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RESEARCH ARTICLE

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Measurement and control of foam generation in a mammalian cell culture

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Abstract

Foam is generated in mammalian cell cultures by excessive agitation or gas sparging. This occurs particularly in cultures that generate recombinant proteins at high cell concentrations. Three antifoam agents were tested for their compatibility with antibody-producing Chinese hamster ovary (CHO) cells. One agent (antifoam 204) was completely inhibitory to growth at a concentration of 10 ppm, one agent (antifoam C) showed partial inhibition and a third (antifoam SE-15) showed no inhibition at this concentration. A novel foam image analyzer (LabCam) was used to evaluate two antifoams (C and SE-15) for their ability to dissipate foam generated in cell culture media by enhanced agitation. The presence of antifoam in the media reduced significantly the foam layer that was generated and this was shown to be rapidly dissipated in the presence of 10 ppm SE-15. The antifoams were also tested for foam dissipation in cultures of CHO cells at $>10^6$ cells/mL. Supplementation of the cultures with SE-15 resulted in dissipation of foam generated by excessive gas sparging within 2 min. Under equivalent conditions 75% of foam dissipated in the presence of antifoam C, within 2 min but there was a residual foam layer up to 25 min. This study showed the value of an optical monitoring system (LabCam) for measuring foam generation and dissipation in a bioreactor to assess the efficiency of antifoam agents to reduce foam in a bioreactor. This has the potential for use as a control system that could be designed for continuous monitoring and foam control in a mammalian cell bioprocess.

KEYWORDS
antibody, antifoam, bioprocess, CHO, mammalian cells

1 | INTRODUCTION

Mammalian cells are routinely used in commercial bioprocesses for the production of biologics including recombinant proteins such as monoclonal antibodies.[1,2] To maintain efficient and consistent production there are a number of critical process parameters such as temperature, pH, dissolved oxygen (DO), agitation, and pressure.[3] The design, optimisation, and strict control of these parameters are critical to the prevention of process collapse in the production of these biologics from mammalian cells. Foam generation during these cultures can arise intermittently as a result of agitation and on demand gas sparging particularly in the presence of higher protein content in the medium. In this study, we considered the detrimental effects of foam generation in mammalian cultures and investigated a system for online monitoring.

In general, monoclonal antibodies are stable and able to withstand slight variabilities in upstream processing such as harsh pH conditions

and even shear during processing.[4] Although this may be the case, the high costs and complexities in antibody production result in a demand for highly efficient, economical production processes which consistently deliver product with a high specificity.[5] It is widely understood that advances in media and cell culture conditions, as well as enhanced feeding strategies contribute significantly toward increases in protein production.[5] Thus, in process optimisation the selection of components which limit or exhibit no negative effects on cell growth and production is of high importance. Although many factors can exhibit detrimental effects on a bioprocess, foaming presents a unique challenge because of the potential for catastrophic effects on the culture. Foam is generated through aeration/gassing or agitation in a process reactor, where bubbles are formed and subsequently sustained by proteins also present in the reactor.[6] Its composition comprises liquid lamellas filled with gas. Foams with a higher liquid content are unstable and subject to a natural collapse in a relatively short space of time.[7] However, polyhedric foams, where lamellae and foam plateau borders are curved and take the form of a polyhedron, are much more stable and are a product of mechanical stresses.[8]

Some previously reported methods of monitoring foam have largely involved imprecise measurements at selected time points or sampling to determine the ability of liquids to generate foam.[9–11] None of these are suitable for real time visual monitoring of foam during a mammalian cell culture bioprocess. Sensing systems are available to monitor foam in bioprocesses that are typically based on sterile probes inserted into the interior of the bioreactor above the base line liquid surface. Detection in these probes involves a switch that is triggered during the development of foam. These probes can utilize a conductivity, ultrasonic or impedance signal at a point when an electrical or acoustic impulse is transmitted through a gap between electrodes or to a receiver. A major problem is that the active probe tips are vulnerable to coating and fouling during a bioprocess. That can often lead to false positive responses that direct the delivery of excess antifouling agent into the bioreactor.[12] This may in turn result in reduced growth of the cells.

A noninvasive image processing strategy has been attempted at a small-scale with single use bioreactors.[13] This detected foam as binary (foam/no foam) and used machine learning to identify the foam in a four scale fine-grain classification. The purpose of our study reported here was to further investigate the use of an optical system that could monitor foam generation in-real time and potentially be attached through a feedback loop to the introduction of an antifoam agent to the culture.

Physical or chemical means can be employed to prevent or reduce foam formation. Physical barriers range from mechanical foam breakers to ultrasound disruption, whereas chemical means incorporate the addition of chemical antifoam agents either during the bioprocess or during media preparation.[14] Chemical means are primarily employed in bioprocesses and are generally classified by formulation. Antifoams are composed of hydrophobic solids in an oil dispersion, aqueous emulsions/suspensions or liquid/solid components containing surfactants,[7] that is based on soluble or insoluble oils.[15] Denkov[9] reported significant time differences in foam dissipation between oil drops deprived of solid particles versus those composed of oil-solid compounds.[9] Some soluble oil antifoams may have a surfactant conjugated with a polyethylene oxide or propylene oxide moiety to enable a lower surface tension than the media. This results in a viscous film, lower in viscosity than a protein film, inhibiting protein foaming at the liquid air interface.[15] Insoluble oil-based formulations are typically composed of mineral oils or polydimethyl siloxanes. The mode of action here is based on hydrophobic interactions disrupting the foam layer through the bridge-dewetting mechanism.[5,15] Optical observations by Denkov demonstrated a much more rapid dissipation of foam with oil-solid based antifoams as these often enter the foam surface rupturing the foam layer, whereas globular antifoam formulations lacking solid hydrophobic particles require the compression of the plateau border between the liquid and foam interface.[9]

