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An evaluation of the efficacy of Aqualox for microbiological control of industrial cooling tower systems

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Summary: A comprehensive sampling protocol was employed to evaluate the efficacy of Aqualox, a biocide based on electrochemically activated water, against legionellae and heterotrophic bacteria in two industrial cooling tower systems. Both of the towers in the study remained free from evidence of *Legionella* spp. contamination throughout a five-month evaluation period, despite the previously demonstrated presence of legionellae in one of the test towers, and in two other towers on the same site, at levels well in excess of UK Health and Safety Commission (HSC) Approved Code of Practice and Guidance (ACOP) upper action limits. Levels of heterotrophic bacteria were controlled below 10^4 cfu/mL in both towers throughout most of the trial. Results also provided indirect evidence of significant activity against biofilm bacteria, with biofilm removal beginning almost immediately after commissioning of the Aqualox treatment systems. The results were particularly encouraging as the two towers studied had a long history of poor microbiological control using conventional bromine-based biocide products. Significant differences were observed between laboratory measurements of total viable counts on frequent liquid samples and those obtained from dip slides following HSC recommendations.

Keywords: Aqualox; Sterilox; *Legionella*; cooling towers; mixed oxidants.

Introduction

Legionnaires' disease, caused by the bacterium *Legionella pneumophila*, is a severe form of pneumonia.[1] In several hospital outbreaks, patients are thought to have been infected through exposure to contaminated aerosols generated by cooling towers.[2–5] Legionellae resident within biofilms are a particular problem in cooling tower systems,[6] and can prove difficult to eradicate by conventional means. LeChevalier *et al*.[7] reported that biofilm bacteria can be up to 3000 times more resistant to standard chlorine disinfectants than unattached cells. Bradford and Baker[8] and Crayton *et al*.[9] have demonstrated that mixed oxidants, in contrast, exert dramatic activity against biofilms.

Aqualox is an aqueous solution of mixed oxidants, generated on-site by electrolysis of a dilute saline solution in a proprietary electrochemical cell, using the same technology as that employed to produce the Sterilox fluid utilized in medical applications. Aqualox generators are supplied with a concentrated

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saline solution which is diluted, normally using potable mains water, before being fed to the electrochemical cell.

Aqualox has an extremely low toxicity, together with a well-documented broad-spectrum biocidal activity.[10–14] Zinkevich *et al.*[14] reported that the mode of action involves destruction of chromosomal and plasmid DNA, RNA and proteins, and preliminary studies[10,15] indicate that this provides good penetration and control of bacterial biofilms. Aqualox has a history of almost three years usage in cooling tower treatment, and highly satisfactory results have been obtained to date using UK Health and Safety Commission (HSC) Approved Code of Practice and Guidance (ACOP)[6] monitoring protocols with continuous negative test results for *Legionella* spp. Aqualox has demonstrated rapid biocidal efficacy against legionella bacteria in the European EN 13623 standard suspension test. A comprehensive sampling protocol was therefore employed quantitatively to evaluate the efficacy of Aqualox against *Legionella* spp. and heterotrophic bacteria in two industrial cooling tower systems.

HSC action limits for cooling towers define total viable counts (TVCs) of less than 10^4/mL and counts of *Legionella* spp. of less than 100/L as indicators that a cooling system is under microbiological control. TVCs between 10^4 and 10^5/mL and/or legionella counts of up to 10^3/L require a review of control measures and TVCs greater than 10^5/mL and/or legionella counts in excess of 10^3/L require immediate corrective action. HSC also states that oxidant reserves should not consistently be allowed to exceed 2 ppm in order to avoid corrosion problems, though levels in excess of this figure are permitted in exceptional circumstances. HSC recommendations for microbiological monitoring specify weekly analysis of single samples for TVCs in cooling water by dipslide, with make-up water monitored quarterly, together with quarterly analysis of single samples of cooling water for *Legionella* spp., using a United Kingdom Accreditation Service (UKAS) accredited laboratory.[6]

These parameters were therefore used as benchmarks in the present study, except that this study significantly increased the scope and frequency of microbiological analysis. Bentham[16] reported that the variability of legionella counts in cooling tower systems is so high that risk assessments cannot be reliably founded on single or infrequent analyses. The concentration of *Legionella* spp. in cooling tower systems may vary by as much as three orders of magnitude within 10 min.[17] Furthermore, although weekly TVC analyses of cooling waters are specified by HSC as a means of assessing microbiological control, a number of studies has shown that there is no evidence of any correlation between TVC and the presence of legionellae in cooling tower systems.[18–20]

