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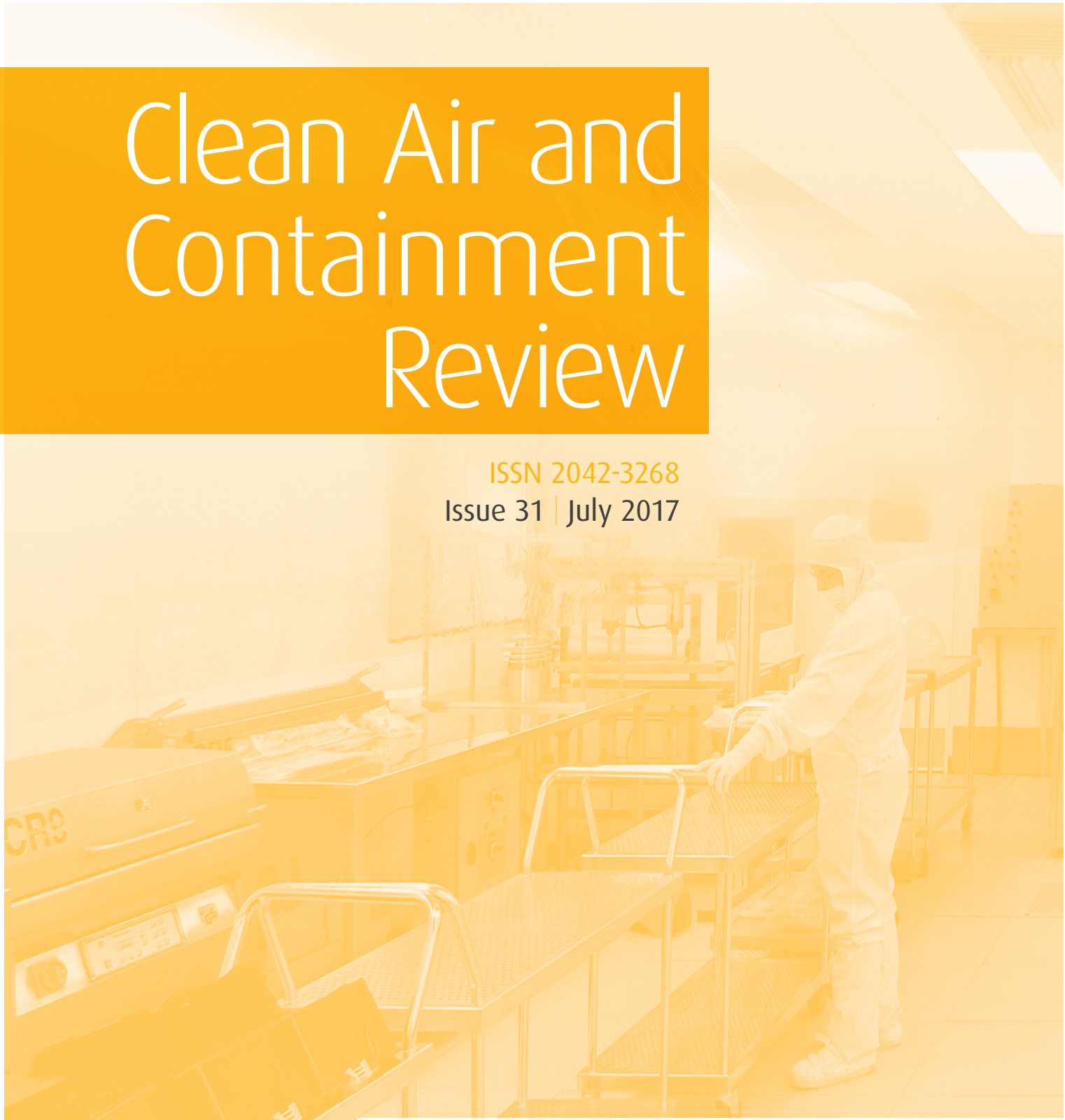

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Clean Air and Containment Review is a quarterly journal aimed at users, specifiers, designers, manufacturers, installers and testers of clean air and containment equipment. It publishes articles of topical, technical and historical interest, updates on standards and regulations, news, views and information on relevant events, especially training.