It is important to consider the multitude of antifoams commercially available. Previous studies have demonstrated negative impacts of antifoams on the growth of not only mammalian cells but also bacterial cells and fungi, such as cell membrane leakage and cytotoxicity, and indeed impacts on protein expression.[16,17] Further downstream considerations should also be assessed as studies have shown impacts on the downstream filtration steps, for instance, certain silicone-based antifoams have been shown to clog filtration apparatus during the downstream processing.[5] Antifoam 204, antifoam C, and antifoam SE-15 are three examples of commercially available antifoaming agents. Antifoam 204 is an organic antifoam mixture of non silicone polypropylene-based polyether dispersions. On the other hand, antifoams C and SE-15 are silicone-based antifoams containing 10–40 μm diameter particles, and both are prepared with nonionic emulsifying agents. Although the removal or reduction of foaming in a bioprocess is critical, antifoam addition can lead to undesirable effects, both upstream and downstream. Reducing the intrinsic and inherent undesirable properties of antifoaming agents, provides a means of not only reducing undesirable effects on cells and protein expression but improving the efficacy of the process as a whole.[18]

The LabCam system, investigated in this manuscript, presents a novel means of tracking and logging changes in foam levels in real time, allowing for early intervention. This capability allows for the optimisation of antifoam agent dosage, thus reducing challenges presented in the downstream process. The camera measures foaming events in situ beside the bioreactor vessel (1 L glass) and reports at frame rates of 1 frame per second (1 fps). Data are presented in real time and is accompanied by video recording for playback and review. In this study, the suitability of the LabCam was explored for monitoring the generation and dissipation of foam in media, for the cultivation of Chinese hamster ovary (CHO) cells expressing a human-llama chimeric antibody. The determination of a suitable antifoam candidate and dosage concentrations (antifoam SE-15, antifoam C or antifoam 204) was determined using the system.

2 | MATERIALS AND METHODS

2.1 | Materials

Cells were grown in a CHO serum free culture media (Biogro Technologies Inc. Winnipeg, Canada). Three antifoams were purchased from Merck, Ireland: antifoam SE-15 (A8582; 10% aqueous silicone

emulsion), antifoam C (A8011; 30% aqueous silicone emulsion) and antifoam 204 (A8311; 100% non silicone polypropylene-based polyether dispersion). Stock solutions of 10,000 ppm (1% v/v) of each antifoam were prepared under sterile conditions in culture media. Trypan blue (0.4%) and anticlumping supplement were purchased from Fujifilm Irvine Scientific, Netherlands. L-glutamine (200 mM) was purchased from Biosciences, Dublin, Ireland.

2.2 | Cell line

CHO-EG2 cells which express a human-llama chimeric monoclonal antibody (Mab) against epidermal growth factor receptor (EGFR) were kindly provided by Yves Durocher of the NRC, Canada. The stock cultures of suspension cells were maintained in shake flasks in Biogro CHO media supplemented with 4 mM L-glutamine (Gibco) and 0.2% anti-clumping agent (Irvine Scientific) under standard cell culture conditions of 37°C, 5% CO_2, 80% humidity and 120 rpm. Cells were passaged three times from vial thaw before bioreactor inoculation (working volume 1 L). Viable cell densities were measured by trypan blue dye exclusion assay on a Luna II automated cell counter (Logos Biosystems). All assays were carried out in biological triplicate.

2.3 | Titer assay of IgG production

The production of IgG 1 was monitored via an immunosorbent IgG assay conducted on a Roche Cedex Bio analyzer (I&L Biosystems, Dublin). The assay reagent was calibrated using a Roche calibrator and quality control standards were used to verify test calibration. Daily samples (0.3 mL) were taken from flasks of CHO-EG2 cells in culture. Each sample was centrifuged at 1000 rpm for 5 min and the supernatant titer was collected for analyses. Supernatants were frozen and stored at −20°C prior to analysis.

2.4 | Cell specific productivity

Q_{MAb} was calculated in pg/(cell×day) using the equations below, where N represents the viable cell density (cells/mL), t represents the time point (h) and antibody titer (pg/mL).

$$\text{Growth rate}\,(\mu) = \left(\frac{(\ln N2 - \ln N1)}{t2 - t1}\right)/24,^{19} \quad (1)$$

$$Q_{MAb}\,(pg/cell.day) = \left(\frac{\text{titer2} - \text{titer1}}{N2 - N1}\right) \times \mu.^{19} \quad (2)$$

2.5 | Bioreactors

Applikon bioreactors (1 L) with "My-Control" controllers were used to test foam generation and dissipation in culture media in the presence or absence of cells. Sodium bicarbonate (0.5 M) and sterile filtered CO_2 were used for pH control of bioreactor cultures, DO was maintained at 30% using sterile compressed air. An agitation speed of 400 rpm was maintained throughout by an agitator secured at 16 cm from the headplate. The temperature of 37°C was maintained with a heating jacket. Aeration was enabled by compressed air through a 0.22 μm sterile filter and a sintered metal porous tip with pore sizes of 15 μm. A single bioreactor controller and agitator were used for all experiments with rinsing with sterile water in between to prevent carryover of antifoam residues.

