# Under Pressure

Many machines that perform mechanical lysis and homogenisation of cells are sold based on the pressure they are capable of reaching. However, recent research demonstrates there is much more to mechanical cell disruption than just one number

Dr Matthew Lougher
at Constant Systems

Since the late 1940s, high-pressure homogenisers have been developed and applied as a method of mechanically disrupting cellular membranes, thereby releasing the intra-cellular components for further purification and study. In order to do this, the machines are traditionally designed and operated in such a way that a hydraulic ram applies high pressure to a liquid sample, which is then forced through a narrow orifice. The pressure differential that forms across both sides of this orifice causes the plasma membrane to rupture, lysing the cells as desired without causing damage to fragile structures and molecules within the cell. This ability to disrupt the cells, while preserving molecules such as proteins, has led to different brands of high-pressure homogenisers becoming a staple item found in many labs around the world – for example, those working on purifying recombinant proteins overproduced through a host organism like *Escherichia coli*.

Although the original manufacturers of the traditional model of high-pressure homogenisers have ceased production, there are several companies today that offer alternative, often faster, products based around similar principles: some use pneumatics to lower the production cost of the machine; while others maintain the use of hydraulics in order to provide more reliable and repeatable results across a range of cell types and species. However, despite there being variations in exactly how they operate, pressure is still the predominant term used to establish the capabilities of each brand and model.

Recent research has begun to explore what else can be used to quantify lysis capability. In order to validate the following discussions in the context of pre-existing technology, Figure 1 illustrates that constant cell disruptors can offer the same lysis efficiency as a traditional homogeniser when operated at the same pressure (1).

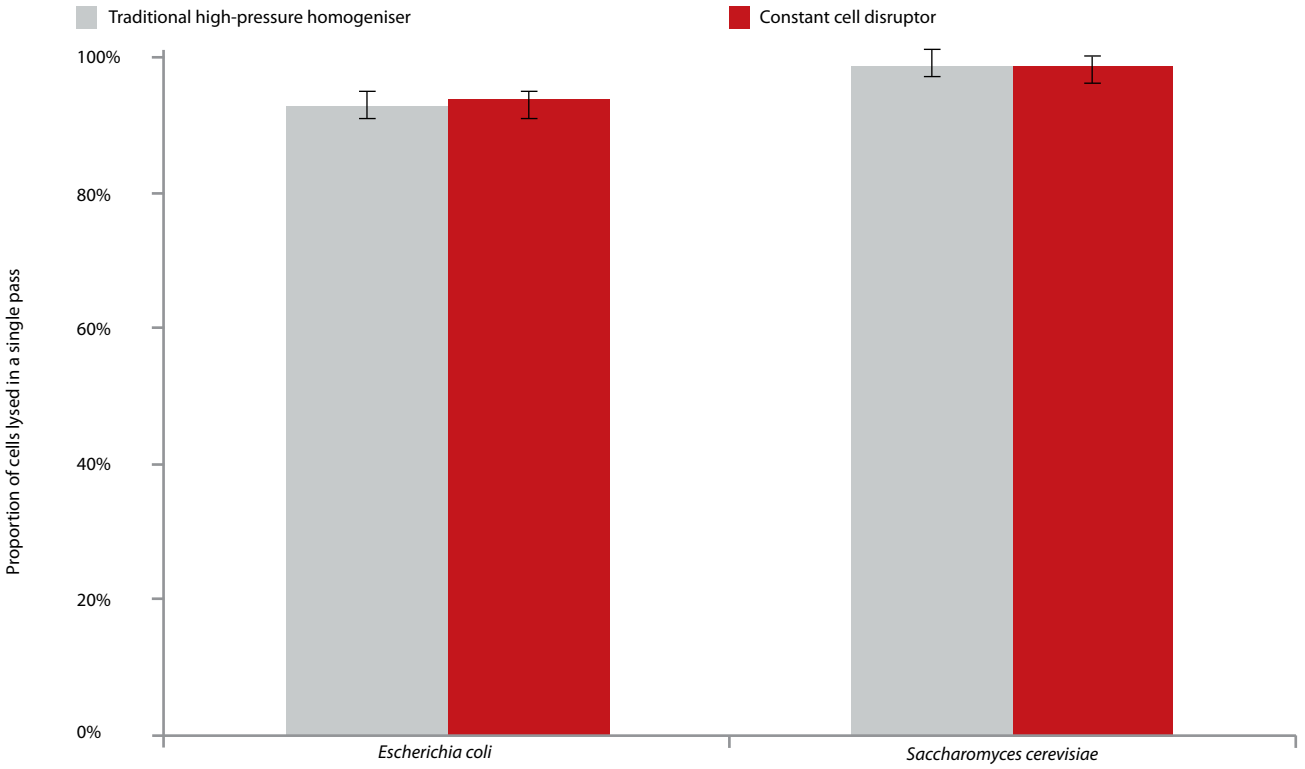


Figure 1: Comparative lysis efficiency of traditional high-pressure cell homogeniser (grey) and a constant cell disruptor (red) when processing *E. coli* at 35kpsi and *S. cerevisiae* at 40kpsi