Enzyme indicators (EIs) – an advanced replacement for biological indicators (BIs) in the qualification of vapour phase hydrogen peroxide bio-decontamination

Tim Coles

Abstract

This paper introduces the newly available enzyme indicators which have the potential to speed up cycle development and cycle verification, in the hydrogen peroxide vapour bio-decontamination process. The recently published work of scientists at PHE Porton Down is reviewed, and the advantages of enzyme indicators over conventional biological indicators are put forward.

Introduction

The use of vapour phase hydrogen peroxide in the bio-decontamination of a variety of enclosures, including pharmaceutical isolators, has become commonplace since the introduction of the first production gas generators by Amsco, now Steris, in the mid-nineteen eighties. Trademarked "VHP" by Steris, the process is more accurately described by the term "micro-condensed hydrogen peroxide" (MCHP) as explained by the author in various earlier papers.[1, 2, 3, 4] However described, the process is almost invariably qualified by using biological indicators (BIs) as the measure of efficacy. BIs may take various forms but the commonly-used version has spores of *Geobacillus stearothermophilus* deposited onto a stainless steel coupon, placed inside a Tyvek® envelope. The growth or non-growth of BIs subjected to MCHP is used, via various techniques, to develop and then to confirm the acceptability of the MCHP cycle. It has become the norm to specify log 6 reduction of BIs as proof of bio-decontamination, although recent work suggests that this is unnecessarily rigorous.[5] Nevertheless, such BIs do present a robust and convincing challenge to the MCHP process. They do, however, suffer from three significant disadvantages:

1. It takes up to 14 days, to get a result from BIs following exposure.
2. The physical nature of high log BIs leads to a certain percentage occurrence of "rogues" which will not be deactivated even after long exposure to MCHP.
3. Such BIs exhibit considerable variability, both within and between batches.

In practice, rather than wait the full 14 days, most operators are content with 7-day incubation of BIs. Even so, this inevitably makes the process of MCHP cycle development slow and laborious.

The issue of rogue BIs is tackled in two ways. The first is to use triplicate BIs at each test site. It can be shown that if two out of three BIs at one site are killed, then log 6 has been achieved. Alternatively, a rogue policy can be declared at the start of studies allowing for example, up to 5% of positive growth BIs to be counted as rogues. The former solution is expensive; the latter is open to debate.

The variability of BIs can be considerable, with the declared D-value of one batch being as much as 100% different from another batch. This factor casts doubt on the capability of BIs to demonstrate the repeatability of a series of MCHP cycles.

Overall, failure to understand the MCHP process properly, combined with the disadvantages presented by standard BIs, has made the development of MCHP cycles and subsequent validation and re-validation, a lengthy and a costly process. It is clear that a reduction in the time, cost and complexity of establishing an MCHP process would be very welcome in the industry.

Thermophile Bacteria

It is well-known that some species of bacteria, the so-called "thermophiles" can tolerate extraordinarily high temperature environments. Such bacteria may be found in hydrothermal vents and hot springs, thriving in water at 120°C. Scientists at Public Health England (PHE) Porton Down, UK, have studied these organisms for a number of years. One aspect of their research concerned the qualification of methods used to deactivate extremely robust infectious agents, such as the abnormal prion protein which is the causative agent of Creutzfeldt Jakob Disease (CJD) in humans and Bovine Spongiform Encephalitis (BSE or 'mad cow disease') in cattle. A spin-off from the work produced an enzyme designated *Thermostable Adenylate Kinase* (tAK) derived originally from the thermophile bacterium *Sulfolobus acidocaldarius*. As the name indicates, this enzyme, as a result of its tightly-packed protein structure, is stable at very high temperatures. It was also found to be stable when subjected to other methods of bio-decontamination, and this included the MCHP process.

This discovery prompted further research which then revealed a very useful property possessed by tAK. It is progressively, and predictably, deactivated over time when exposed to bio-decontamination processes. The level of activity of the enzyme can be measured relatively easily, using a luciferin-luciferase reaction. This, therefore, clearly offered the potential for a direct and immediate measure of the efficacy of any given MCHP bio-decontamination process. Such a measure would not be subject to the sort of disadvantages inherent in the use of BIs.

