Microbial Cell Factories

Poster Presentation

Evaluation of antifoams in the expression of a recombinant FC fusion protein in shake flask cultures of *Saccharomyces cerevisiae &Pichia pastoris*

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Background

Optimisation of culture conditions for the expression and production of important therapeutic biologics such as recombinant proteins, antibody-fragments and fusion proteins is a key element in the rapid and cost effective manufacture of these important molecules [1]. The factors to be considered when producing proteins from microorganisms such as *Saccharomyces cerevisiae* or *Pichia pastoris* include: pH, temperature, carbon and nitrogen sources and the essential oxygen requirement. The demand for oxygen by a microorganism can be met by aerating the medium that it is growing in, which is most often done by sparging sterile air through the medium.

An unfortunate effect of both sparging gas through culture media at high rates and intense agitation is the formation of foam. This is a particular problem when surface active species such as proteins are present at high concentrations. Foams are gas/liquid dispersions with >95% gas content [2]. Foam formation can reduce the efficiency of gas exchange at the surface of the culture, as a barrier is formed between the culture and the gases in the headspace of the vessel. Foaming can also be detrimental to the cells: when bubbles burst they exert sheer forces, which can damage cells and/or any secreted proteins.

Additionally cells and culture medium are lost to the foam phase which can lead to a decrease in process productivity. In extreme cases a 'foam out' situation can lead to loss of process sterility [2].

In order to minimise the deleterious effects of foaming, antifoam agents are used which prevent foam forming by reducing the surface tension of the culture [1]. There is a wide range of antifoam agents available from various suppliers. Examples of commonly used antifoams include compounds from the following chemical types: polyalkylenglycols, alkoxylated fatty acid esters on a vegetable bases, polypropylene glycol (PPG), siloxane polymers, mineral oils and silicates.

For this investigation a secreted recombinant protein expressed by both *P. pastoris* and *S. cerevisiae* was used as a marker of protein production yield. The product protein was produced using the following expression systems; in *P. pastoris* the gene had been inserted into a methanol-inducible expression cassette. In *S. cerevisiae* (Uracil autotrophic strain) protein expression was under the control of the *TPI1* promoter. The protein itself has a molecular weight of approximately 48 kDa.

We examined the effectiveness of four antifoam agents; Schill & Schelinger's Struktol SB2121 (Polyalkylenglycol), Schill & Schelinger's Struktol J673A (an alkoxylated fatty acid ester on a vegetable base), Sigma Antifoam C (Siloxane polymer) and Fluka P2000 (Polypropylene glycol), for use with both *P. pastoris* and *S. cerevisiae*. The effect on the growth rate and the protein production yield for all antifoam types at varying concentrations was determined by monitoring the growth and target protein production in *S. cerevisiae* and *P. pastoris*.

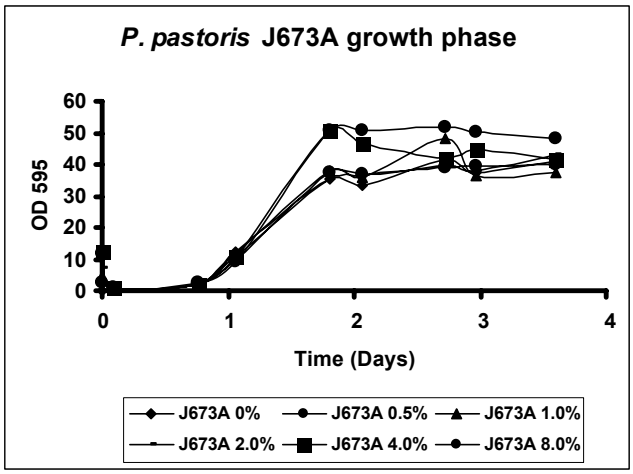

Figure 1
Growth curves for *P. pastoris* in YPD medium at 30°C with J673A antifoam.

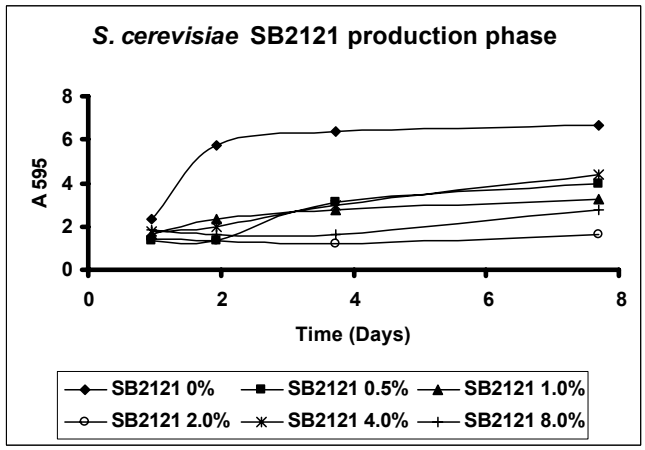

Figure 3
Growth curves for *S. cerevisiae* TM6* in SD^{-URA} medium at 30°C with SSB2121 antifoam.