## Why Categorise by Pressure?

Readers unfamiliar with this technology may wonder why pressure has become the dominant term when referring to mechanical lysis. Simply put, when using a traditional homogeniser, the only aspects that can be altered are the driving pressure of the hydraulic ram and the size of the orifice through which the sample passes. The pressure at the ram can easily be converted to the pressure experienced by the sample through scaling the dimensions of the ram to the piston in the disruption chamber, and so a specific target pressure can easily be set.

The same cannot be said for the orifice size though, as during operation of the most common and traditional model of homogeniser, this size must be almost continuously adjusted to allow sample to flow. However, care must be taken not to open it too far, otherwise all of the generated pressure will disperse. As such, it is the main quantifiable operating principle, namely pressure, which has become synonymous with the ability of a mechanical disruption technique to lyse cells.

Not only this, but users of these machines often base their protocols around using pressures that they know lyse the organism they are working with to the desired level, demonstrating just how ingrained this terminology truly has become within the cellular biology community. It is for this reason that manufacturers of mechanical means of cell disruption typically advertise and sell their machines based on this number.

## Why Does Pressure Cause Cells to Burst?

In order to work towards a better method of quantifying disruption capabilities, one must first understand what causes cells to burst across the pressure differential generated at the orifice of a high-pressure homogeniser. As cells are forced through the orifice, they experience compression forces upon entering and expansion upon exiting. These forces can cause cells to simply burst in ways analogous to compressing or overinflating a balloon and contribute to the overall lysis efficiency.

As well as this, they are also exposed to a high degree of lateral shear while traversing the orifice, as individual cells are dragged across the external surface of the orifice, which – sticking with the analogy of an inflated balloon – is comparable to pinching and pulling a section of the balloon wall, ultimately tearing a small section off and causing the balloon to burst. The combination of these forces, as well as other minor factors, leads to the effect shown in Figure 2 (see page 14), which

