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Foam Control in Fermentation Bioprocess

From Simple Aeration Tests to Bioreactor

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Abstract

In this article, we describe the development of a simple laboratory test for the effective screening of foam control agents on a selected fermentation system, the mass production of *Yarrowia lipolytica*. Aeration testing is based on sparging air in the foaming medium allowing partial reproduction of the gas–liquid hydrodynamic encountered in bioreactors. "Dynamic sparge test," for which measurements are made during foam formation, was used to compare the capacity of three antifoams, based on different technologies, to control the foam produced in the fermentation broth. The selected foam control agents were: (1) an organic antifoam (TEGO AFKS911), (2) a silicone-based emulsion containing in situ treated silica (DC-1520) and (3) a silicone/ organic blend silica-free formulation. The testing results demonstrated dramatic differences among them and showed that the capacity of TEGO AFKS911 and DC-1520 to control the foam generated in the fermentation broth decreases as a function of fermentation time. This occurred to a much lesser extent for the silicone/ organic blend formulation. These results were correlated with the change of the foam nature and the increase of foam stability of the fermentation broth with culture time. The increase in protein content as a function of growth time was correlated with an increase in foam stability and antifoam consumption. A "synthetic fermentation broth" was also developed, by adding both proteins and microorganism to the culture medium. This allowed us to mimic the fermentation broth, shown by the similar antifoams behaviour, and is therefore a simple methodology useful for the selection of appropriate antifoams.

Index Entries: Antifoam; aeration; bioreactor; silicone.

Introduction

Fermentation is often accompanied by foam formation because of the high foaming tendency of solutions containing biomaterials such as

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proteins (1). These proteins can act as surfactants owing to their amphiphilic structure. They can adsorb at the interface, partially unfold and form strong intermolecular interactions. This produces a visco-elastic, irreversibly adsorbed layer at the air/liquid surface, which stabilises the foam (2). These kinds of films are not easily breakable. Restraining the formation of foam in a bioreactor is a crucial point for two reasons: on one hand it allows the control of the fermentation itself and on the other hand the fermentation equipments can be optimised and it therefore minimises the production costs. Screening of a range of antifoams during complete fermentation batches is one way to select the most appropriate product to control the foam produced during a specific process. This methodology presents major disadvantages such as the time it takes, and then the number of materials that can thus be tested is very limited. The selection of the proper antifoam will then often be limited to the availability of the material and on previous experiences.

The aim of this study was to develop a laboratory test for the effective screening of foam control agents. Previous work with the same aim used either a synthetic system made of bovine serum albumin (3) or a dynamic surface tension measurement (4). However, at the beginning of the fermentation, the foam stabilisation will be caused by the proteins already present in the growing medium, whereas at the end of the fermentation, by the proteins produced by the microorganisms (5). It was important to assess whether the overall protein type and content, as well as the microorganism content could modify the relative antifoam efficiency obtained in this laboratory test. Biomass production of *Yarrowia lipolytica* was selected as a fermentation system, as we experienced it to be especially foamy (6). This specific system was used to assess the effect of the yeast mass production on the absolute and relative antifoam efficacy.

In this article, we describe the development of a laboratory test, which enabled us to compare the efficiency of three antifoams based on different technologies. The mechanism of action of antifoam was the subject of recent studies, which often concentrated on synthetic surfactant solutions (7). Antifoams are often divided into two categories, depending whether they are based on soluble or insoluble oils (8). The "soluble" antifoams are often based on surfactant containing polyethyleneoxyde or polypropylene oxide moieties. Having a lower surface tension than the solution, the antifoam molecules adsorb at the interface, forming a low viscosity film, which is weaker than the proteins visco-elastic film because of lower intermolecular interactions. The second type of antifoams is based on insoluble oils such as polydimethyl siloxane or mineral oil. They are most usually formulated with hydrophobic particles, which help the small antifoam droplet to enter the solution surface (9,10). It has been already described that silicone-based antifoams based on simple polydimethyl

siloxane formulated with silica have reduced efficacy when very viscoelastic protein films are the cause of the foam formation (11). In this work, we compared the relative efficacy of:

1. an organic antifoam: a soluble antifoam based on polyglycol moieties (TEGO AFKS911 from Goldschmidt). This foam control agent is currently used to control the foam produced during *Y. lipolytica* mass production in our laboratory;
2. a silicone-based foam control emulsion: an emulsion of polydimethyl siloxane-based antifoam formulated with *in situ* treated silica (a solid hydrophobic particle), DC-1520 from Dow Corning. In the formulation, the active is 20% meaning that for the same quantity of material, this product contains five times less active that the two other formulations used in this work. We have however selected the approach to work at equivalent antifoam formulation volumes instead of working at constant antifoam active content, as it is more likely to be the practice used during antifoam selection;
3. a silicone/organic blend: a custom blend of polyglycol enriched with silicone materials having surface-active properties. The formulation contains 25% of Pluronic PE 6100 (BASF), 25% polypropylene glycol (Mw2000, as P2000 from BASF), 25% of a low Mw hydroxy-terminated PDMS (PA Fluid from Dow Corning) and 25% of a silicone polyether (FS 1270 from Dow Corning). This material was found especially efficient for the control of the foam during the fermentation of sugar cane molasses for the production of fuel ethanol (12). This formulation, although based on silicone, does not contain silica and was especially interesting to add to this study, to cover a wider range of antifoam materials.

Materials and Methods

Strain

Yarrowia lipolytica strain 6481 was supplied by CWBI (6) and was stored at –80°C before utilization.

Culture Medium

The culture medium used for studying biomass production contained glucose (2%), peptone (1%), yeast extract (1%), and chloramphenicol (0.05%).

Evaluation of the Microbial Population

Microbial populations were evaluated by counting the yeast colonies on a jellified medium. After appropriate dilution (in sterile tubes containing a solution of 0.5% NaCl, 0.1% of peptone, and 0.2% of Tween-80), the cells suspension was spreaded onto Petri dishes containing a jellified

growing medium made of glucose (2%), peptone (1%), yeast extract (1%), and Agar (1.4%). Petri dishes were incubated at 30°C for 48 h.

Bioreactor

A 20-L Biolafitte bioreactor fitted with two RDT4 (rushton disk turbine with four blades) impellers was employed for studying biomass production. The bioreactor was filled with 12 L of culture medium and then sterilized at 121°C for 20 min. A regulation system was used to control temperature at 30°C throughout the experiment after sterilization. A pH of 7.0 was maintained constant during the process using acidic (H3PO4 85% diluted twice) and basic (KOH 50%) regulation. The airflow rate was set to 1 vvm and the stirring speed was fixed at 200 rpm. A mechanical foam breaker was used when no antifoam addition was required for certain experiments. The bioreactor was inoculated with a 8% vol of inoculum under aseptic conditions. Precultures were made in 1-L Erlenmeyer flask containing glucose (2%), yeast extract (1%), and peptone (1%) and were inoculated 24 h before the beginning of bioreactor tests. During this time, preculture flasks were shook on an orbital agitator and maintained at 30°C.

Protein Assay

Protein assay was determined using the method developed by Lowry et al. *(13)* using the Folin-Ciocalteus reagent. The assay was performed on the precipitate formed by the addition of trichloroacetic acid to the solution to be analysed. The calibration line straight was completed with BSA (Albumin Fraction V from Merck) in concentrations varying from 0 to 200 μg/mL.

Sparge Test

Sparge tests were performed in a glass column (diameter, 9.2 cm; height, 30 cm). Airflow was supplied at the base of the column at a rate of 3 L/min. Fine bubble dispersion was obtained by forcing the air through a porous diffuser. The volume of foaming medium was 400 g. All experiments were performed at room temperature on "fresh" foaming medium (maximum 24 h storage at 4°C) or synthetic foaming medium.

Dynamic Sparge Test

In each experiment, testing was performed using the same amount of antifoam (for DC-1520, the amount of active antifoam is five times less than in TEGO AFKS911 and in the silicone/organic blend) added at the first stage of the test and during further aliquot addition. Aliquot volumes were however adapted from experiment to experiment, but they are mentioned in the legends accompanying the figures. An initial quantity of antifoam was deposited on the inner side of the graduated sparge column with a micropipette (Nichirya, Model 800) before the foaming medium was poured into the column. The mixture was stirred for 30 s before the

gasflow was flowed in the liquid and the rise of foam height monitored. Under these conditions, very fast generation of stable foam was obtained. When the foam reaches the top of the column (arbitrarily set at 30 cm), an additional antifoam drop was added on top of the column. The collapse time of the foam was then recorded. The test ran for 10 min and the overall quantity of antifoam required to control the foam, during the experiment enabled comparison of antifoam efficiency.

Static Sparge Test

The foaming medium (400 g) was poured into the column before the airflow was started. Once the foam reaches the top of the column, the airflow was stopped. The foam level was recorded as a function of time.