Cooling towers comprise complex ecological niches, and even different towers of identical design on a single site will generally behave quite differently microbiologically. This was the case on the test site, as shown by historical HSC ACOP records, which were carefully considered in the design of this study. The towers chosen for the study were selected because they had a long history of poor microbiological control using bromine, but data from other on-site towers of identical design, albeit under similar control, was insufficiently comparable to justify use of those towers as controls for the test towers, and it was therefore considered that pre-trial monitoring of the test towers would provide the most appropriate control data under these circumstances.

Materials and methods

Test site and towers

Both of the test towers were located on a major UK manufacturing site. Prior to the Aqualox study, both had been treated with bromine, supplied as bromodichlorohydantoin, at levels typically in the range 0.5 to 2 ppm, but with periodic episodes where dosing reached the 3–5 ppm range, with the potential for corrosion above acceptable industry standards.

Tower 1 provided direct cooling to a refrigeration compressor plant and consisted of a spray tower of metal construction with no mechanically assisted airflow. Make-up water was from a potable mains supply.

Tower 2 provided direct cooling to a plate heat exchanger and comprised a pair of induced draught cooling towers of fibreglass construction. Make-up water for this tower came from a low-grade non-potable source.

Treatment

Towers 1 and 2 were treated using the Aqualox system (Sterilox Technologies International Ltd, Abingdon, UK). Standard Aqualox generators were fitted to each tower, with the dosing controlled via Sentek in-line redox probes, with a redox set point of 580 mV. Potable mains water was fed to the Tower 1 Aqualox generator for Saline dilution, but in the case of Tower 2, initially only non-potable make-up water with a hardness of 120–360 ppm as $CaCO_3$ was available for this purpose. This caused operational problems with the Aqualox system as a result of physical fouling of the electrode chambers. A new potable mains supply to the Aqualox generator was therefore installed for saline dilution at the beginning of March 2001, though make-up water for the tower continued to be supplied from the original, non-potable, source.

Towers 1 and 2 were drained and disinfected, though not manually cleaned, in August 2000, more than four months prior to commissioning of the Aqualox systems. Pre-study monitoring of the towers commenced on 1 November 2000 (day 1), 58 days prior to commissioning of Aqualox systems, whilst the towers were still under bromine treatment. Aqualox systems were commissioned on 28 December 2000 (day 58), and monitoring continued until 25 May 2001 (day 206) when the towers were again cleaned down and chlorinated to HSC recommendations.

Throughout the trial, in addition to the bromine or Aqualox treatments, each tower was also dosed on the 1st of each month with 50 ppm active ingredient of a non-oxidizing isothiazolone biocide, maintaining this concentration for a minimum of 4 h, in accordance with HSC ACOP recommendations.

Microbiological and chemical analyses

Towers were sampled weekly during the pre-study monitoring period, and twice weekly thereafter by taking 1 L volumes from the sumps into sterile vessels containing sufficient sodium thiosulphate to bring the final concentration to 1% (v/v). Separate 1 L samples were taken for *Legionella* spp. and TVC analyses, and this sampling procedure was repeated after 10 min, providing duplicate samples for both *Legionella* and TVC analysis. Samples were maintained at 4°C in transit to the laboratory and delivered there on the day of collection. All microbiological analyses of water samples were performed by a UKAS-accredited laboratory participating in the Public Health Laboratory Service Water Microbiology External Quality Assessment Scheme. Legionella analyses were performed using the membrane filtration method specified in BS 6068-4.12:1998 with a lower limit of detection of 100 cfu/L, and *L. pneumophila* positives were serotyped. Heterotrophic TVC analyses were performed by pour plating appropriate dilutions to yeast extract agar (Oxoid) and incubating at 30°C for three days.

Samples of cooling water for chemical analysis were also collected periodically, and the oxidant reserve was determined by the N,N-diethyl-p-phenylene-diamine (DPD) method.[21]

Tower 2 make-up water

From day 94 of the trial onwards, single samples of the non-potable water that supplied the Tower 2 Aqualox generator system up until day 121, and that provided make-up water to the tower throughout the trial, were also taken weekly. These were analysed for TVC and *Legionella* spp., using the above methodology.