In the absence of cells, bioreactors were rinsed initially with sterile deionized water (di H_2O) and media (500 mL) added to each of three bioreactors. For cell cultures, bioreactor assemblies were autoclaved with calibrated pH probes and Lumisens optical DO probes in situ, prior to aseptic media addition. A sterile hold was conducted for a minimum period of 12 h before inoculation. Cells were inoculated at a seeding density of 3×10^5 cells/mL into 1 L media via a sterile inoculum bottle welded onto the media addition line, and L-glutamine (4 mM) was supplemented into the media just before inoculation.

2.6 | Optical analyzer

Foam production and dissipation were monitored in each bioreactor using an external optical analyzer (LabCam from JM Canty Inc., Buffalo, NY). The LabCam comprises a camera connected via ethernet to a processing system (Canty VCM). The LabCam was set up beside a benchtop bioreactor and positioned to visualize the liquid surface. The LabCam was raised 10 cm off the bench to accommodate measurements of the surface of the media at 500 mL. The camera was positioned at a fixed distance of 35 cm from the vessel and was calibrated using the volumetric markings on the bioreactor vessel, where each 100 mL marking represented an increment of 1.5 cm (Figure 2).

2.7 | LabCam data output and analysis

The LabCam used digital imagery to capture the lower and upper layers of the foam that was generated in our experiments. This allowed the associated software to track the rising and falling edges of the foam layers over time and to determine the height of the foam layer above the media surface in the bioreactor. Data points were collected by the camera at a rate of 1 frame per second (fps). The dissipation of foam was determined from the camera data which was normalized into percentages of the initial foam layer thickness. Data were processed and graphed using GraphPad Prism, Version 9.3.1 (471).

3 | RESULTS

3.1 | Impact of antifoam on Viable Cell Density and IgG production

The impact of three commercially available antifoams on the growth of CHO-EG2 cells was determined at a range of concentrations.

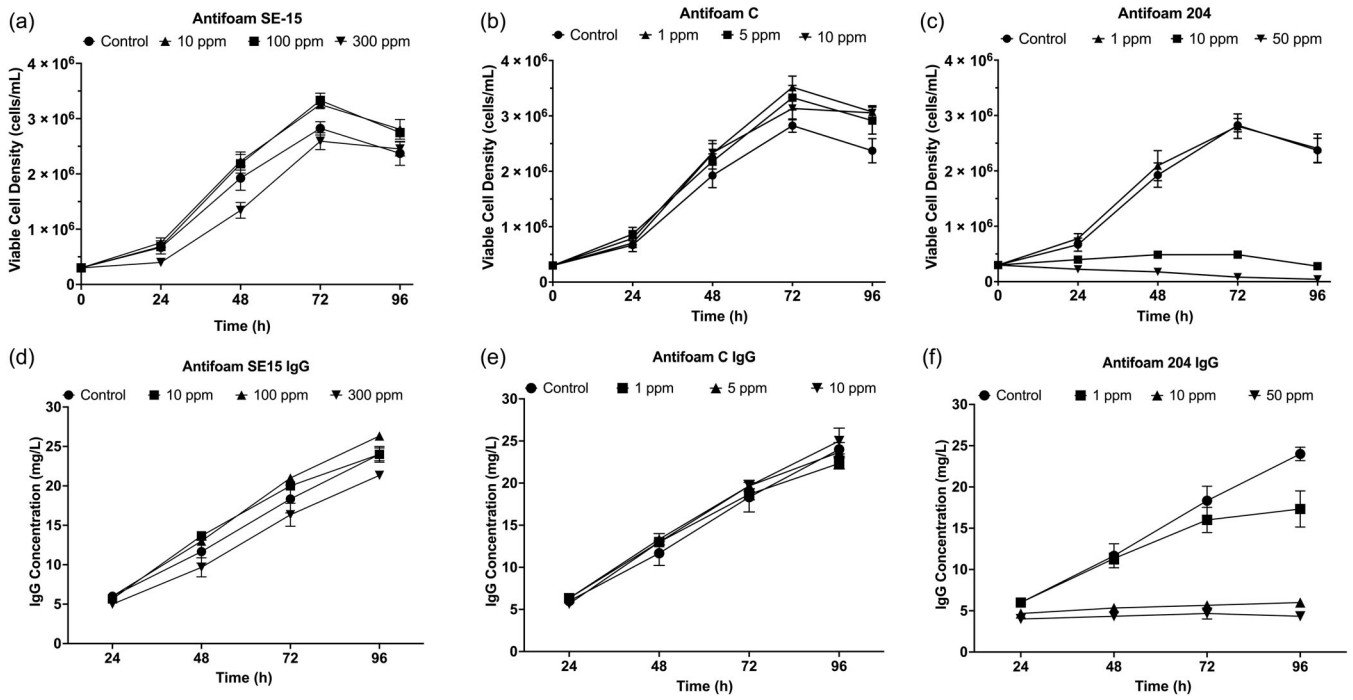

FIGURE 1 (a–c): The impact of antifoam C, SE-15, and 204 on the growth of CHO-EG2 cells (Biogro CHO media). The concentration range tested was antifoam SE-15 (10–300 ppm), antifoam C (1–10 ppm), and antifoam 204 (1–50 ppm) and (d–f): the impact of antifoam C, SE-15, and 204 on the antibody production of CHO-EG2 cells in Biogro CHO media. Sample analysis was based on the mean of three biological replicates ($n = 3$), $+/-$ the standard error of the mean.

