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BEYOND DE-FOAMING: THE EFFECTS OF ANTIFOAMS ON BIOPROCESS PRODUCTIVITY

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Abstract: Antifoams are often added to bioprocesses with little knowledge of their impact on the cells or product. However, it is known that certain antifoams can affect the growth rates of both prokaryotic and eukaryotic organisms in addition to changing surface properties such as lipid content, resulting in changes to permeability. This in turn can be beneficial to a recombinant protein production system for soluble proteins, as has been demonstrated by increased secretion of α-amylase and GFP, or achievement of greater yields of protein due to increased biomass. However, in some cases, certain concentrations of antifoams appear to have a detrimental effect upon cells and protein production, and the effects vary depending upon the protein being expressed. These findings emphasise the importance of optimising and understanding antifoam addition to bioprocesses.

MINI REVIEW ARTICLE

Foaming in bioprocesses

Foam occurs in bioprocesses due to the introduction of gases into the culture medium, and is further stabilised by proteins produced by organisms in the culture[1]. Foam is made up of liquid lamellas which are full of gas. Foams with high liquid content are unstable, while dry polyhedric foams are more stable and usually formed due to mechanical stresses[2]; both types can be found in bioprocesses. Examples of undesired foam formation is seen in bioprocesses used for paper, food, beverage and drug production such as the synthesis of antibiotics[3]. Unwanted foaming can also occur during water purification, blood transfusions, and in the dyeing of fabrics[3,4]. In this review, I focus on the foaming that typically occurs in bioprocesses producing recombinant proteins.

The production of recombinant proteins on large scales is essential for the development of drugs as well as the engineering of antibodies[5], the identification of functions and interactions of proteins[6] and also in the production of enzymes[7]. Valuable proteins such as insulin[8] and human growth hormone[9] have been produced recombinantly on an industrial scale in bioreactors and have enabled treatment and understanding of many diseases. In these formats, foaming is a problem that is particularly acute due to gassing used to maintain appropriate dissolved oxygen (DO) concentrations. Foaming can lead to reduced process productivity since bursting bubbles can damage proteins[10], result in loss of sterility if the foam escapes the bioreactor[11] or lead to over-pressure if a foam-out blocks an exit filter. To prevent the formation of foam, mechanical foam breakers, ultrasound or, most often, the addition of chemical antifoaming agents (or "antifoams")[11] are routinely employed in bioreactors and large shake flasks. There is a well-established literature on antifoams, highlighting their importance in bioprocesses, but relatively little information on how they affect the biology of the process itself[11]. In this review, the effects of antifoams, both positive and negative, on bioprocess productivity are discussed.

Antifoams

Antifoams can be classified as either hydrophobic solids dispersed in carrier oil, aqueous suspensions/emulsions, liquid single components or solids[12-14] and may contain surfactants[15]. Many antifoaming agents are commercially available, with 43 currently being sold by Sigma-Aldrich alone. While little information is routinely given about the composition of antifoaming agents, their specific defoaming properties have been thoroughly investigated. These include their effects on foam height with time, their influence on the volumetric oxygen mass transfer coefficient (k_La) of the system, their gas hold-up characteristics and their globule size and distribution in relation to their action upon foams. Much of the literature available on antifoams in bioprocesses in bioreactors documents their effects upon the DO and the volumetric mass oxygen transfer coefficient (k_La) in a system[16-24], rather than upon cells and recombinant proteins.

Antifoams can be split into two categories of fast and slow antifoams, depending on their mechanism of foam destruction: slow antifoams are often oils which destroy foam over a longer period of time, while fast antifoams, are usually mixed agents which enter the foam film[25]. Some simple methods of determining the ability of antifoams to reduce foam are the Bartsch shaking test[26] and the Ross-Miles pouring test[27].

De-foaming mechanisms

Several mechanisms explaining the action of antifoams have been suggested which include bridging-dewetting, spreading fluid entrainment and bridging-stretching[25]. For oil-based antifoams, bridging-dewetting and bridging-stretching mechanisms are known to occur and are illustrated in Fig. 1. Bridging-dewetting (Fig 1A) occurs when an oil drop enters the surface of the foam film and is deformed into a lens shape (Fig 1A (c)). When the film thins, the lens enters the opposite surface of the foam film and forms a bridge. The film is

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dewetted away from the oil bridge by capillary forces causing the film to rupture (Fig 1A (d)). With bridging stretching (Fig 1B), the oil particle bridges the foam film surface (Fig 1B (a) and (b)). This leads to the formation of an oil bridge which stretches over time, becoming an unstable film that ruptures at the thinnest region so that the entire foam structure is destroyed (Fig 1B (c) and (d))[3,28]. Mixed agents enter the foam and destroy it in this manner (Fig 1B)[25].

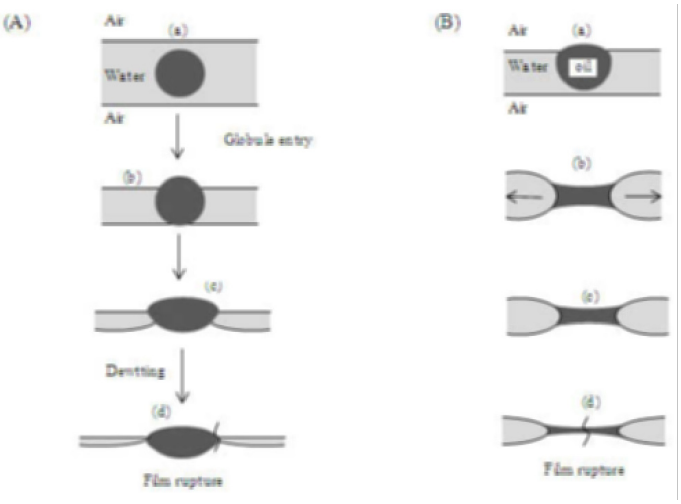

Figure 1. Bridging-dewetting and bridging-stretching antifoam mechanisms. (A) Bridging-dewetting, where an oil drop becomes a lens, rupturing the film, and (B) bridging-stretching where the oil particle bridges the foam film surface forming an oil bridge; this stretches forming an unstable film, eventually rupturing the foam. Adapted from Denkov and Marinova 2006[3].