Dip-slide methodology

Single dip-slide samples (OXOID, DS147A) for both towers were taken twice weekly from the sumps following manufacturers instructions. All dipslides were incubated for 48 h at 30°C.

Results

Analysis for Legionella *spp.*

L. pneumophila serogroup 2–14 was isolated from Tower 2 at a level of 150 cfu/L two weeks prior to the start of the pre-study evaluation, during the course of routine HSC ACOP monitoring, whilst the tower was still under bromine treatment. HSC ACOP monitoring also detected *Legionella* spp., at a level of 1900 cfu/L, and *L. pneumophila* serogroup 2–14 at a level of 4000 cfu/L in two other towers on the same site, in the first and second week of the Aqualox pre-study monitoring period respectively. No legionella bacteria were detected in any of the Aqualox-treated towers during the five-month period of this study.

TVCs

Figures 1 and 2 show the duplicate TVC results from both towers as the average of the two measurements on each day. The higher and lower of the two individual values are also indicated by the upper and lower bars, respectively.

Throughout January, Tower 1 TVCs varied widely and considerable differences were observed between two samples taken 10 min apart (Figure 1). Three samples showed counts in excess of 10^4 cfu/mL, but duplicates of these showed counts of less than 10^2 in one case and less than 10^1 in the other two.

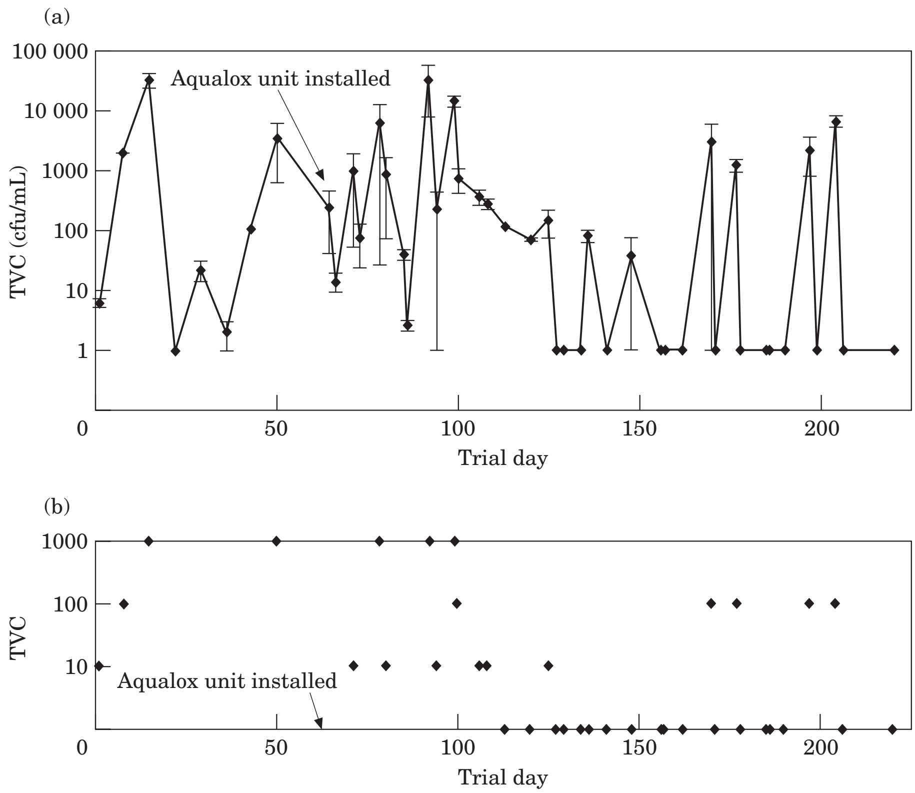

Figure 1 Total viable count (TVC) (a) and dip-slide counts (b) for Tower 1.

Such high variability indicates discontinuous distribution of bacteria within the cooling water, and fluctuations of this magnitude within a short time frame are consistent with biofilm release, providing high intermittent counts against a generally lower background.

From the beginning of February (day 93), TVCs showed a consistent downward trend, and from the beginning of March until the beginning of April (days 121–152) higher counts were of the order of 10^2, with lower counts that were often zero. From early April until the end of the Aqualox study, intermittent TVCs were recorded in excess of 10^3, but none exceeded 10^4, whilst the majority of the lower counts were again zero.