Results and Discussion

Test Protocols

In laboratory studies, foam can be generated in different ways *(2,14)*. Several air incorporation systems can be tested: sparging, whipping, shaking, or pouring. In the frame of this study, we selected the sparging of air, a simple aeration test, which allows partial reproduction of the gas–liquid hydrodynamic encountered in bioreactors. This method, commonly used as a research tool, involves forcing gas through the liquid via a sparger to create bubbles and hence foam, once surface-active materials are present in the liquid. It enables the delivery of controlled volumes and flow rates of a chosen gas to form a foam of a specific volume or for a specific time.

Foaming methods can be divided between the static and the dynamic methods. In static methods measurements are made after the foam has been formed whereas in dynamic methods measurements are taken during foam formation. Foam volume is often measured in static methods whilst time is often taken as the variable in dynamic measurements. Both types of methods were used in this work. Foam stability can be estimated by measuring the foam evolution as a function of time, after a given volume of foam is produced. The gasflow is stopped when the foam reaches the top of the column (arbitrarily set at 30 cm in our study) and the foam height progression is recorded during a definite time. This is referred as the "static sparge test" in this document. On the opposite, in the dynamic method, bubbling is maintained during the time of the experiment. Foam level is recorded as a function of sparge time, and the effect of different variables (for instance, level and type of antifoam, type of foaming medium) can be assessed by comparing what we will call the "foam profile." In a typical experiment, before the beginning of the measure, an initial quantity of antifoam was deposited inside the column. The liquid was stirred for half a minute to ensure proper dispersion of the antifoam. Once the flow of air started, foam was generated. Owing to the addition of antifoam, the foam level raised much more slowly than in its absence. Once the antifoam became progressively

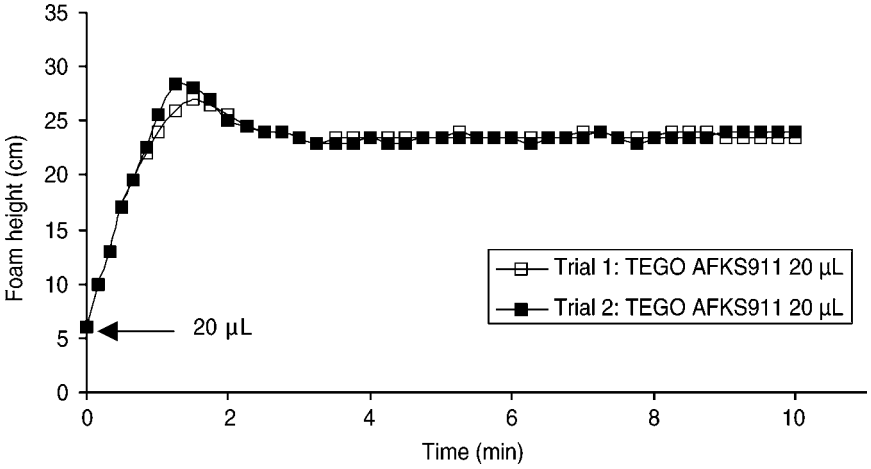

Fig. 1. TEGO AFKS911 ability to control the foam as a function of aparge time: reproducibility test—foam height as a function of sparge time (1 drop–arrow = 20 µL).

deactivated *(15)*, the foam reached the top of the column, arbitrarily set at 30 cm. An additional quantity of antifoam was then added in top of the foam column, which enabled the assessment of the so-called "knock down" capacity of the antifoam. The test was stopped after 10 min and the total amount of antifoam needed to control the foam during this interval allowed comparison of the different antifoams efficiency. This will be referred as the "dynamic sparge test" in this document. The test was found to be reproducible, as presented in Fig. 1, when the same, fresh, foaming medium was used.

Foaming Tendency of Fermentation Broth

Before making any experiment in the presence of antifoam, it was important to assess the foaming behavior of the selected fermentation system in order to characterize the pattern of foaming during fermentation *(5)*. Owing to the foaming tendency of the fermentation broth, only a static test could be selected. Mass production of *Y. lipolytica* was realized in flask culture and growth was quenched by cooling after different time periods. The fermentation broth samples were then tested by the "static sparge test" to assess the foaming tendency and foam stability after different stages of mass production.