Figure 1a–c shows the effect of antifoam SE-15, antifoam C, and antifoam 204 on the growth profiles determined by viable cell density. Stock solutions of 10,000 ppm (1% v/v) of each antifoam were prepared under sterile conditions in culture media at varying concentrations based upon the manufacturer's guidance. Cultures (50 mL) were seeded at 3×10^5 cells/mL on day 0 and cells grown for 96 h. A control flask (no antifoam) was cultured in parallel with test flasks supplemented with varying concentrations of each antifoam. The cultures were maintained under standard conditions in shake flasks and samples (0.5 mL) were taken at daily intervals to determine viable cell densities. All assays were carried out in biological triplicate. The concentrations of the antifoams that were tested were based within the manufacturers' recommended ranges. Our objective was to determine the maximum concentration of each that could be used without affecting cell growth. Figure 1a shows that antifoam SE-15, up to a concentration of 100 ppm, did not impact the growth of cells compared with the control. However, at 300 ppm the growth rate and maximum cell density decreased compared with the control. Antifoam C also appeared to improve the growth of CHO-EG2 cells up to concentrations of 10 ppm compared with the control with no antifoam added, although growth rates did not show a significant difference overall, Figure 1b. In viability studies carried out for antifoam 204, 1 ppm of antifoam showed no impact on CHO-EG2 cell growth over the 96 h compared with the control. However, at concentrations >10 ppm, no cell growth was evident.

Concentrations of antifoam SE-15 up to 100 ppm showed no significant reduction in anti EGFR IgG1 production over 96 h, in fact slightly higher levels of IgG were detected in cell titer at the 72 and 96 h time points (Figure 1d–f). Q_{MAb} calculations revealed cell specific productivity were increased by 10 and 100 ppm SE15 at the 48 h time point, but only appeared to be increased by 100 ppm SE-15 at 72 h. Although both SE-15 and C at 48 h increased cell specific productivity, all antifoams tested showed negative impact on cell specific productivity at 96 h. Antifoam 204 impacted the production of IgG from 72 h at the lowest concentration tested (1 ppm) and showed no production at concentrations of 10 and 50 ppm (Figure 1f).

3.2 | Foam formation and dissipation

In order to assess the ability of antifoam agents to dissipate foam, media (500 mL) was added to each bioreactor with continuous agitation at 400 rpm and at a temperature of 37°C. Foam was then artificially created by increasing the agitation to 2000 rpm for 30 s, after which the agitation rate was reduced to 400 rpm. The appearance of a foam layer at this stage is shown in Figure 2. Once the foam layer (3.5 cm) had settled (5–8 s), an antifoam agent (0.1–5.0 mL) was injected via the sampling line into the media. In the absence of antifoam, the foam layer took 80 min to completely dissipate. Two of the antifoam agents (antifoam C and SE-15) were chosen to evaluate their ability to dissipate the foam layer by using the image analyzer (LabCam). Figure 3 shows the decrease in the depth of the foam layer over time. Antifoam SE-15, at 10 or 100 ppm completely dissipated the foam layer in 3 min (Figure 3a). However, a higher concentration

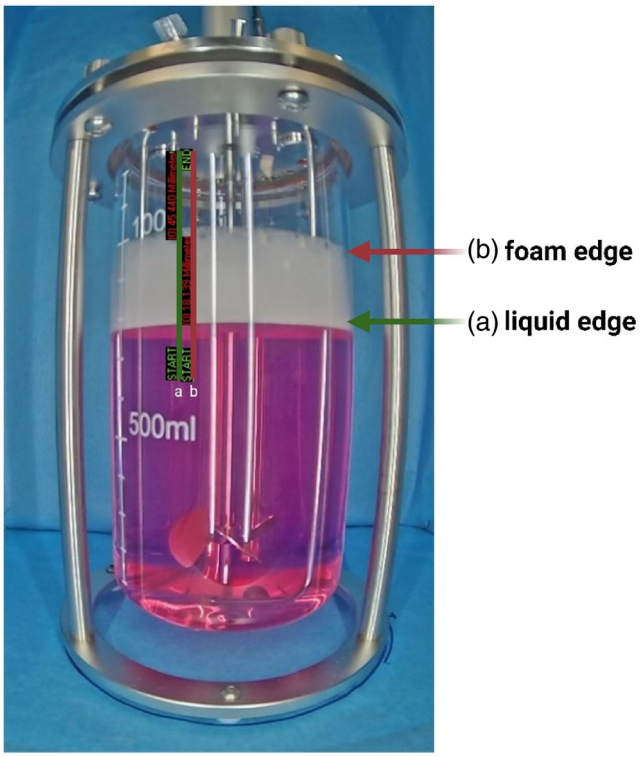

FIGURE 2 The photograph shows the camera image of the bioreactor after foam generation. The LabCam identifies the foam edge line and the liquid edge line to enable a continuous measurement of the depth of the foam head. The green and red graduation markings are those superimposed by the camera software as a measure of the liquid levels at the start and end of a time interval. The vertical white line to the left is the volumetric graduation on the bioreactor turned away from the camera so as not to interfere with the liquid edge determinations.

(100 ppm) of antifoam C was required to dissipate the equivalent amount of foam which took up to 6 min (Figure 3b). A lower concentrations of antifoam C (10 ppm) did not completely dissipate the foam layer and only slightly reduced the rate of foam layer decrease compared with the control.

