Total Organic Carbon Analysis of Residual Contaminants in Bioreactor Equipment: A Cleaning Verification Study using Surface Swabbing

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1. Introduction

Biopharmaceuticals are often produced using bioreactors. A variety of ingredients, processing materials and cleaning agents are used during the production process. These substances can remain on equipment surfaces and contaminate the next manufactured product (1). Cross contamination can negatively impact the safety and efficacy of a biopharmaceutical product. Therefore, bioreactors must be meticulously cleaned to remove residual contaminants and avoid cross contamination.

Cleaning validation is necessary to ensure residual contaminants are reduced to the lowest acceptable limits. Bacterial cells represent the "worst-case scenario" in manufacturing equipment cleaning because they are composed of insoluble organic contaminants (2). Total organic carbon (TOC) analysis is a fast, highly sensitive, and inexpensive analytical technique commonly used to evaluate the approximate level of organic contamination (3).

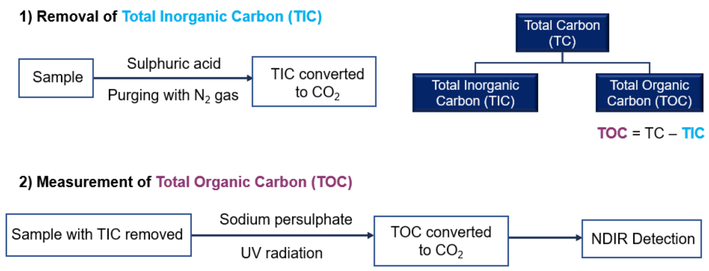

Figure 1. TOC analyser principle of operation schematic.

2. Aim

To assess the effectiveness of a cleaning procedure in the removal of contaminants from bioreactor equipment surfaces by measuring the total organic carbon concentration of swab samples.

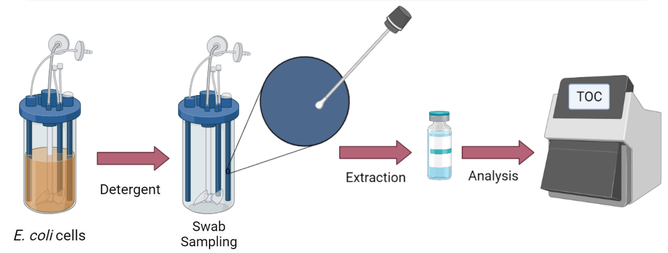

Figure 2. Graphical representation of the method used.

3. Experimental Details

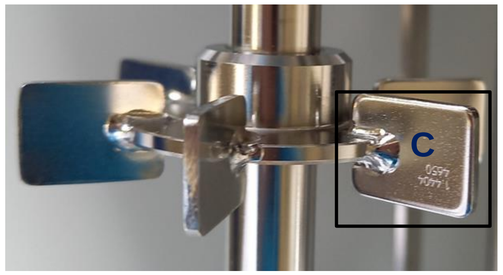

Figure 3. Bioreactor stainless-steel impeller blades (surface area swabbed = 5.60 cm^2).

Three different areas of the bioreactor were selected for swab sampling. These areas included the upper side (**A**) and bottom (**B**) of the glass vessel and the stainless-steel impeller blades (**C**).

The bioreactor was used to obtain a high-density culture of *Escherichia coli* JM109 cells. A 1 % (w/v) enzyme-active anionic detergent solution was used to clean the bioreactor equipment surfaces. The swab sampling of the upper side of the glass vessel and the stainless-steel blades was performed in triplicate. Each swab head was placed into a TOC vial with 30.0 mL of ultrapure water. The adsorbed contaminants of each swab head were desorbed using a vortex mixer. The TOC concentration of each vial was measured using a Multi N/C® Pharma UV TOC analyser. The TOC analyser was calibrated using a series of sucrose standard solutions. Range: 0-100 mg/L, r =0.99911, LOD = 3.46 mg/L, LOQ = 12.89 mg/L

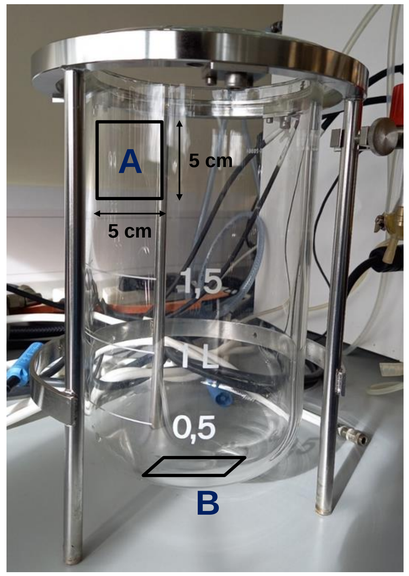

Figure 4. Bioreactor glass vessel labelled with swab sampling areas.