The foam levels are plotted as a function of time in Fig. 2, which allows the determination of the influence of growth time on foam stability. The early parts of the curves (when air is still flowing) are all similar, showing that increasing culture time (i.e., yeast population) had no effect on medium foamability, foam was produced rapidly within a few seconds, independently of the fermentation medium composition. Foam capacity *(16,17)*, which is related to the volume of foam normalized by the volume of air injected at the same time, was already high at the early stage of the culture and was not modified by the culture time. The foam stability was not

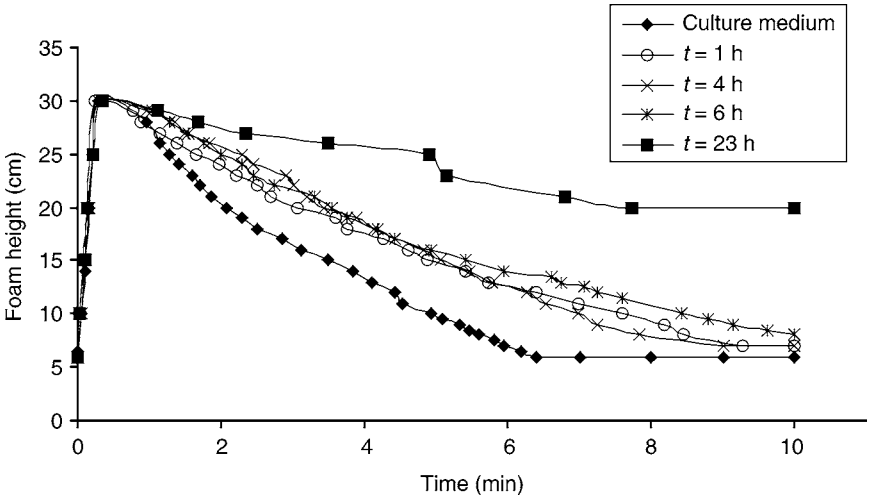

Fig. 2. Evolution of foam stability at different stages of *Y. lipolytica* mass production (culture medium, t = 1, 4, 6, and 23 h)—foam height as a function of time.

identical between the samples although the foam capacity was very similar, which is not uncommon for protein-stabilized foam *(18)*. Foam stability was assessed when air was stopped and by looking at the decay of foam as a function of time. Figure 2 clearly shows that the foam became more stable when culture time increased. During the lag phase in which no increase of the yeast population was recorded, the foam stability was constant but started to increase after 6 h of culture. These results highlight the fact that the nature of the foam changed with culture time and that yeast mass production significantly increased the foam stability of the fermentation broth. The foam is usually stabilised by proteins or other surface-active molecules present in the culture medium. The foaming capacity of the culture medium clearly showed this (*see* Fig. 3 as well). Proteins are furthermore produced during the process, *(19, 20)*, leading to more stable foam. The foaming pattern that this specific mass culture showed was then relatively simple to explain, as the foam stability had a tendency to increase as a function of fermentation time.

Efficiency of Antifoams in Fermentation Broth

In order to compare the efficiency of the three antifoams in the fermentation broth, the "dynamic sparge test" was used. Yeast mass production was run in a 20-L bioreactor using a mechanical foam breaker in order to enable the production of "antifoam-free" samples of fermentation broth after different times of culture. Samples of the culture broth were taken directly from the tank in a sterile manner at regular intervals. "Dynamic sparge tests" were performed on the different samples, directly after collection from the tank.

Figure 3 shows the foam profile obtained with the three antifoams, when tested in the culture medium (with no microorganisms). The foam

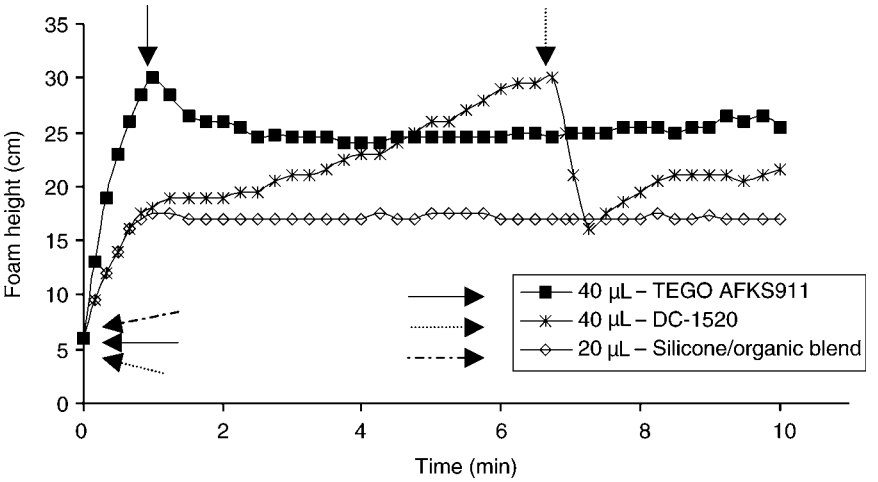

Fig. 3. Antifoam efficiency of selected materials on culture medium—foam height as a function of sparge time (1 drop–arrow = 20 µL).

