,15] As such, it was determined that comprehensive characterization of the effects of antifoam supplementation on bioreactor oxygen transfer could be highly valuable to scientists and engineers who need to balance both foam mitigation and sufficient oxygen transfer within their upstream processes.

Antifoam C is a widely used silicone-based antifoam emulsion. This supplement has been demonstrated to be suitable for controlling headspace foaming in mammalian cell culture bioreactors at concentrations of up to 30 ppm.[16] For this work, we characterized the effect of this emulsion on the oxygen mass transfer within two types of 5-L single-use bioreactor systems. The dose response of Antifoam C on the system $k_L a$ was analyzed within BIOne SUBs with either drilled-hole sparger or microsparger components. The decrease in oxygen transfer previously described in the literature was observed within the drilled-hole sparger system. However, comparable results were not seen within the microsparger system. These data suggest that the use of a microsparger element may be appropriate to ensure oxygen transfer is consistent within upstream bioprocesses where antifoaming supplementation is necessary for foam control.

Methods and Materials

Two types of BIOne Single-Use Bioreactor (SUB) models were utilized for this study: 2022-1005 and 2022-1006. The 2022-1005 model has a 5-L working volume, a single right-handed pitch blade impeller, and a microsparger. The porous sparging element for this model has hundreds of holes with diameters of 20 – 40 microns. The 2022-1006 model is a nearly identical model with the same volumetric geometry and impeller configuration. However, this model contains a drilled-hole sparger with 7 × 1.5 mm holes in a single linear arrangement.

During this evaluation, the static gassing-out method was used to determine the system $k_L a$, as described in de Ory, Romero, and Cantero, 1999.[17] The evaluation was performed using a model medium which included common cell culture media components. The composition of the model medium was 1.6 g/L sodium bicarbonate, 7.6 g/L sodium chloride, and 0.1% Pluronic F-68 (Gibco, 24040032). Concentrations were selected based upon previously described mass transfer characterization methods.[18] The antifoam supplementation media used during this study was prepared using Antifoam C Emulsion (Sigma, A8011).

During the evaluations, the bioreactor working volumes were maintained at 3000 mL. The bottom air sparge rate was set at 150 sscm for both systems (0.05 vvm). Two different agitation rates were evaluated under of scope of this study. The agitation rate of 232 rpm (P/V = 30 W/m³) was selected as it represented a typical power input for high-density mammalian cell cultures.[19] An additional set of conditions was analyzed at 400

rpm (P/V = 150 W/m^3) to determine if any effects seen at the 232 rpm setting were dependent on the overall power-input within the bioreactor system. Bioreactor operational temperature during the entire study was maintained at 37°C. Bioreactor operation was performed using the BIOne 1250 Single-Vessel Controller (Distek, 2022-8100).

During testing, the bioreactor k_La was evaluated at Antifoam C emulsion concentrations ranging from 0 – 30 ppm. Testing for each set of conditions was performed in duplicate. Upon completion of experimental testing, data were normalized using the formula shown in **Equation 2**.

$$Normalized\ kLa\ (X\ ppm\ Antifoam\ C) = \frac{kLa\ X\ ppm\ Antifoam\ C}{Average\ kLa\ 0\ ppm\ Antifoam\ C} \times 100$$

(Eq. 2)

