

Modification of a French pressure cell to improve microbial cell disruption

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Abstract A procedure for modification of the valve stem of a 40 K French pressure cell is described. The modification should be done by a machinist and requires a metalworking lathe. After modification of the valve stem, a torlon 4203 plastic ball is used between the valve stem and valve seat to control the pressure within the cell. The torlon plastic ball is a key component needed to obtain the high pressures required for efficient disruption of microbial cells.

Keywords French press · Bacterial lysis · Photosynthetic bacteria

Results and discussion

The French pressure cell for disruption of microbial cells was invented by Stacey French in the 1950s (French and Milner, 1955). Since that time, there have been suggested modifications to both the press and the cell in attempts to improve on the original design (Walters and Stahly 1968; Vanderheiden et al. 1970; Strauss 2003).

The fundamental principle underpinning the high efficiency of the French pressure cell relates to subjecting a suspension of microbial cells to a high pressure (>10,000–

20,000 psi), and then abruptly reducing the pressure by using a valve to dropwise bleed off material from disrupted cells, which is collected for further use.

Older models of the French pressure cell (such as the 20 K cell; see below) have a plastic ball that is located between the valve stem and the valve seat, to control the size of the valve orifice, and hence, the pressure on the suspension of microbial cells. Although the plastic ball must be replaced frequently, in our hands these cells have worked well for decades; on the other hand, we do not know of a source for replacement plastic balls. However, Thermo Fisher Scientific (<http://www.thermofisher.com/global/en/home.asp>), currently the source of French pressure cells, no longer offers the 20 K cell with a plastic ball-type of valve closure. Instead, a 40 K cell that has a metal-on-metal needle valve is available. We have found that the metal-on-metal needle valve does not work well because after processing a few samples, imperfections in the needle of the valve stem or the valve seat develop and interfere with valve closure, and so it is not possible to obtain the >10,000–20,000 psi needed for efficient microbial cell disruption.

An alteration of the 40 K-type of valve was suggested by Walters and Stahly (1968), in which the valve stem was ground flat by 9/32 inch to yield a modified valve stem. This modified valve stem was used to screw in and force a 3/16 inch diameter stainless steel ball bearing to alter the conformation of the valve stem and seat. After these changes, a 3/16 inch diameter nylon ball was placed between the valve stem and seat, to provide a flexible valve closure and maintain pressures of 10,000–20,000 psi on microbial cell samples, as disrupted cells were bled off by manipulation of the valve (Walters and Stahly 1968).

There may have been some changes in the 40 K-type of valve in the last 40 years. Regardless, we found that the

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Walters and Stahly (1968) method did not work well, using a 40 K cell that was purchased in 2003. The problem was that in our hands, the use of a 3/16 inch diameter nylon ball did not allow closure of the valve to reach pressures needed for efficient breakage of a variety of microbial cells (i.e., above 10,000 psi).

Since the Walters and Stahly (1968) publication, in which the authors recommended the use of a nylon ball to modulate the French pressure cell valve closure, many new plastics have been devised. In an attempt to find an alternative 3/16 inch diameter ball that would allow use of our 40 K French pressure cell modified similarly to the Walters and Stahly (1968) approach, we investigated the relatively new plastic torlon (Engineering Laboratories Inc.; <http://www.plasticballs.com/torlon.htm>). Torlon is a very strong plastic and comes in several varieties. We chose 3/16 inch diameter balls of the torlon grade 4203 that has a relatively high “tensile elongation at break” value, in the hope that this would allow flexibility to fill imperfections in the metal valve stem and seat. Furthermore, instead of grinding the valve stem flat, we used a lathe to remove the tapered portion of the valve stem, and then a drill bit to produce a cavity at the tip of the valve stem (Fig. 1A–C). This cavity facilitates the centering of the plastic ball directly over the orifice of the valve seat.

The modified French pressure cell equipped with a torlon ball was tested by disruption of *Rhodobacter sphaeroides* (a purple, non-sulfur photosynthetic bacterium) cells suspended in a buffer of 10 mM Tris–HCl pH 8, 150 mM NaCl,

1 mM MgCl₂, ~10 µg/ml of DNase I (Sigma). We were delighted to find that the use of torlon grade 4203 3/16 inch diameter balls results in very smooth operation of the modified 40 K cell up to a pressure of 20,000 psi. Higher pressures may be obtained, but we did not attempt to go above 20,000 psi. Clogging of the valve was minimized by: (1) using a Dounce homogenizer to reduce the number of cell clumps, (2) the inclusion of ~10 µg/ml of DNase I and 1 mM MgCl₂ to the cell suspension, (3) passing the sample through the French pressure cell a second time. The efficiency of breakage was equal to the efficiency of a 20 K cell equipped with a plastic ball.