profile enabled the differentiation of the antifoam efficiency of the different materials, and showed how a specific antifoam performed in the system. The silicone/organic blend showed excellent foam control. The foam remained low and controlled for the duration of the experiment. DC-1520 showed a gradual loss of efficiency, which lead to the need of an additional aliquot addition once the foam reached the top of the column. The consumption of antifoam required to control the foam during 10 min was then higher, reflecting poorer antifoam persistency. TEGO AFKS911 showed some difficulty to control the foam at the early beginning of the experiment, leading to the addition of an aliquot very early. Foam was then very well controlled during the rest of the time. This illustrated more a slower dispersion of the antifoam at the beginning of the experiment.

Figure 4 shows the foam profiles obtained with TEGO AFKS911 in fermentation broth of different culture times. Yeast density started at 2×10^6 cells/mL and reached 1.2×10^8 cells/mL after 24 h of culture. We can see that only one addition of TEGO AFKS911 (20 µL of antifoam) was required to control the foam of the fermentation broth at the early stage of the culture (0 and 6 h) for 10 min. The maximum foam height reached a limit of 25 cm. It is interesting to note that the addition of the micro-organism and the autoclave step seemingly improved the dispersion of the TEGO antifoam, leading to a more progressive foam profile (compared with the foam profile of TEGO in the culture medium, in which an early second addition was required). As the microorganism population started to rise, the amount of antifoam used to control the foam during the experimental time increased from 20 µL (t = 6 h) to 40 µL (t = 9 h) and reached 160 µL at the end of the culture (t = 24 h). The same trend was observed with the silicone emulsion DC-1520 (i.e., the quantity of antifoam required to control

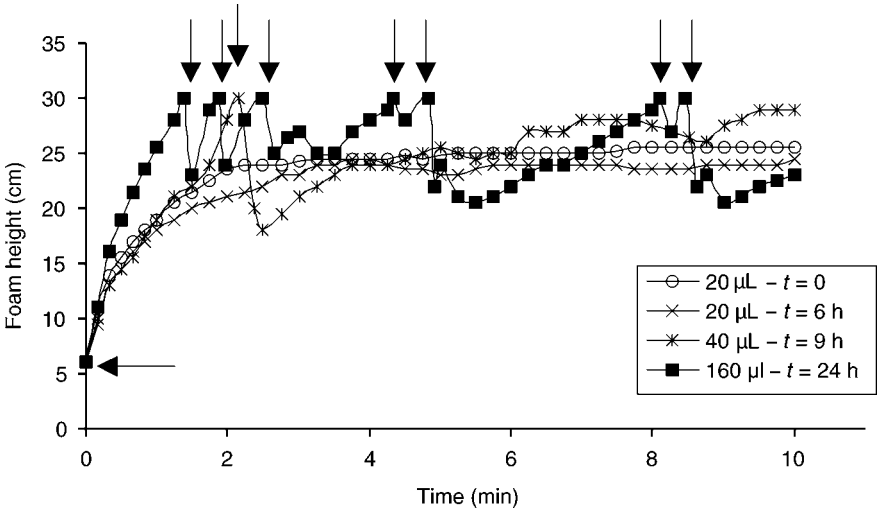

Fig. 4. TEGO AFKS911 ability to control the foam as a function of culture time ($t = 0$, 6, 9, and 23 h)—foam height as a function of sparge time (1 drop–arrow = 20 µL).

the foam increased as a function of growth time) but for the silicone/organic blend the quantity of antifoam required was constant and was not as strongly dependent on the yeast growth (results not shown). These results show that the yeast mass production decreased the capacity of the antifoam material (TEGO AFKS911 and DC-1520) to control the foam. Screening a wide range of antifoam materials should ideally be made with fermentation broth collected after 24 h of growth, as this corresponds to the more stringent testing conditions.

This kind of screening was exemplified with the three selected materials. Their ability to control the foam produced in the *Y. lipolytica* fermentation broth after 24 h of culture is illustrated in Fig. 5. The fermentation broth was produced in a 20 L bioreactor using a mechanical foam breaker to control the foam during the fermentation (this allows to obtain "antifoam-free" fermentation broth). After 24 h of culture, cell density reached 2.9×10^8 cells/mL.