Dip-slide results for Tower 1 showed consistently lower microbial counts than the TVC study data with 83.9% of the counts being lower than both the TVCs from the same day and 96.8% lower than the higher of the two TVCs (Figure 1).

Results for Tower 2 are presented in Figure 2. TVCs recorded before commissioning of the Aqualox system varied between 10^3 and 10^7, with a significant proportion in excess of 10^4. From commissioning of the Aqualox system until the beginning of March (days 58–121), TVCs varied between 10^2 and 10^6, apart from a single sample providing a count of zero, and another in excess of 10^6. A significant proportion of samples provided counts in excess of 10^5. Following the installation of a supply of potable mains water to the Aqualox generator in early March, as indicated on Figure 2, there was an immediate and significant reduction in TVCs, which fell to levels of less than 10^3, and remained there throughout the rest of the Aqualox study, apart from two occasions where counts approached 10^4.

Dip-slide results for Tower 2 showed that 62.3% of the counts were lower than both the TVCs from the same day, with 69.8% lower than the higher of the two TVCs (Figure 2). Figure 3 shows the TVCs of the non-potable water supplying the Tower 2 Aqualox system up until March (day 121), and providing make-up water to the Tower throughout the course of the Aqualox study. Counts varied between 10^1 and in excess of 10^5, with a significant proportion in excess of 10^4.

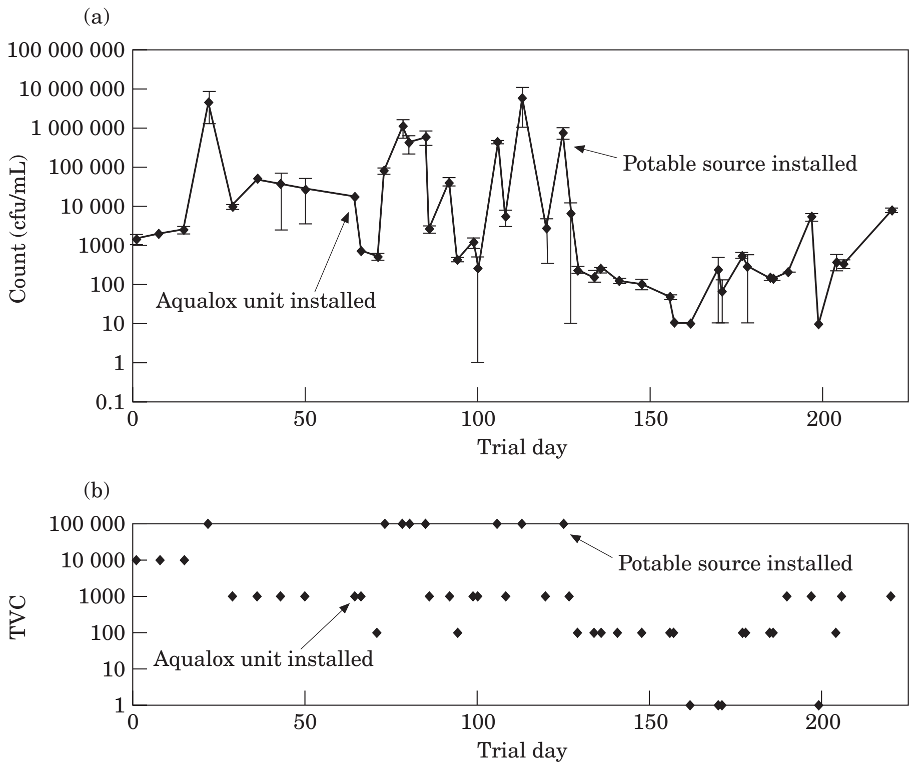

Figure 2 Total viable count (TVC) (a) and dip-slide counts (b) for Tower 2.