3.3 | Pre-addition of antifoam for foam prevention

In order to examine the effect of pre-addition of an antifoaming agent to media prior to foam generation, culture media containing SE-15 or C were subjected to the same conditions of a high agitation rate as in Section 3.2. Bioreactors were set up containing the antifoam supplemented media at the preferred concentrations established in Figure 3: 100 ppm of antifoam C and 10 ppm of antifoam SE-15. Each bioreactor was agitated at 400 rpm for 1 h prior to an increase to 2000 rpm for 30 s, before reduction back to an agitation of 400 rpm (Figure 4). In the absence of antifoam, there was a gradual decline in the foam layer after the high agitation intervention but at least 50% of the foam head remained after 30 min. The foam layer generated in the presence of either antifoam agent was significantly less than

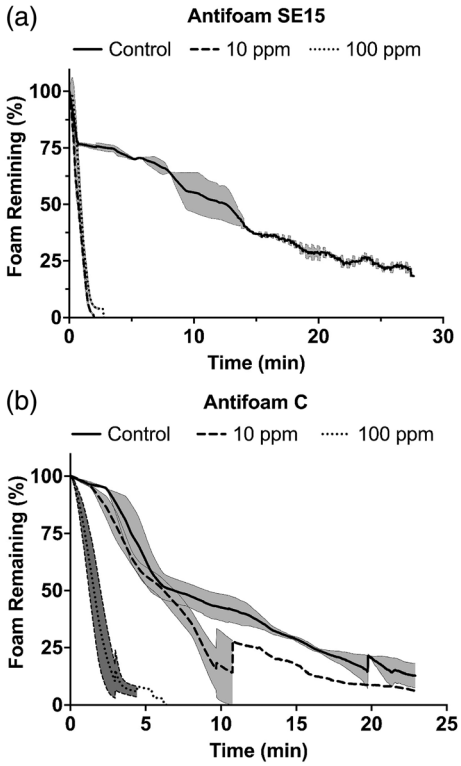

FIGURE 3 Foam dissipation in media. Media (500 mL) was agitated at 400 rpm for 1 h, then exposed to 2000 rpm for 30 s and subsequently returned to 400 rpm. Antifoam was added 5–8 s after returning to 300 rpm. The shaded areas represent the standard error of the mean and the lines the means ($N = 3$).

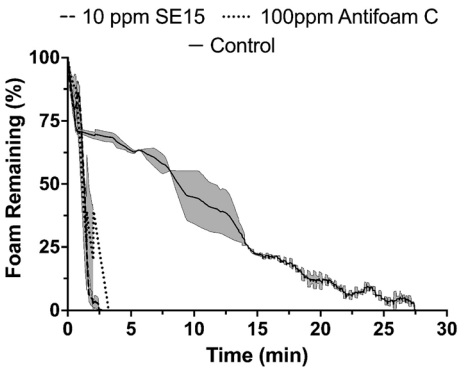

FIGURE 4 Foam dissipation in media. Media (500 mL) containing 10 ppm antifoam SE-15 or 100 ppm antifoam C was agitated for 400 rpm for 1 h prior to exposure to increased agitation at 2000 rpm for 30 s. The graphs show the decrease in the foam layer immediately after exposure to high agitation. The control represents media with no antifoam present, the data are based on an average of three independent analyses ($N = 3$).

in the control and at around 0.5 cm height. Both antifoams greatly increased the foam dissipation rate. The presence of antifoam SE-15 resulted in a rapid dissipation of the foam layer to about 25% within the first minute with a gradual reduction to an undetectable level over 30 min. The presence of the antifoam C resulted in slower but more erratic decline in the foam layer which was undetectable after 15 min.

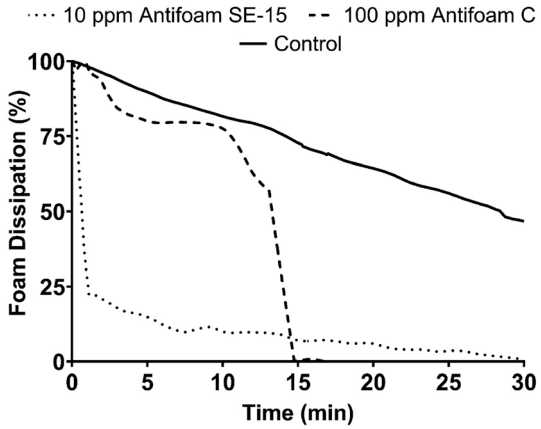

FIGURE 5 Foam dissipation in cell cultures. Cell culture (500 mL) grown to 3×10^6 cells/mL with an agitation rate of 400 rpm was sparged with an air flow of 20 mL/min for 60 s to generate a foam layer before immediate addition of either 10 ppm antifoam SE-15 or 100 ppm antifoam C. The graphs show the dissipation of the foam layer in the bioreactors. Control represents no antifoam administration. The data are representative of a single bioreactor culture.