Figure 5 demonstrates the significant difference between antifoam efficiencies. It should be noted that the antifoam aliquot used here was 100 µL compared with the 20 µL used for establishing antifoam testing protocol. A different volume of the aliquot was selected, which was used to discriminate efficiency of different materials, as foam profiles have to be obtained in appropriate conditions. If too small or too large aliquots were used, meaningless data could have been obtained (such as flat foam profiles or little to no foam control with each of the materials). With the commercial silicone-based emulsion DC-1520, an overflow was observed. Under these experimental conditions this material was not able to control the foam during the time of the experiment. The silicone/organic blend formulation was obviously more efficient than the TEGO AFKS911 material: 100 µL required

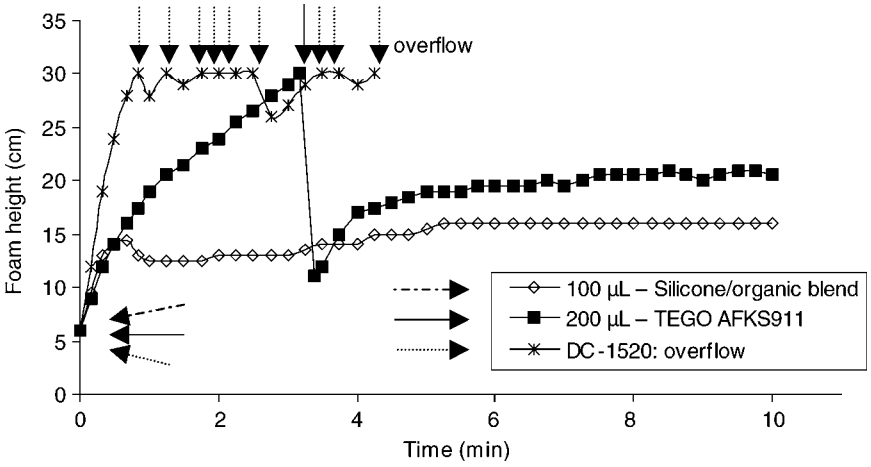

Fig. 5. Antifoam efficiency of selected materials on culture broth after 24 h of culture—foam height as a function of sparge time (1 drop–arrow = 100 µL).

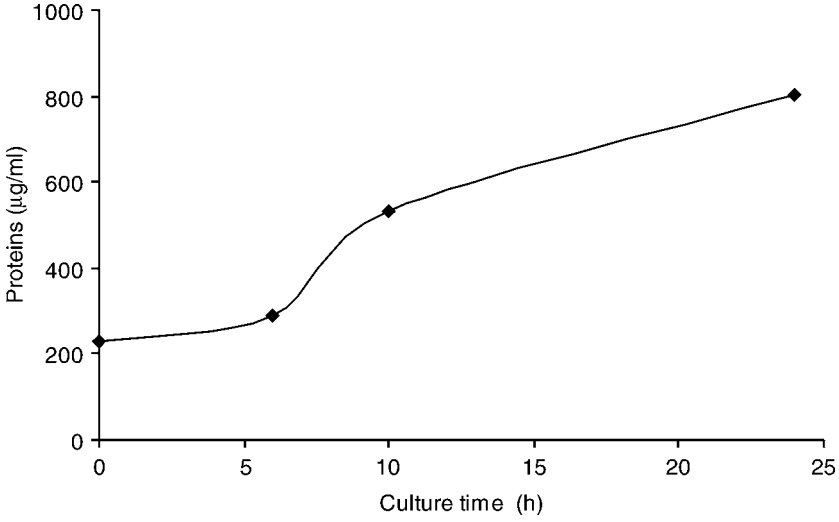

Fig. 6. Evolution of the protein concentration in the culture medium as a function of culture time.

to control the foam for 10 min for the former compared with 200 µL for the latter antifoam. Moreover, the foam profile obtained with the silicone/organic blend was flatter, suggesting longer lasting antifoam efficiency. The following antifoam efficiency ranking can be established: DC-1520 << TEGO AFKS911 << silicone/organic blend.