Results and Discussion

The described effects of silicone-based emulsion addition on oxygen mass transfer were observed within the drilled-hole sparger bioreactor (**Figure 1**). The k_La within this system decreased approximately 50 – 60%. When these data were analyzed with one-phase exponential decay curves, results demonstrated a high degree of fit for both sets of agitational conditions evaluated (R^2 = 0.982, R^2 = 0.984).
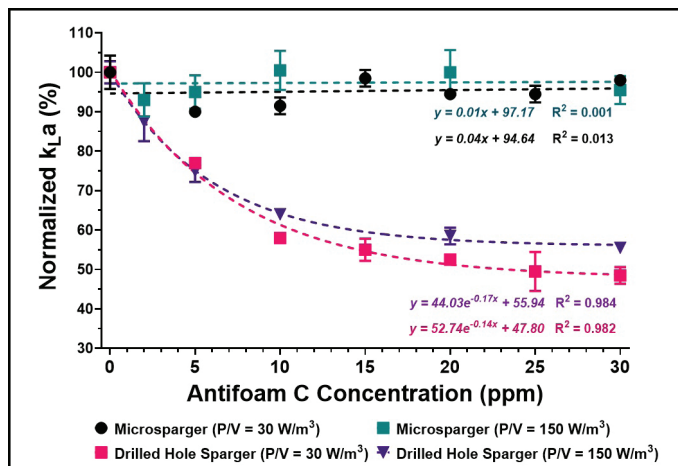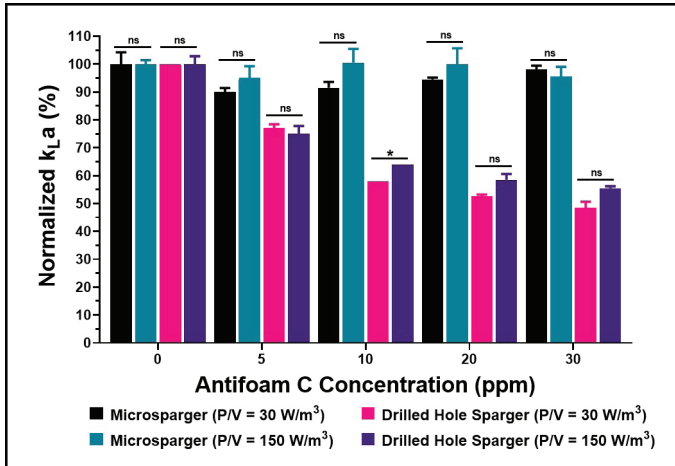

Figure 1: Dose response of Antifoam C Emulsion on kLa in both Microsparger and Drilled-Hole Sparger BIOne Single-Use Bioreactors. Dose-response decrease in kLa observed in drilled-hole sparger system. No dose effect observed in microsparger system. Drilled-hole sparger system data analyzed with one-phase decay model. Microsparger system data analyzed with linear regression. Data are mean values from n = 2 trials. Bars shown represent standard deviation.

In contrast to the what was observed with the drilled-hole sparger, no Antifoam C emulsion dose-dependent k_La response was observed within the microsparger system. When analyzed with a linear regression, the correlation coefficients for both agitation settings supported the absence of any effect of the emulsion addition on the rate of oxygen transfer within the system (R^2 = 0.001, R^2 = 0.013).

It has been suggested that the antifoam-dependent k_La reduction described in the literature is due to increased coalescing of air bubbles within the medium.[1,6] This coalescence ultimately decreases the interfacial surface area (*a*) available for oxygen transfer within the system. As such, the value of the k_La term for the bioreactor is reduced and the oxygen transfer rate is decreased.

The pore diameters on the microsparger are approximately two orders of magnitude smaller than the hole diameters found on the drilled-hole sparger. At consistent air flow rates, this attribute results in a considerable increase in the air bubble interfacial surface area available for oxygen transfer.[20] Previous work performed by our team demonstrated this interfacial surface area increase supports a 2 – 4 fold k_La increase when using the microsparger system under standard process conditions.[21] Data from this study demonstrates that the increased interfacial surface area may also help to reduce the effects of emulsion-driven air bubble coalescence.

The results from this study also suggest that the respective effects of antifoam supplementation on oxygen transfer within both the drilled-hole sparger and microsparger systems are likely not dependent on overall system agitation. Comparable responses in system k_La were observed upon antifoam addition at both normal (P/V = 30 W/m^3) and amplified (P/V = 150 W/m^3) agitation rates for both bioreactor systems. As shown in **Figure 2**, the multiple unpaired t-tests demonstrated no significant difference between agitation settings at nearly all testing conditions. The only exception was the difference observed with the drilled-hole sparger system at the 10 ppm Antifoam C condition. The low variance recorded in k_La measurements for this condition may have been the reason for this single inconsistency. Despite this lone difference, the overall comparability across the results suggests that agitation rate likely does not significantly influence the effects of antifoam supplementation on oxygen mass transfer.