Results

The different types of antifoam affect *S. cerevisiae* and *P. pastoris* growth in different ways depending on the concentration and medium type being used. When Struktol J673A is used with YPD medium for *P. pastoris* growth, increasing antifoam concentration increases optical density (OD) at 595 nm (see Figure 1). Conversely when Struktol SB2121 is used with SD^{-URA} medium for S. cerevisiae (strain: ALCOFREE™ Yeast 01) [3] protein production, increasing antifoam concentration reduces OD measurements of the cultures (see Figure 2). When Antifoam C is used with YPD medium, S. cerevisiae growth is not affected by Antifoam C concentrations up to 8% (see Figure 3). The effect on protein production is less variable, with the trend being that concentrations over 1% total volume decrease the yield of recombinant protein in the cultures (See Figure 4).

Conclusion

The data indicate that antifoam agents can be used at concentrations up to 1% total volume. Higher concentrations can lead to higher optical densities being obtained but with a decrease in protein yield. Additionally some of the antifoam agents become difficult to work with at higher concentrations, producing precipitates which interfere with sampling and analysis. Table 1 highlights the main conclusions for each individual antifoam and application.

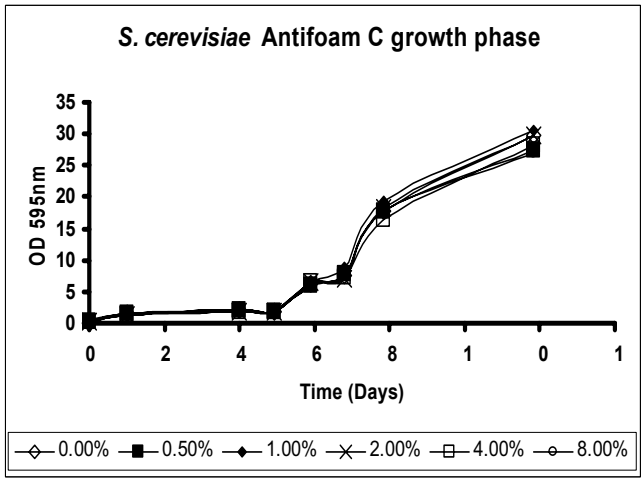

Figure 2
Growth curves for *S. cerevisiae* TM6* in YPD medium at 30°C with Antifoam C

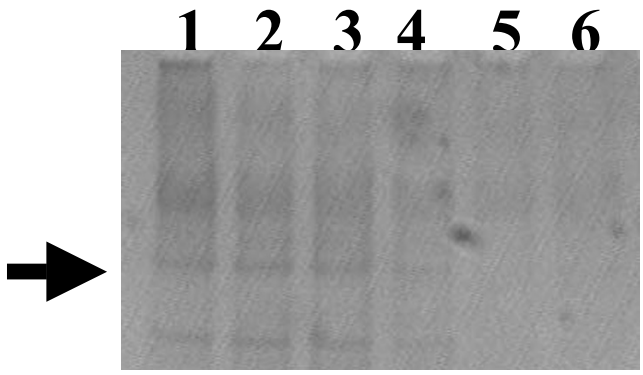

Figure 4
Silver stain of *P. pastoris* production phase samples 120 hr post methanol induction with Struktol J673A antifoam: Lane **1** 0% J673A, **2** 0.5% J673A, **3** 1% J673A,**4** 2% J673A,**5** 4% J673A,**6** 8% J673A. The arrow indicates the recombinant protein produced in these experiments.

Table 1: Summary of main conclusions

Antifoam name	Antifoam type	*Pichia pastoris*		*Saccharomyces cerevisiae*	
		Growth	Production	Growth	Production
SB2121	**Polyalkylenglycol**	No effect < = 8% [SB2121]	No effect < = 8% [SB2121]	Optimal when [SB2121] >0% <4%	Decreased by [SB2121] >1%
J673A	**Alkoxylated fatty acid ester on a vegetable base**	Increases with [J673A]	Decreased by [J673A] >1%	Increases with [J673A]	Decreased by [J673A] >1%
Antifoam C	**Siloxane polymer**	No effect < = 8% [Antifoam C]	TBC	No effect < = 8% [Antifoam C]	TBC
P2000	**Polypropylene glycol**	Decreased by [P2000] >1%	TBC	Increases with [P2000]	TBC

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