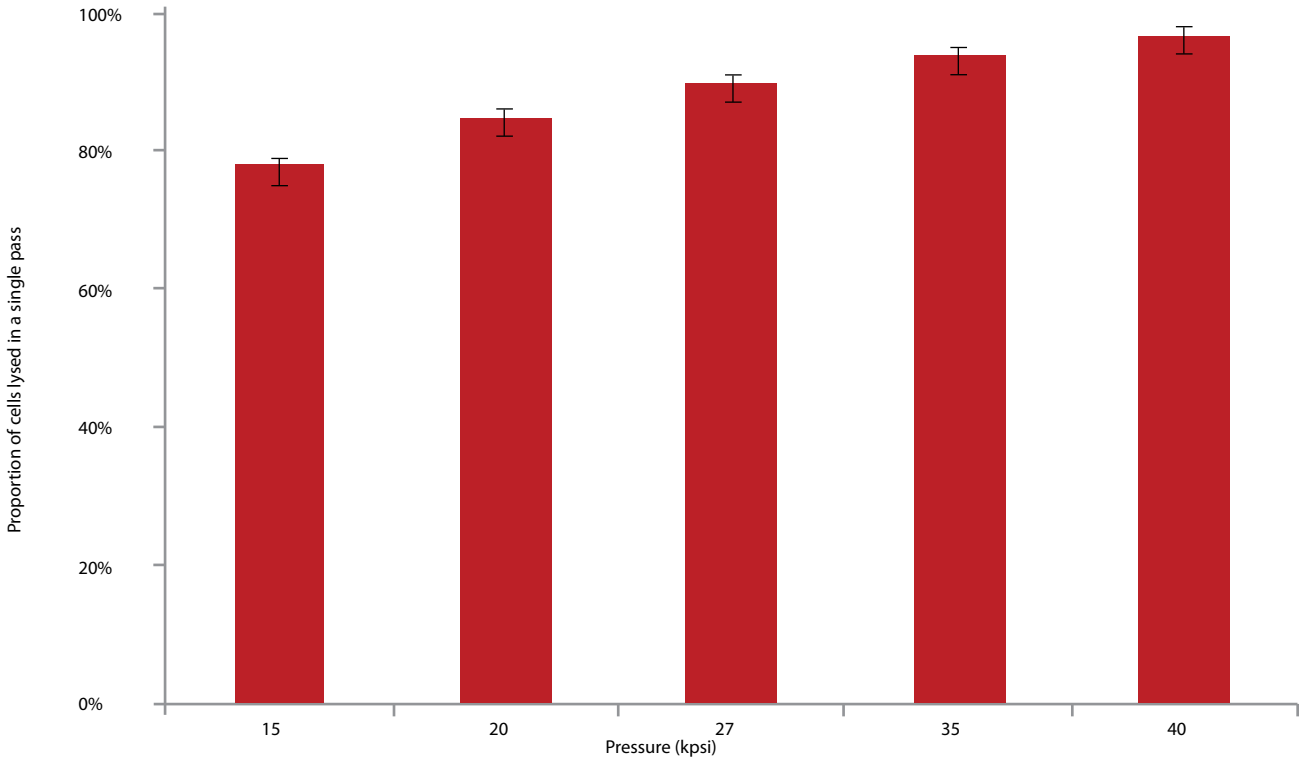


Figure 2: Increase in lysis efficiency of *S. cerevisiae* by a constant cell disruptor system when operated at increasing pressures

demonstrates the increase in lysis efficiency of *Saccharomyces cerevisiae* when processed at increasing pressure.

After the applied pressure, it is the shear force experienced within the orifice that is the easiest to manipulate, as the amount of force also depends on the interactions between cells with both the surface of the orifice and, indeed, other cells in the solution, as well as pressure. As such, it is this aspect of the lysis process that is currently being researched by manufacturers of hydraulic cell disruptor systems.

## Replicating High-Pressure Effects at Low Pressure

It is easy to see why the prospect of achieving the same lysis results at a lower pressure is desirable. Not only does the running cost of the machine (in terms of energy consumption) lessen, but also the degree of wear and tear on it will be reduced as well. This means that machines operating at lower pressures could operate for longer between services and would likely require consumable parts to be replaced less frequently, further reducing operational costs.

The idea is also appealing to manufacturers due to the possibility that production costs would also be lowered which, given the current economic restrains felt by researchers and manufacturers around the globe, could make this technology available to more potential customers. As such, obtaining high-pressure results by coupling low pressures with other optimisations can benefit both customer and supplier.

If operational pressure is to be reduced, then some other factor must be altered in order to compensate for the reduction in force acting on the cells. It has been hypothesised that the amount of shear force experienced by each cell could be increased by reducing the cross-section of the orifice through which the cells are passed. The construction of the Constant Systems Cell Disruptor – which uses constant hydraulic technology – is such that only a single component, the jet – which contains an orifice of fixed size – would need replacing with one with a different diameter orifice. An experiment was therefore carried out processing a sample of *S. cerevisiae* at 20kpsi using smaller orifices, the results of which can be seen in Figure 3 (see page 16).

It can be observed that decreasing the orifice diameter does indeed increase the lysis efficiency with the result that, to within experimental error, an orifice with a diameter 44% of the standard jet gives comparable lysis at 20kpsi to the typical Cell Disruptor configuration at 40kpsi. Similar work has also been carried out using a jet with an orifice dimeter 56% of standard with *E. coli*, again giving comparable outcomes. This demonstrates that pressure need not be the be all and end all of mechanical cell disruption and homogenisation, and that other factors can contribute to the same final conclusion.

Readers may ask why this result has not been shown previously, due to the fact that, as stated earlier, orifice size was the only aspect apart from pressure that could be altered in traditional high-pressure cell homogenisers. The reason is linked to the processing time required by the different models of machine. For example, using a traditional high-pressure cell homogeniser, it could take up to 20 minutes to process approximately 35mL of sample at a set pressure with the orifice starting small and being gradually increased. If the orifice was closed further, then it could be possible that lysis results would be replicated at a lower pressure, but it would take even longer to process.

However, as is often the case when working with biological samples, this equates to more time outside of a refrigerated