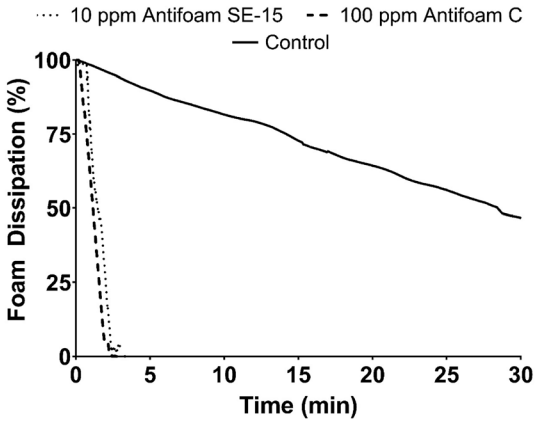

FIGURE 6 Foam dissipation in cell cultures supplemented with 10 ppm antifoam SE-15 or 100 ppm antifoam C. Cells were grown in cultures (500 mL) to 3×10^6 cells/mL in the presence of either antifoam agent before exposure to an enhanced air flow of 20 mL/min for 60 s to generate a foam layer. The graphs show the dissipation of the foam layer in the bioreactors. Control represents no antifoam administration. The data are representative of a single bioreactor culture.

3.4 | Antifoam screening in a CHO-EG2 bioreactor culture

In order to determine the effect of antifoam agents in the presence of cells, an experiment was established in which cells were grown in culture (1 L) to 3×10^6 cells/mL over 8 days in a bioreactor prior to exposure to artificially generated foam. In this case it was decided to use gas sparging rather than high agitation rates that would likely damage the cells. Foam was generated by sparging the CHO cell culture with sterile compressed air at a high flow rate of 20 mL/min for 60 s through a sintered porous metal tip with pore sizes of 15 μm. In control cultures established in the absence of antifoaming agents, a 4.5 cm foam layer was generated, which was significantly greater than the control in the absence of cells. This foam head took >30 min to dissipate after the sparging was stopped. In the presence of either antifoam the foam layer generated in the cultures by the high sparging rate was approximately 0.5 cm and equivalent to that measured in the absence of cells. Results showed that antifoam SE-15 completely eradicated the foam layer 2.5 times faster than if the layer was allowed to dissipate without chemical intervention and was 54 times faster in reducing the foam layer by 75% compared with the control (Figure 5). Antifoam C completely eradicated the foam layer five times faster than that of the control (Figure 5). Both antifoam SE-15 and C were equally effective in preventing the generation of a foam layer in the CHO-EG2 culture (Figure 6).

3.5 | Antifoam longevity studies

The longevity of the antifoam agent efficacy in culture media was evaluated up to 8 days using the LabCam system. Antifoam SE-15 (10 ppm) or antifoam C (100 ppm) was pre-dosed into a volume of 1 L CHO media under sterile conditions in Applikon bioreactors. Foam was generated using high-speed agitation (2000 rpm, 30 s) on day 0 and every subsequent day up to 8 days. The LabCam was used to monitor the generation of foam in the media with and without (control) either antifoam SE-15 or antifoam C. Any foam that generated dissipated rapidly in the presence of antifoam each day up to day 8. Figure 7 shows the surface of the culture media at day 8 after high-speed agitation in the presence and absence of antifoam SE-15 or antifoam C. A sizeable foam layer (3.5 cm) was observed in the absence of antifoam but minimal (<0.5 cm) or no foam layer in the presence of antifoam SE-15 (10 ppm) or antifoam C (100 ppm). This indicates the efficiency of the antifoams to prevent foam generation over a period of at least 8 days.

4 | DISCUSSION

The purpose of the present work was to evaluate a novel, optical method for monitoring foam generation and dissipation in mammalian cell cultures. The LabCam system monitors digital images that recognize the edges between the lower foam layer above the culture media and the upper foam layer edge with the headspace of the bioreactor. The system facilitates noncontact foam detection in a glass bioreactor vessel and allows the depth of any foam layer to be monitored continuously. The system was used to evaluate differences in the performance of commercially available antifoaming agents under various conditions. In the experiments described in this paper, the antifoam was added manually following a measurement by the LabCam of the foam generated in the bioreactor. However, there is of course a potential for developing an interface

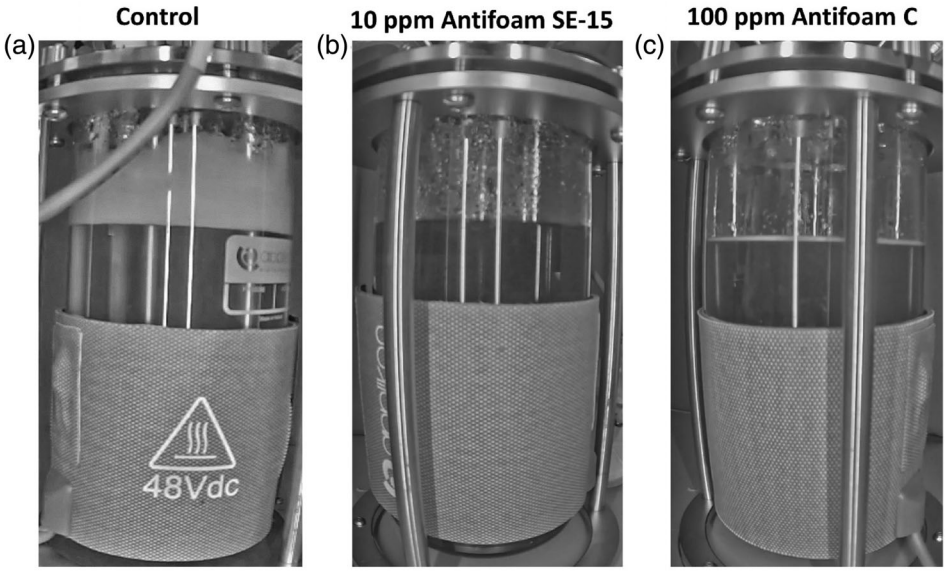

FIGURE 7 Longevity of the antifoaming capacity. The photographs shown were taken by the LabCam of the surface of the cultures in the bioreactors at day 8 after a high agitation rate (2000 rpm, 30 s) was applied. (a) Control culture without antifoam, (b) with 10 ppm antifoam SE-15, and (c) with 100 ppm antifoam C.

connection to enable a feedback loop and automated delivery of the antifoam.