Foamability and Protein Content

Concentrations of protein in the fermentation broth were assessed by the method of Folin *(13)* and protein concentrations are plotted as a function of culture time in Fig. 6. Protein content increased as a function of

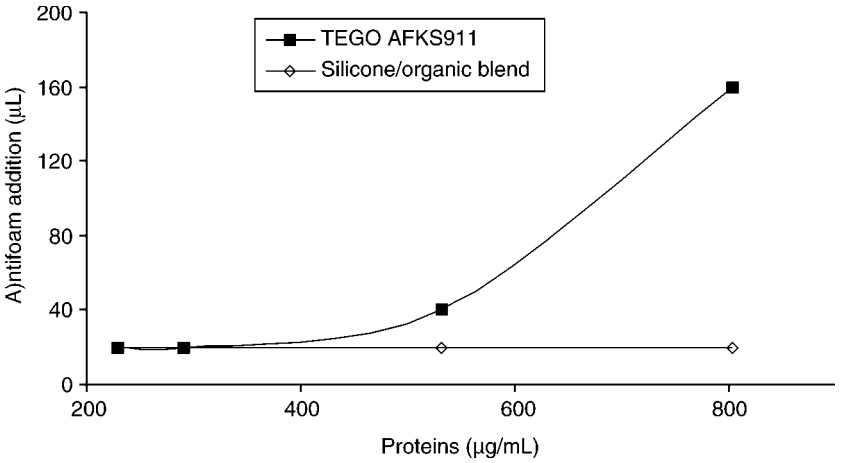

Fig. 7. Quantity of TEGO AFKS911 and silicone/organic blend needed to control the foam as a function of the protein concentration during *Y. lipolytica* mass production.

time, which correlated with an increase in foam stability and a decrease in efficiency of antifoam. Figure 7 shows that the increase of protein concentration in the broth is correlated with a lowering of the foam control efficiency (which was translated by an increased quantity of antifoam required to control the foam for 10 min) and an increase in antifoam consumption. The relation is obviously not linear. At some point, further increase of the extracellular concentration lead to a more significant decrease of antifoam efficiency and to a much higher antifoam consumption. Interestingly, the shape of the curve obtained with the TEGO AFKS911 and the silicone/organic blend was very different, showing that the silicone/organic blend formulation was much less sensitive to the protein content in the foaming medium.

Sparge Test on the Fermentation Broth vs Simple Synthetic Medium

We have shown that a simple dynamic sparge test can help to discriminate between antifoams of various efficiencies. This was exemplified with the *Y. lipolytica* mass production. This methodology is useful for comparative testing of antifoams but it was questioned if a simpler test could be designed. Other systems, which would mimic fermentation broth, were investigated to elaborate a simpler methodology useful for the selection of appropriate antifoams. Figure 3 shows the foam profiles obtained with the three antifoams for the culture medium free of microorganisms. The three antifoams were found to efficiently control the foam of the culture medium. The foam produced in the culture medium was less difficult to control than when produced in the fermentation broth after 24 h and the ranking of the antifoam efficiency was also different. The ranking in the culture medium is TEGO AFKS911 ± DC-1520 < silicone/organic blend, which is different from the one in the 24-h fermentation broth: DC-1520 << TEGO

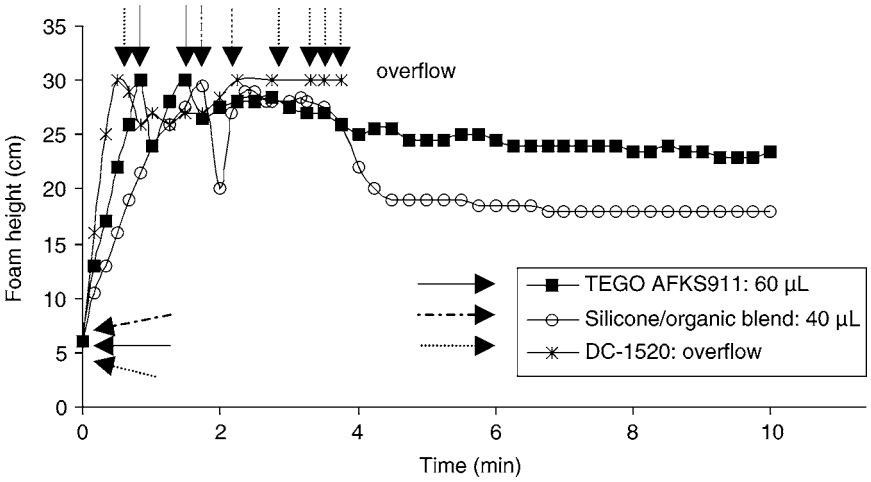

Fig. 8. Anitfoam efficiency of selected materials on culture medium containing additives (0.5% albumin and 13% baker yeast)—foam height as a function of sparge time (1 drop–arrow = 20 µL).