After use in the French pressure cell at 20,000 psi, the 3/16 inch diameter torlon 4203 ball appears by eye sight to be as it was before use except for a circular groove, apparently from the valve seat (Fig. 1D, right). This minimal amount of deformation needed to obtain 20,000 psi is in great contrast to the extreme deformation of the 3/16 inch diameter nylon ball used to obtain a maximum of 10,000 psi (Fig. 1D, left). Nevertheless, we suggest that a new torlon ball be used each time the valve stem is unscrewed far enough to allow a change in the orientation of the ball.

In summary, it appears that modification of a French pressure cell along the lines of the procedure described by Walters and Stahly (1968), modified by drilling a cavity at the end of the valve stem along with the simple change from a nylon to torlon 4203 3/16 inch ball, results in a change from a non-functional apparatus to an efficient apparatus. The specific modifications are:

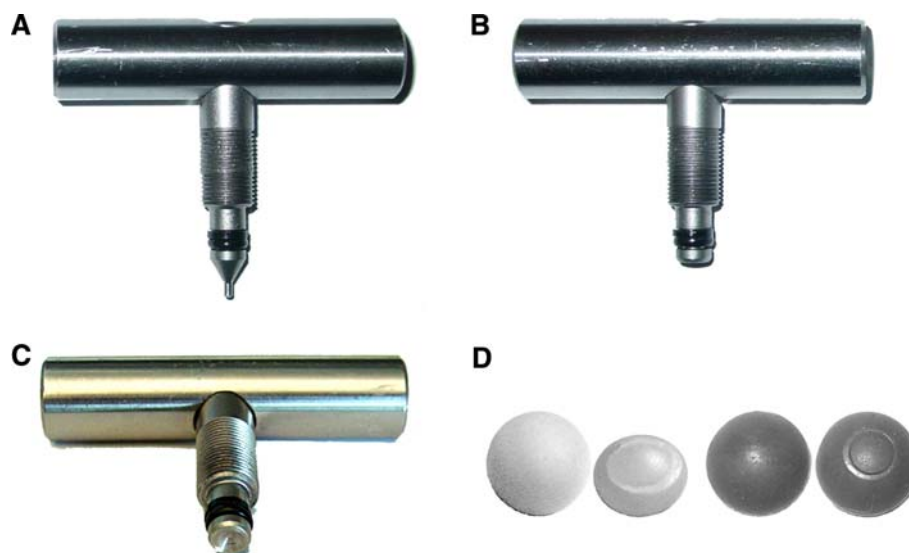


Fig. 1 Valve stems and plastic balls. **A** The 40 K valve stem provided by the manufacturer showing the needle tip. **B** The 40 K valve stem modified by removal of the tip and drilling a depression at the end, as described in the text. **C** An oblique view of the 40 K valve stem modified as described in the text, showing the cavity drilled into the

end. **D** *Left pair* appearance of a 3/16 inch nylon ball before (*left*) and after (*right*) use in a modified French pressure cell to reach a maximum of ~10,000 psi; *Right pair* the appearance of a 3/16 inch torlon 4203 ball before (*left*) and after (*right*) use in a modified French pressure cell to reach ~20,000 psi

- (1) Remove the tip from the valve stem of a 40 K cell by turning down in a lathe until the thickness of the tip flange is approximately 0.1 inch (measured from the edge of the O-ring slot; see Fig. 1A–C).
- (2) Use a 1/16 inch centering bit to drill a small pilot hole in the end of the valve stem.
- (3) Use a 3/16 inch (60° point angle) centering bit to drill a tapered cup approximately 7/32 inch in diameter in the end of the valve stem. The cup should be deep enough so that a 3/16 inch torlon ball does not wobble, but also leave as much metal as possible so as not to overly weaken the valve stem itself (See Fig. 1C).
- (4) Thoroughly clean any metal shavings from the valve stem by washing copiously with water and drying

with compressed air, to ensure the absence of shavings in the O-ring area.

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