4. Results

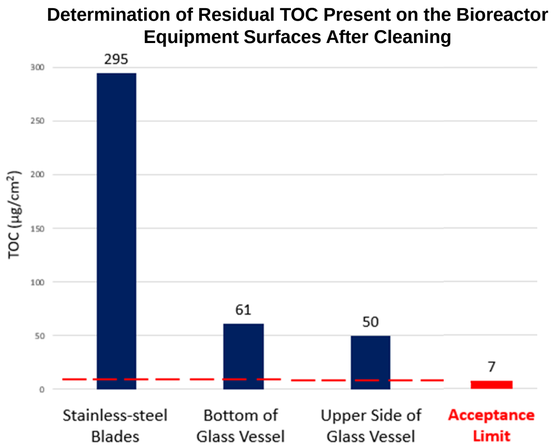

Figure 5. Bar chart showing the quantities of organic contaminants present on the bioreactor surfaces.

The total surface area of the bioreactor was calculated to be 1489 cm^2. The acceptance limit for the quantity of residual contaminants present on the surfaces of the bioreactor was calculated to be 7 µg/cm^2. The TOC values obtained from this study were all significantly above this threshold. Strikingly, the stainless-steel blades were found to have the highest levels of contamination with a value of 295 µg/cm^2.

5. Discussion

An investigation was conducted to identify the source of the high levels of contamination. Despite the widespread use of the chosen enzyme-active anionic detergent, there are currently no papers published describing its use in cleaning verification studies. It was noted that the TOC vials used in the study were composed of borosilicate glass — the same type of glass as the bioreactor vessel. Therefore, a scaled-down experiment was conducted using these vials in place of the bioreactor to determine whether the enzyme-active anionic detergent was the source of organic contamination. This study was based on the principle that the TOC concentration of the ultra pure water within these vials would be solely due to residual detergent. The results of this study verified that the detergent had remained on the equipment, even after the rinsing procedures had been performed.

6. Conclusion

It was determined that the cleaning process was not effective in the removal of organic residues from the surfaces of the bioreactor. The high quantities of organic contaminants present on the equipment surfaces were attributed to the enzyme-active anionic detergent that was used during the cleaning procedure. This finding suggests that the use of this detergent in bioreactor equipment cleaning could potentially result in the contamination of the subsequent manufactured product, negatively affecting its safety and quality. In conclusion, the results of this study highlight the importance of cleaning verification, small-scale experimentation, and detergent selection.

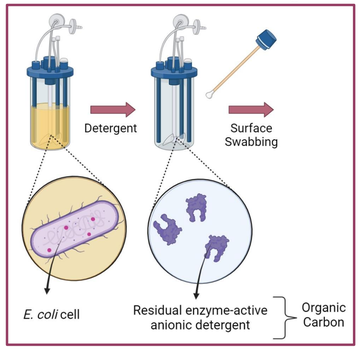

Figure 6. Image showing the residual detergent present on the bioreactor.

7. References

(1) Sajid, S.S., Arayne, M.S. and Sultana, N. Validation of cleaning of pharmaceutical manufacturing equipment, illustrated by determination of cephradine residues. *Analytical Methods*, 2010, **2**(4), pp.397-401.

(2) Strege, M.A., Stinger, T.L., Farrell, B.T. and Lagu, A.L. Total organic carbon analysis of swab samples for the cleaning validation of bioprocess fermentation equipment. *Biopharm International*, 1996, **9**(4), pp.42–45.

(3) Juarbe, N. and Strege, M. Validation of a method for the determination of polysorbate 20 residue for the support of the cleaning of pharmaceutical vial closures. *Journal of Validation Technology*, 2007, **13**(2), pp.114–123.