AFKS911 << silicone/organic blend. Simple culture medium was thus not representative of the fermentation broth after 24 h.

Enrichment of the starting culture medium was carried out by protein addition (albumin) and microorganism addition (baker's yeast). The ability of the three antifoams to control the foam of the culture medium was decreased by the addition of both proteins and yeast.

Adjustment of the protein and yeast content lead to a "synthetic fermentation broth" containing 0.5 % albumin and 13% baker yeast. The foam profiles obtained with the three antifoams in this "synthetic fermentation broth" are illustrated in Fig. 8. It is interesting to see that the ranking of antifoam performance is then similar to the one obtained with the 24 h-old fermentation broth: DC-1520 << TEGO AFKS911 << silicone/organic blend *(20)*.

Conclusions

The relative antifoam efficiency of various materials can be assessed by using a simple sparge test, reproducing the aeration conditions of bioreactors. The capacity of antifoams to control the foam generated in culture medium or fermentation broth, decreases as a function of fermentation time. A fermentation broth obtained after 24 h of mass production is then the best foaming system to use to perform an antifoam screening exercise. The volume of antifoam required to control the foam during the test duration is then used as a measure of the antifoam efficiency. Efficiency of these antifoam was found to be very different and the silicone/organic blend formulation shows a longer lasting performance, i.e., a lower dependence of the antifoam efficiency on the proteins content.

A "synthetic fermentation broth" was developed, by adding both protein and baker's yeast to the culture medium. The behavior of the antifoams in this "synthetic fermentation broth" was approaching that of the fermentation broth after 24 h of culture.

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References

1 Wilde, P. and Clark, D. (1996), In: *Methods of Testing Protein Functionality*, Hall, G., ed., Blackie Academic & Professional, London, pp. 110–152.
2 Wilde, P. (2000), *Curr. Opinion Colloid Interval Sci.* **5**, 176–181.
3 Sie, T. and Schügerl, K. (1983), *Eur. J. Appl. Microbiol. Biotech.* **17,** 221–226.
4 Abribat, B., Molitor, J. -P., De Haut, C., Merlet, S., and Claessens, P. Patent WO 03/040699A1, Cognis, France S. A.
5 Hall, M., Dickinson, S., Pritchard, B., and Evans, J. (1973), *Prog. Industr. Microbiol.* **12,** 169–234.
6 Destain, J., Roblain, D., and Thonart, P. (1997), *Biotech. Let.* **19,** 105–107.
7 Garrett, P. (1993), In: *Defoaming: Theory and Industrial Applications*, Garrett, P., ed., Marcel Dekker, New York.
8 Lee, J. and Tynan, K. (1988), *2nd International Conference on Bioreactor Fluid Dynamics*, 353–377.
9 Wasan, D. and Christiano, S. (1997), In: *Handbook of Surface and Colloid Chemistry*, Birdi, K. S., ed., CRC Press, New York.
10 Denkov, N. (2004), *Langmuir* **20,** 9463–9505.
11 Christiano, S. and Fey, K. (2003), *J. Ind. Microbiol. Biotech.* **30,** 13–21.
12 Lecomte, J. -P., Cazaroto, M., Rivs, M., and Mivrat, T. (2004), Patent PCT/2004/035370 filed on October 21, 2004.
13 Lowry, O., Rosebrough, N., Farr, A., and Randall, R. (1951), *J. Biol. Chem.* **193,** 265–275.
14 Domingo, X., Fiquet, L., and Meijer, H. (1992), *Tenside Surf. Det.* **29,** 16–22.
15 Denkov, N., Marinova, K., Hristova, H., Hadjiiski, A., and Cooper, P. (2000), *Langmuir* **16,** 2515–2528.
16 Baniel, A., Fains, A., and Popineau, Y. (1997), *J. Food Sci.* **62,** 1–5.
17 Razafindralambo, H., Paquot, M., Baniel, A., et al. (1997), *Food Hydrocolloids* **11,** 59–62.
18 Popineau, Y., Loisel, W., Bertrand, D., and Gueguen, J. (1993), *Traitement Industriel des Fluides Alimentaires Non-Newtoniens* **27–29,** 72–81.
19 Ghildya, N., Lonsane, B., and Karanth, N. (1988), *Adv. Appl. Microbiol.* **33,** 173–222.
20 Schügerl, B. (1985), *Proc. Biochem.* **20,** 122, 123.