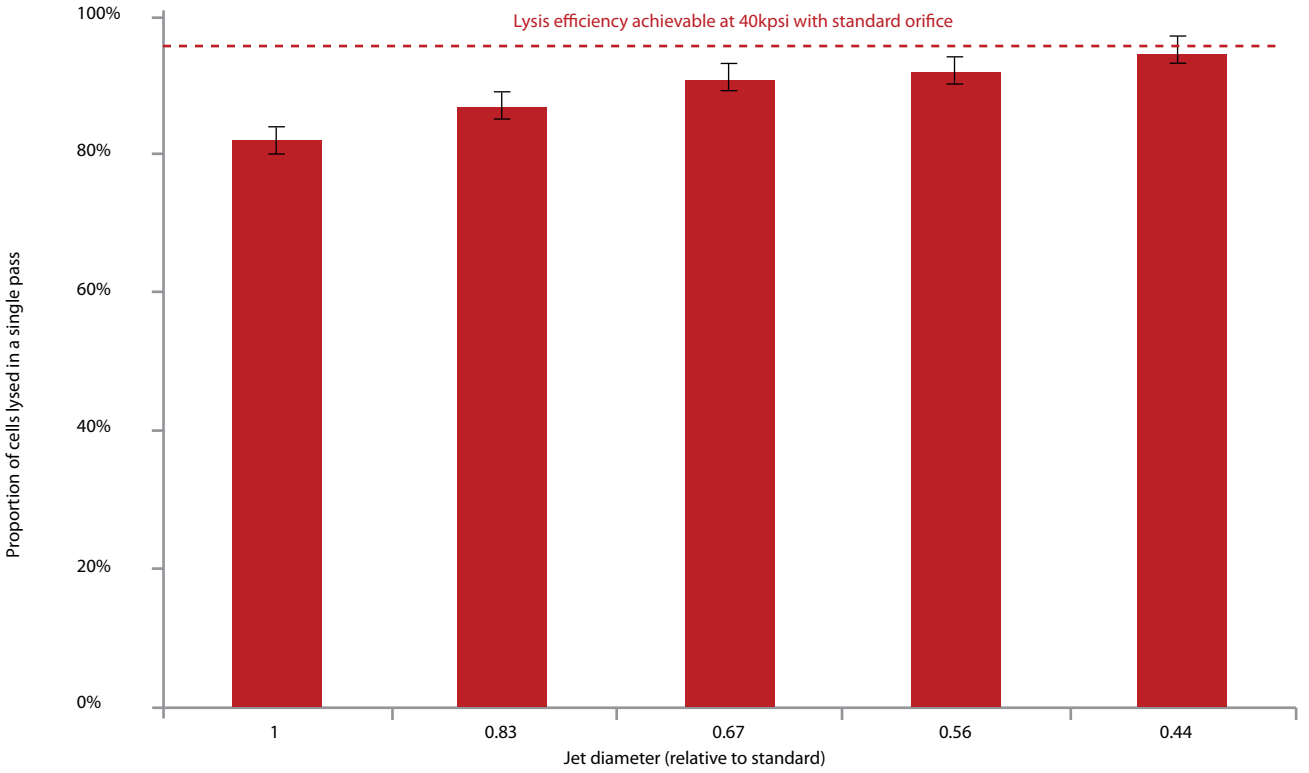


Figure 3: Demonstration of lysis efficiencies achievable at 20kpsi using different orifice (jet) diameters

environment, which could damage the function and yield of the desired biomolecule. Conversely, with the latest models of cell disruptors capable of processing the same 35mL sample in as little as 10 to 60 seconds (or even faster using a newly developed high flow rate system, which can process up to 150 litres an hour), reducing the orifice size no longer has to have the same impact as it would in traditional high-pressure cell homogenisers and does, in fact, only increase the processing time by approximately two seconds per 10mL of sample.

## Multi-Variable Approach

As detailed above, it has been shown that lysis efficiency need not be determined solely by the pressure at which a sample is processed, and that there are also other aspects which can be adjusted in order to achieve lysis results previously associated with high pressures but at much lower values. One such factor is the orifice diameter, as demonstrated, and ongoing research has also unveiled other factors that can affect the lysis efficiency of samples.

Examples include further manipulating jets by introducing multiple orifices, or altering the path the sample takes immediately after processing – thus affecting the friction and shear forces experienced by cells not lysed within the jet itself. Eventually, this research could lead to a multi-variable approach to cell lysis, with results and capabilities no longer governed by pressures but, instead, on the maximum lysis achievable under different conditions which, at the end of the day, is the main concern for most users.

Further work is also being carried out to look at the effect that these factors have on lysis of other, tough-to-break samples such as Gram-positive bacteria. As such, it is thought that there are combinations of components contributing to overall lysis efficiency that can be optimised for different groups or families of organisms. This would then allow lysis to be carried out at lower pressures than previously thought for many different organisms, which would not only cut operational costs as previously described, but would also reduce energy consumption while maintaining the same lysis results as long-established and accepted technologies.

Reference
1. Research conducted by Constant Systems

## About the author

Dr Matthew Lougher is the Principal Scientist at Constant Systems, where he manages the on-site laboratory in order to aid in the development of new products, as well as provide support to customers and the sales and marketing team. He has a Master's degree in Physics, plus another Master's and PhD in Mathematical Biology and Biophysical Chemistry, all from the University of Warwick. During his PhD, he worked towards solving the functional and structural properties of a transcriptional repressor identified as part of an antibiotic resistance pathway.
Email: matt.lougher@constantsystems.com