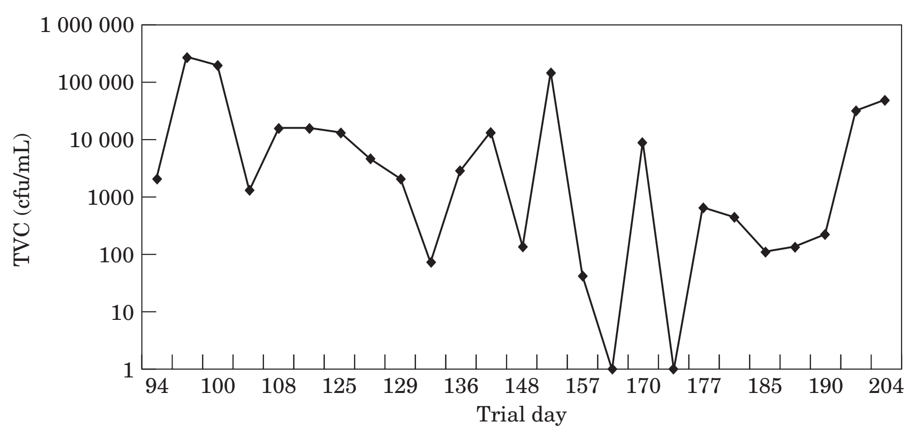

Figure 3 Total viable count (TVC) of non-potable make-up water for Tower 2.

Isothiazolone effect

No evidence of any significant reduction in TVCs from either tower was noted as a consequence of the monthly addition of the non-oxidizing isothiazolone biocide.

Oxidant reserve

Free oxidant reserve in both towers varied between 0.3 and 1.8 ppm, with the majority of results lying between 0.3 and 1.0 ppm.

Discussion

Most studies of microbiological control measures in cooling tower systems employ a minimalist sampling protocol, usually based on HSC ACOP recommendations for routine surveillance of microbial quality in cooling systems, which according to Bentham[16] is unlikely to produce findings of much scientific value. The sampling regimen used in the present study, in contrast, provides comprehensive replicated data, which facilitates far more meaningful conclusions than would normally be the case.

Given the nature of the sampling regimen employed, the absence of legionellae from any of the towers following Aqualox commissioning is a significant finding, particularly so when Tower 2 provided evidence of legionella colonization in the pre-study period. Bentham[21] and Fliermans[22] report that a positive *Legionella* spp. culture at any point in time indicates that a system is contaminated, regardless of subsequent culture results, and that once colonization has occurred, legionellae cannot be eradicated from cooling tower systems. Both authors consider that emphasis should be placed on control, rather than eradication, of legionellae, and suggest that this can be achieved through control of appropriate criteria, including choice of biocide and biocide dosage method. Legionellae were also isolated from two other towers at levels well in excess of HSC ACOP upper action limits, thus demonstrating that legionellae were present on-site at the start of the study period. The findings of the present study therefore support those of earlier laboratory studies, and provide further evidence of the efficacy of Aqualox for control of legionellae in cooling tower systems.

The TVC results showed a consistent pattern of high counts and high variability immediately following Aqualox commissioning, followed by a decline to lower baseline levels with sporadic individual higher counts. This is consistent with biofilm removal as a consequence of Aqualox commissioning, supporting the contention of other workers[8,9] that mixed oxidant solutions are particularly effective in removing biofilms. In addition, the trial towers had not undergone full manual clean down prior to commencement of the trial, and the Aqualox systems therefore performed well against a significant challenge. Results obtained from Tower 2 were especially noteworthy, since this tower presented a severe challenge to any treatment/control system due to the constant biological loading present in the make-up water.

The TVC results illustrate the value of duplicate sampling, since they reveal a level of variability between samples taken 10 min apart which would have gone unnoticed using a less rigorous protocol, with a concomitant reduction in the potential significance of the data obtained. Comparison of the sampling techniques shows that dipslide counts from Tower 1 were generally 10–100 fold lower than corresponding TVC results. Data for Tower 2 also shows dipslide counts were generally lower than TVCs. These results demonstrate that whilst dipslides can be used to measure the underlying microbial growth trends within a cooling tower system, they are not a substitute for quantitative laboratory-based TVCs.

The results of this study clearly demonstrate that Aqualox is an effective alternative to conventional bromine-based products for treatment of cooling tower systems. TVCs in all of the test towers were controlled well within HSC ACOP limits, with free oxidant reserves also within recommended limits throughout the course of the study. Further corroboration of these findings could, perhaps, have been provided by a crossover study, but in a commercial setting this was not permissible, as once the superiority of the Aqualox system over bromine had been demonstrated, health and safety considerations effectively proscribed a return to bromine.

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