Figure 2: Comparison of Antifoam C emulsion dose response at both normal (P/V = 30 W/m³) or amplified (P/V = 150 W/m³) agitation rates. Results demonstrate that observed Antifoam C dose-response is likely not dependent on overall system agitation rate. Difference observed at 10 ppm for drilled-holed sparger system may be due to low variance recorded during this condition. Data are mean values from n = 2 trials, analyzed with multiple unpaired t-tests. Bars shown represent standard deviation. *ns = no significant difference, * = p < 0.001*

Conclusions

Excessive headspace foaming can be incredibly problematic for upstream bioprocesses. Rupture of foam bubbles can induce apoptotic pathways in cultures, resulting in decreased viable cell densities and increased concentrations of potentially degradative enzymes within the product pool. Additionally, accumulated foam has the potential to decrease the dCO_2 stripping capacity of a bioreactor system, which can negatively impact the overall culture growth. High levels of foam can also foul bioreactor exhaust filters, potentially compromising the integrity of the system and causing the loss of the production batch. Such severe consequences often necessitate the use of antifoam emulsion supplementation within upstream mammalian bioprocesses.

A decrease in bioreactor oxygen mass transfer due to the addition of silicone-based antifoam emulsion supplementation has been well-described within the literature. During this study, this described effect was consistently observed within the drilled-hole sparger system at multiple agitation rates. The system k_La demonstrated a repeatable decrease upon treatment with Antifoam C emulsion. However, no comparable response was observed with the same treatment within the microsparger

system. Conversely, the addition of the Antifoam C emulsion did not appear to have any significant effect on oxygen mass transfer within this bioreactor system.

It is the belief of the authors that the differences observed regarding the effects of antifoam on oxygen mass transfer within bioreactor systems of different sparger types has not been previously reported. This new information may be valuable for process engineers and scientists who continue to develop, characterize, and improve upstream bioprocesses. Consistent oxygen transfer within a bioreactor system has the potential to drive improved cell growth, productivity, and product quality. As demonstrated in this work, the implementation of a microsparger within a bioreactor processes may support oxygen mass transfer consistency even when antifoam supplementation is required.

Acknowledgements

References

1. Routledge, S. J. (2012). Beyond De-Foaming: The effects of antifoams on bioprocess productivity. *Computational and Structural Biotechnology Journal*, 3(4). doi:10.5936/csbj.201210014

2. Grilo, A. L., & Mantalaris, A. (2019). Apoptosis: A mammalian cell bioprocessing perspective. *Biotechnology Advances*, 37(3), 459-475. doi:10.1016/j.biotechadv.2019.02.012

3. Frahm, B., Brod, H., & Langer, U. (2009). Improving bioreactor cultivation conditions for sensitive cell lines by dynamic membrane aeration. *Cytotechnology*, 59(1), 17-30. doi:10.1007/s10616-009-9189-9

4. Gramer, M. J. (2013). Product Quality Considerations for Mammalian Cell Culture Process Development and Manufacturing. *Mammalian Cell Cultures for Biologics Manufacturing Advances in Biochemical Engineering/Biotechnology*, 123-166. doi:10.1007/10_2013_214

5. Obom, K. M., Magno, A., & Cummings, P. J. (2013). Operation of a Benchtop Bioreactor. *Journal of Visualized Experiments*, (79). doi:10.3791/50582

6. McAndrew, J. & Kauffman, G. (2020). Upstream Process Development with a k_La Criterion Within the BIOne Single-Use Bioreactor [White paper]. Distek, Inc. https://www.distekinc.com/wp-content/uploads/2020/10/BIOne-SUB-Upstream-Process-Development-with-a-k_La-Criterion_WP.pdf

7. Mitchell-Logean, C., & Murhammer, D. (1997). Bioreactor Headspace Purging Reduces Dissolved Carbon Dioxide Accumulation in Insect Cell Cultures and Enhances Cell Growth. *Biotechnology Progress*, 13(6), 875-877. doi:10.1021/bp970078s

