Sample Foaming

This document is to explain the known causes of foaming and the known solutions when processing samples on a Constant Systems Cell Disrupter.
The Cell Disrupter design results in the sample being forced through a small orifice at consistent high pressure where the sample can be accelerated to over 500 m/s in less than 200 ms. After which the sample is then passed through air until it hits the target and spreads over a heat exchange surface. Under normal conditions foaming is not experienced with sample preparations, however, there are some conditions which can encourage foaming. Foam formation can be attributed to the nature of the microbe itself or a number of changes in the sample and their treatment, these factors are reviewed below:

1. **Cell concentration, types of protein & protein release.**

Although there are many proteins in cell lysates, when breaking systems used for the production of recombinant proteins a large quantity of this single protein may be expressed and may significantly influence the protein profile within the lysate. Typically, cells contain in the order of 50% of their dry weight as protein. In recombinant systems the levels of protein can be substantially higher. The type of protein present can also effect foaming, again when using a recombinant, the physical properties of the protein upon release may favour foaming. Typically, the protein release is closely associated with the cell density with an almost linear relationship being observed when tested on these systems to over 200 g dry weight per litre of sample. At the upper end of this concentration the rheology of the cell suspension changes significantly, and this can cause a substantial change in fluid behaviour (non-Newtonian). Under these conditions the release of soluble protein is reduced. Typically, cell concentrations of 200g/l dry weight can normally be disrupted. Above these levels the physical nature of the system can change and will be in a very concentrated form and the extract may be in the form of a gel rather than a free-flowing liquid. Typically, if the cells have been disrupted successfully there is low viscosity as the large DNA molecules have been sheared and the proteins are in solution. If very concentrated cell pastes are used, then there is a possibility of a gel formation.

Recommendation

A simple way to reduce foaming without adding or removing chemicals to the sample preparation is to reduce the concentration of protein present in the system. Once the level of protein is reduced normally the amount of foam will also decline. We would recommend disrupting at a series of diluted samples and observe the effects by measuring the levels of foam volume. The cleanliness of the apparatus (especially the One-Shot equipment) prior to adding cell suspensions could also affect performance.

2. Protease Inhibitors in the sample preparation

It has been found that protease inhibitors Ethylenediaminetetraacetic acid (EDTA) and Phenylmethanesulfonyl fluoride (PMSF) can be a contributing factor regarding foam formation.

Recommendation

Constant Systems recommends using EDTA-free Protease Inhibitor Cocktails such as cOmplete manufactured by Roche®. Alternatively, EDTA or PMSF should be added to the sample preparation after cell lysis and this can be achieved safely by adding to the receiving flask/beaker* prior to processing the sample. This method of adding the EDTA/PMSF to the receiving flask/beaker* is used often by customers with no detrimental effect to the sample.

* Inner Cup if using MC equipment or Receiving Cup if using OS equipment.

3. Sample preparation & Sample storage

Sample preparations may also affect the production of foam. The use of surface-active agents, Triton and Tween may affect the amount of foaming. Similarly, some suspension buffers may change the foaming properties. If growth medium is present in substantial levels this may well contain components that can cause foaming e.g. yeast extract, peptones, and extra cellular products from the microbe. The samples, if not used directly, can typically be frozen and stored prior to disruption. If this is not done carefully, with rapid freezing and thawing, there is potential for substantial disruption of the sample prior to putting it through the disrupter. The presence of large quantities of free protein prior to disruption can significantly alter the amount of foaming. We recommend that cells initially are washed in a suspension buffer and disrupted before they are frozen. The effects of freezing should then be investigated as to its effects on foaming to ensure that is not a contributory factor.

Recommendation

As above poor storage of the cells prior to disruption and also inadequate freeze/thaw methods may have a significant effect on the amount of foaming. If foaming is experienced we recommend that cells initially are washed in a suspension buffer and disrupted before they are frozen. The effects of freezing should then be investigated as to its effects on foaming to ensure that is not a contributory factor.

4. Antifoam Additive.

If altering the sample preparation or its dilution does not affect the foaming, or if these precautions cannot be taken, then the addition of an antifoam agent does reduce the foaming problem. Both antifoam silicon (sigma antifoam A) and Polypropylene glycol 2000 can reduce foam formation and lead to rapid foam drainage.