Antifoams and oxygen transfer

In order to grow, aerobic organisms require a sufficient concentration of dissolved oxygen in the medium. The oxygen transfer rate (OTR) depends upon the k_La and upon $C_{L\infty} - C_L$, where C_L is the dissolved oxygen concentration and $C_{L\infty}$ is the oxygen saturation concentration in the liquid phase at the gas-liquid interface[29]. The k_La is a measure of how much oxygen is transferred into the medium over a certain amount of time[24]. The k_La of a system can be influenced by several factors such as properties of the medium like viscosity, the presence of organisms and their by-products. Additions to the medium such as antifoams also have an effect[23,24]. It has been observed that low concentrations of antifoam can reduce the k_La but at higher concentrations the k_La may rise[20,22]. To ensure optimum oxygen transfer within a system, the effect of differing concentrations of the antifoam to be used should be assessed, although this is not typically done. Changes to the k_La can be due to effects on kl (m/s) and on a (specific surface area m^{-1})[20,30]. It has been suggested that antifoams enhance bubble coalescence and increase bubble size leading to a reduction in the specific surface area therefore lowering k_La[11,16,17,20,30]. However it has also been observed previously that the k_La rises at higher concentrations of antifoam agents. This may be due to bubble coalescence reducing the surface tension, which then leads to decreasing bubble size and the k_La rises again. Secondly it is possible that antifoams accumulate oxygen from rising bubbles as they have good oxygen solubility, and release it to the aqueous phase. Bubbles bursting at the surface also disperse small drops of the antifoam causing more oxygen to be released[20,22]. In the case of oils which have a greater oxygen solubility than water, oil droplets may increase oxygen permeability in the water boundary layer of the gaseous dispersion[31]. The ability of antifoams to reduce k_L has been suggested to be less for bubble swarms than for a single bubble[21]. It is also possible that surfactants can lead to rippling or eddying which influences the k_La. k_L has not been found to be greatly affected by antifoam agents, with the main effect being upon a[23].

In bioprocesses both positive and negative effects of antifoams upon oxygen transfer have been observed, for example a silicone-based antifoam negatively affected the mass transfer coefficient, gas hold up and gas velocity within the media[16]. However it was found by Koch *et al* that antifoams without silicone oil did not greatly affect the oxygen transfer rate, whereas those containing silicone oil had a significant effect at the beginning of the process, which decreased over the duration[19]. Our research has demonstrated that in shake flasks the k_La was higher at concentrations of 0.4% v/v to 0.6% v/v and decreased with increasing concentration up to 1% v/v. Additionally, DO in shake flask cultures of *P. pastoris* was unaffected by the presence of antifoam, suggesting that any changes to k_La were not great enough to influence the DO in the culture[32]. These DO measurements have been performed in various growth media in both the absence and presence of cultures of prokaryotic and eukaryotic microbes[1,11,13,16,18,19,25]. In contrast, literature on the biological effects of antifoams on recombinant protein production by microbial host cells is more limited, suggesting that this area is not routinely considered.

Antifoams and recombinant protein production in prokaryotes

A study by Koch and colleagues investigated the effects of several antifoams upon foam destruction as well as upon protein production. The agents tested included; silicone oil (S184); polypropylene glycol (PPG) (SLM54474); silicone oil/PPG mixture (VP1133); and an emulsion containing 10% S184 (SE9). The antifoams were added at various concentrations to *E. coli* K12 cultures producing β-galactosidase fusion protein. It was found that at 1000 ppm of PPG/silicone oil mixture, 555 ppm of emulsion and increasing concentrations of PPG, the specific growth rate of the cells was reduced compared to starting concentrations of under 125 ppm. The other antifoams at increasing concentrations appeared to have no significant effect upon the growth of the cells, although the highest growth rates were achieved in the presence of the emulsion. The mass of the cells grown in the presence of the emulsion was also approximately double that of the cells with the other antifoams. The volumetric and specific product activity of β-galactosidase fusion protein increased with increasing concentrations of PPG and PPG/silicone oil mixtures, while decreased with increasing S184 concentration. This study highlights the range of effects different antifoam compositions could exert upon a culture and also that the concentration applied should be considered, although possible mechanisms of action of the antifoams were not explained[19].

The influence of PEGs of two different molecular weights and various concentrations upon *Bacillus subtilis* and *Bacillus amyloliquefaciens* producing α-amylase has been studied by Andersson *et al*. The *Bacillus* species were cultured in a two-phase aqueous system composed of PEG600 at 8% w/v and 20% w/v in addition to PEG3350 at 5% w/v, 9% w/v and 7% w/v. The production of α-amylase by *B. subtilis* was doubled in the presence of PEG600 at 8% combined with 5% PEG3350, but decreased with 9% PEG3350 alone. An increase in production was also reported with 20% PEG600 for *B. subtilis*, but resulted in a decrease for *B. amyloliquefaciens cultures*. A change in the morphology of the cells was also observed using an aqueous two-phase system, and the PEGs