8. Gray, D. R., Chen, S., Howarth, W., Inlow, D., & Maiorella, B. L. (1996). CO2 in large-scale and high-density CHO cell perfusion culture. *Cytotechnology*, 22(1-3), 65-78. doi:10.1007/bf00353925

9. Vardar-Sukan, F. (1998). Foaming: consequences, prevention and destruction. *Biotechnology Advances*, 16(5-6), 913-948. doi:10.1016/s0734-9750(98)00010-x

10. Prins, A., & Riet, K. V. (1987). Proteins and surface effects in fermentation: Foam, antifoam and mass transfer. *Trends in Biotechnology*, 5(11), 296-301. doi:10.1016/0167-7799(87)90080-1

11. Liu, K., Phillips, J. R., Sun, X., Mohammad, S., Huhnke, R. L., & Atiyeh, H. K. (2019). Investigation and Modeling of Gas-Liquid Mass Transfer in a Sparged and Non-Sparged Continuous Stirred Tank Reactor with Potential Application in Syngas Fermentation. *Fermentation*, 5(3), 75. doi:10.3390/fermentation5030075

12. Al-Masry, W. A. (1999). Effects of antifoam and scale-up on operation of bioreactors. *Chemical Engineering and Processing: Process Intensification*, 38(3), 197-201. doi:10.1016/s0255-2701(99)00014-8

13. Morão, A., Maia, C. I., Fonseca, M. M., Vasconcelos, J. M., & Alves, S. S. (1999). Effect of antifoam addition on gas-liquid mass transfer in stirred fermenters. *Bioprocess Engineering*, 20(2), 165. doi:10.1007/s004490050576

14. Qian, Y., Xing, Z., Lee, S., Mackin, N. A., He, A., Kayne, P. S., . . . Li, Z. J. (2014). Hypoxia influences protein transport and epigenetic repression of CHO cell cultures in shake flasks. *Biotechnology Journal*, 9(11), 1413-1424. doi:10.1002/biot.201400315

15. Swiderek, H., Logan, A., & Al-Rubeai, M. (2008). Cellular and transcriptomic analysis of NS0 cell response during exposure to hypoxia. *Journal of Biotechnology*, 134(1-2), 103-111. doi:10.1016/j.jbiotec.2008.01.001

16. Velugula-Yellela, S. R., Williams, A., Trunfio, N., Hsu, C., Chavez, B., Yoon, S., & Agarabi, C. (2017). Impact of media and antifoam selection on monoclonal antibody production and quality using a high throughput micro-bioreactor system. *Biotechnology Progress*, 34(1), 262-270. doi:10.1002/btpr.2575

17. Ory, I. D., Romero, L. E., & Cantero, D. (1999). Laboratory scale equipment for the determination of k L a in bio-reactors. *Bioprocess Engineering*, 20(1), 73-75. doi:10.1007/pl00009036

18. Matsunaga, N., Kano, K., Maki, Y., & Dobashi, T. (2009). Estimation of dissolved carbon dioxide stripping in a large bioreactor using model medium. *Journal of Bioscience and Bioengineering*, 107(4), 419-424. doi:10.1016/j.jbiosc.2008.11.017

19. Xu, S., Hoshan, L., Jiang, R., Gupta, B., Brodean, E., O'neill, K., . . . Chen, H. (2017). A practical approach in bioreactor scale-up and process transfer using a combination of constant P/V and vvm as the criterion. *Biotechnology Progress*, 33(4), 1146-1159. doi:10.1002/btpr.248

20. Ju, L. K., & Sundararajan, A. (1995). The effects of cells on oxygen transfer in bioreactors. *Bioprocess Engineering*, 13(5), 271-278. doi:10.1007/bf00417639

21. McAndrew, J. & Kauffman, G. (2020). Increasing oxygen transfer within the BIOne single-use bioreactor using a microsparger [White paper]. Distek, Inc. https://www.distekinc.com/wp-content/uploads/2020/12/Distek-BIOne-Increasing-Oxygen-Transfer-Using-a-Microsparger-WP_212.pdf