Foaming in bioprocessing has been described as typically being non coalescing, in the form of a stable foam separated by thin liquid walls.[20] It has been documented that antifoam additions can yield both positive and negative effects on mammalian cell growth. Thus before use it is important to elucidate the effect of an antifoam agent on the growth and production of a specific cell line.[21] Foaminess, as explained in a paper from Junker in 2007, is classified as the "equilibrium volume", where the foam height may depend upon the volumetric flowrate of gas in a typical cylindrical bioreactor. It is typically monitored by foam sensors such as conductive probes or admittance probes, which all act on the liquid holding properties of the foam in question and provide an indication of the presence of a foam layer.[20] Traditional modes of foam monitoring/detection in bioreactors, provide a simple "yes" or "no" indication of foam presence, with a lack of any information on the properties or dynamics of the foam layer in question.[22] Importantly, it is critical to the design of a successful process and indeed a successful product, that foaming be controlled or eliminated to prevent drastic impacts on the process or product in question.[18]

Foam presents a major technological challenge because of the consequences of its uncontrolled and potentially intermittent generation in a production process. The formation of foam can result in (1) a breakdown of cell structural integrity, cell death at the foam liquid interface and damage to proteins as a result of bubbles bursting,[6,7] (2) breaches in process sterility should a foaming out event occur, and (3) the blockage of exit filters causing pressure related damage.[5] Many studies have probed the impact of foaming on the production of biologics and the devastating effects these foaming events have on mammalian cells, with impacts not limited to just serum containing media.[23]

Four main assays are currently employed to investigate antifoaming and to characterize the stability and durability of foam layers. These include (1) the Ross-Miles test, (2) the Bartsch method, (3) the automated shake test (AST), and (4) the Bikerman test.[9] The Ross-Miles test has been adopted as the American Society for Testing and Materials (ASTM) standard method for foam analysis (D1173-07 (2015)). In this test a fixed solution volume is passed through a standard orifice onto a bed of the same standard solution in a cylinder located a defined distance from the orifice. The height of foam is measured thereafter.[11] The Bartsch method characterizes the millisecond differences allowed for surface adsorption preceding new bubble collisions. The basis of this test is a shaking cylinder, where foam is typically formed at the moment where the solution hits the top and bottom ends of the cylinder when the direction of motion changes.[10] It is controlled by the coverage of bubble surface by surfactant and by the surfactant changes which induce electrostatic repulsions between the ionic surfactant and the entrapped bubble.[24] The AST is based on a similar principle to the Ross-Miles test where a solution is subjected to cycles of shaking followed by quiescent periods where the defoaming time is measured. The foam durability is determined by the number of cycles taken before "antifoam exhaustion". In this assay, the period where foam has completely dissipated is determined visually by the appearance of a clear air–liquid interface.[9] Unlike the aforementioned tests, the Bikerman test utilizes a monodisperse array of gas bubbles generated by sparging via capillaries or porous glass; here, fast acting antifoams are investigated with continuous bubbling, and slow acting antifoams are investigated with alternating cycles of bubbling and quiescence. The steady state height scales of the formed foam align linearly with the flow rate allowing for the calculation of Σ, "the unit of foaminess".[25] These commonly used methods only provide crude quantitative measurements of foam generation. There remains a need for an improved method of foam detection and monitoring during a bioprocess.

Although foaming contributes to decreased hydrodynamic and oxygen mass transfer mechanisms in bioreactors, these effects can be mitigated by the addition of antifoams. However, antifoam agents can

also exhibit detrimental effects with regards to cell viability, growth and protein production.[26] We examined the impact of three commercially available antifoams on cultures of antibody-producing CHO cells. Antifoam 204 (a polyether) exhibited a high degree of toxicity when tested at a concentration as low as 10 ppm in cell culture. In another study, antifoam, 204 showed toxicity and reduced growth of an IgG1-producing CHO DG44 cell line at concentrations of 30 ppm.[5] Interestingly, it was observed that while 1 ppm antifoam 204 did not impact cell growth it did impact protein expression as noted by IgG analysis. Although IgG titer analysis did not show strong evidence to suggest negative impacts of antifoam SE-15 and C down to concentrations of 100 ppm and 10 ppm, respectively, data indicated a reduced cell specific productivity in the presence of all antifoams at the concentrations tested. These results highlight the importance of a degree of control over antifoam dosage. It was decided that further investigation of the antifoaming effects of antifoam 204 be discontinued, and the antifoaming potential of only antifoam SE-15 and antifoam C became the focus of our work with bioreactor cultures. The subsequent foam dissipation studies were designed with the LabCam system to investigate (1) dosing of antifoam agents into a bioprocess post foaming, (2) antifoam addition to media to prevent foaming, and (3) the longevity of antifoam efficacy in CHO media up to 8 days.