McLeod et al.

Recognising the potential for the rapid and accurate evaluation of bio-decontamination processes, scientists at Public Health England (PHE) embarked on a direct comparison of the performance of classical BIs and tAK based enzyme indicators (EIs). The EIs consist of a polyester substrate carrying a small quantity of the active enzyme, mounted in a plastic carrier. The EI is fairly similar in size and shape to a classical BI, and is presented inside an isolator or other enclosure in much the same

way. This work has been described by McLeod et al[6] and it is that draft paper which forms the basis of this review.

Describing the work briefly, BIs and EIs were exposed to MCHP cycles in a two-glove flexible film isolator using a commercial hydrogen peroxide system. In this study, three BIs and four EIs were placed together on the base tray of the isolator and exposed to VHP for cycle times of 0, 2, 4, 6, 8, 10, 12 and 14 minutes. Positive controls (non-treated) and negative controls (reagent/growth media) were also included for both BIs and EIs. This process was carried out on six independent occasions. Following exposure, the BIs were suspended, serially diluted and placed on agar plates ("plated out"). The plates were incubated for 48 hours before enumeration of total viable counts (TVC) to establish the log reduction in each case. The EIs were placed in tubes containing ATP-Luciferase solution and then ADP solution was added. The residual activity of each EI was measured as relative light units (RLU) in a luminometer.

The results were used first to show a clear correlation between the RLU output of each EI and the time of exposure to VHP. The activity of the enzyme declined logarithmically with time of exposure.

The results were then used to show the correlation between the log reduction of BIs and the RLU of the EIs. The BI log reduction and decreasing EI RLU values were plotted alongside each other and demonstrated a close relationship between the two. This relationship is then used to plot an equation by which EI RLU can be used to predict BI log reduction values (plus and minus the 95% confidence level).

In the course of the study, the great variability of response from the BIs was confirmed, in marked contrast to a more stable response from the EIs. Within cycles, tAK indicators showed the average percentage coefficient of variance between readings for each time point to be 19%, but for BIs it was 96%. The between cycle variance was shown to be 26% for tAK and a rather large 179% for BIs. This is an indication of the overall unpredictability of BIs, which places some degree of doubt on the robustness and reliability of MCHP cycles developed using BIs.

The authors conclude that, under the conditions of the study, there is a strong correlation between tAK activity and BI log reduction.

Conclusion

From the work of McLeod et al, it would seem that enzyme indicators, based on the enzyme tAK, wholly outperform classical biological indicators in the evaluation of MCHP (VHP) bio-decontamination cycles.

The results for EIs can be obtained in a matter of minutes from harvesting, against a minimum seven days for BIs.

EIs are shown to have a much more predictable response to the MCHP process than BIs, with less variance both within and between cycles. Indeed, the observed variance with EIs may well truly reflect the intrinsic variance within the MCHP process itself in a way that is not possible with BIs. EIs are not prone to an underlying and unknown "rogue" rate.

Whilst the current range of luminometers display the results for EIs in RLUs (Relative Light Units), it should only take some relatively simple software changes to display the results in terms of equivalent log reduction, a measure of performance familiar to current MCHP operators.

The cost of each EI is thought to be significantly more than that of a BI. However, since triplicate BIs could potentially be replaced with a smaller number of EIs, subject to validation in any given MCHP system, the consumable cost may not be increased overall.

A luminometer will also be required, either a simple dual tube manual reading device or an automated multi-tube reading machine. These have significant cost; however, the time saving which the tAK-based EI can offer, may well pay back the cost of the reader quite quickly.

It is suggested that MCHP cycle development and re-qualification based on good-quality chemical indicators (CIs) and enzyme indicators (EIs), may present a more predictable, and thus more robust, process, and a significant saving in time. All of which potentially represents an increase in quality, combined with a reduction in costs. Such a combination is surely a compelling attraction for the industry.

Protak Scientific is the global exclusive licensee (from Public Health England) for the commercialisation and development of thermostable Adenylate Kinase for verification of decontaminations within chambers and open volumes.

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