The studies were conducted in Biogro culture media, which has been reported extensively for growing CHO cells. It was decided to maintain the presence of the difunctional block copolymer Pluronic F-68 in the Biogro CHO media, as this is routinely utilized in mammalian cell culture as a media additive. The surfactant is employed for its protectant properties against mechanical stress caused by the bursting of foam bubbles at the surface interface of cultures on cells.[27] Although this addition has been documented to reduce foaming by up to 30%, it has been shown that CHO cells can internalize this component degrading it in lysosomes, resulting in a need for additional antifoaming agents.[26] Our experiments were designed in batch cultures with cells growing to 3 to 4 $\times$ 10^6 cells/mL in a relatively low protein media. Nevertheless, these cultures were susceptible to foam generation with excessive agitation or aeration. Under manufacturing conditions CHO cells may be grown to 20 to 50 $\times$ 10^6 cells/mL with protein concentrations ranging to 10 g/L or above through a combination of Mab titers and host cell proteins. Under these conditions, the susceptibility for foam generation would be even greater and therefore the monitoring and control of foam becomes even more important.

In order to determine the suitability of the LabCam system in bioprocessing, it was critical to develop a robust means of generating a model for bioreactor foam. Here, the foam layer was generated by means of high-speed agitation in cell culture media, in the absence of cells. Although agitation can contribute to the generation of foam in a bioprocess, it is important to note that this harsh means of generating foam was used for demonstrative purposes rather than process imitation. The LabCam software required measurement calibration, achieved by fixing the bioreactor in place, and using the volumetric markings on the bioreactor vessel to calibrate the distances. Next, lines of detection are drawn over a freeze frame image from the LabCam software. The "lines" correspond to two parameters, (1) the liquid line, which measures the fall of the meniscus upon foam generation, and (2) the foam line, which measures the rising line of the top of the foam layer (Figure 2). From this point, the bioreactor frame and LabCam remained in the same position on the bench for consistency.

The LabCam software successfully tracked, recorded, and reported the generation and dissipation of the foam from changes in the distance between the detected edges of the foam layer. The initial suitability studies were based on foam dissipation of Biogro CHO media, in the absence of cells. From the results obtained, it was shown that a concentration of antifoam SE-15, up to 10 times lower than that of the manufacturers recommended starting concentration (100 ppm) was sufficient for the dissipation of artificially generated foam. The SE-15 appeared to dissipate foam almost seven times faster than antifoam C at the same concentration. However, both antifoams were equally as effective at preventing the formation of a foam layer, even after high-speed agitation, compared with the control (no antifoam). Our data showed that a supplementation in culture media of SE-15 or antifoam C at 10 ppm and 100 ppm, respectively, was able to dissipate generated foam for at least 8 days. Quantitative data, generated by the LabCam system provided powerful insight into antifoam concentration versus foam dissipation rates. The purpose of investigating antifoam longevity was to explore different strategies of preventing foam generation. One could add antifoam (manually or automatically) once foam has developed at some point in the bioreactor run. Alternatively, one could add antifoam at time 0 at a predetermined concentration that would not inhibit cell growth but knowing that the level of antifoam would be sufficient to reduce foam generating at some later time point in the bioreactor run. We showed that the antifoam added at the beginning of the bioprocess was still effective at day 8.

The present article has described experiments associated with foam monitoring in mammalian cell cultures, specifically Mab-producing CHO cells. However, the requirement for monitoring and control of foam is applicable to all types of aerated bioprocessing. In a recent paper dealing mainly with beer production Tiso et al.[28] highlighted the need for advanced online sensors and an imaging techniques in beer fermentation, in which the control of foam is a massive challenge. It is hoped that the optical system described in our research may contribute to ongoing developments in this area of foam monitoring.

5 | CONCLUSION

Three antifoaming agents were evaluated in CHO cell media and cultures by the LabCam image analyzer. Of these, antifoam SE-15 proved the most suitable in dissipating foam generated by either excessive agitation or gas sparging. In our experiments, we determined that the antifoam agent was able to completely dissipate a foam layer in around 3 min in the presence or absence of cells. This effect was produced at a relatively low concentration of 10 ppm, which was shown

to be a concentration that did not inhibit cell growth when added to CHO cultures. The LabCam system provides a novel optical method that was used at-line to monitor the dissipation of foam in a bioreactor. This technology has significant potential as an added tool in the development of bioprocesses particularly in situations where foam generation could occur, for example at high cell densities or high protein concentrations where there might be a demand for enhanced gas sparging or culture agitation.

AUTHOR CONTRIBUTIONS

Michael Butler: Conceptualization; project administration; resources; funding acquisition; writing – review and editing. **James Flynn:** Conceptualization; methodology; validation; formal analysis; investigation; data curation; writing – original draft; visualization. **Laura Breen:** Conceptualization; methodology; validation; formal analysis; investigation; data curation; writing – original draft; visualization; writing – review and editing; project administration. **Shankara Narayanan:** Data curation; formal analysis.

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CONFLICT OF INTEREST STATEMENT

The manufacturers of the LabCam, JM Canty provided technical support, equipment, and funding to the project.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in ResearchGate at https://r.search.yahoo.com/_ylt=AwrFQJMp2Hhlug0ERBTrFAx.;_ylu=Y29sbwNiZjEEcG9zAzEEdnRpZAMEc2VjA3Nj/RV=2/RE=1702447273/RO=10/RU=https%3a%2f%2fresearchgate.net%2f/RK=2/RS=9KS8006j4n45PGH53ltAxY3Jjh4-.

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