Endotoxin release from biologically active bench-scale drinking water anthracite/sand filters

William B. Anderson, Colin I. Mayfield and Peter M. Huck

ABSTRACT

Endotoxin release from biologically active dual-media (anthracite/sand) bench-scale drinking water filters was investigated. The biological filters were typically net producers of endotoxin during normal operation with mean concentrations increasing from 6 endotoxin units (EU)/mL to 16 EU/mL in filter influent and effluent, respectively. The filter operating condition resulting in the greatest release of endotoxin occurred upon shutdown and subsequent restart, with the highest observed filter effluent endotoxin concentration being 745 EU/mL in the first pore volume following the return of flow through the filter. Effluent samples collected following filter shutdowns were chlorinated to determine the effect of bacterial cell death on endotoxin release. Chlorination did not induce immediate 'dumping' of endotoxin, nor did holding the chlorinated samples for 5 d result in an increase in aqueous endotoxin concentration. In addition to endotoxin, measurable quantities of potassium were detected in the interstitial water in the biofilter during shutdown periods. In order to reduce potential risk to dialysis patients and humidifier users, it is recommended that, following even short biofilter shutdowns, filters should be immediately backwashed or, failing this, the first five pore volumes of effluent water be discharged to waste to allow endotoxin levels to return to pre-shutdown values.

Key words | biofilters, drinking water, endotoxin, HPC bacteria, potassium

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INTRODUCTION

A growing trend in drinking water treatment has been the intentional incorporation of biologically active rapid filters into the treatment train, particularly following ozonation. A disinfectant is not normally applied prior to these filters, except in the case of ozone, which usually dissipates prior to reaching the filter or shortly after contact with the upper media layer. Indigenous bacteria that enter the filter from the raw water source establish biofilms and begin to consume easily biodegradable organic matter. This is advantageous since it lowers disinfectant demand, reduces disinfection byproduct formation and avoids uncontrolled biological 'regrowth' in distribution systems. Even without prior ozonation, rapid filters used for particulate removal will develop biofilms (e.g. Brewer & Carmichael 1979).

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Many of the organisms that inhabit these biofilters that have been identified are Gram-negative bacteria (e.g. Burlingame *et al.* 1986; Anderson 2004). Gram-negative bacteria differ from Gram-positive bacteria in that they have an outer cell wall layer that contains lipopolysaccharide (LPS). This LPS is also known as endotoxin, although it is actually the lipid A portion of the LPS that induces the adverse health responses typically associated with endotoxin (Prescott *et al.* 2002). It has been estimated that a single Gram-negative bacterium contains 3.5 million LPS molecules occupying an area of about 4.9 μm^2 (Williams 2001). Ingraham *et al.* (1983) estimated that an average *E. coli* cell contains 1.2 million LPS molecules, weighing 10×10^{-15} g and making up about 3.4% of the cell's total dry weight.

LPS molecules are released from bacteria during multiplication, death and lysis of whole cells, as well as from constant sloughing, which has been described as being analogous to the body shedding small pieces of skin or hair (Williams 2001). Many Gram-negative bacteria produce LPS-laden bilayered spheres known as membrane vesicles or blebs, which range in diameter from 50–250 nm and are actively shed (Beveridge 1999).

Published research involving endotoxin occurrence in raw and treated drinking water, and removals through treatment processes, is very limited by today's standards of available data regarding information on known contaminants of human health concern. What is available is confounded by potential methodological difficulties/limitations in early studies. In addition, since the late 1980s there has been a move to report endotoxin concentrations (or, more accurately, endotoxin activity) as endotoxin units (EU) rather than in units of weight (typically ng/mL). This form of reporting reflects the fact that endotoxin potency depends upon the genus of bacteria and, within species, on the specific lot or batch from which the reference endotoxin is isolated (Dawson 1994). Much of the literature available, however, does not use the newer terminology. In interpreting the literature where concentrations are reported in weight units (typically ng/mL) it is therefore important that conversion factors be provided. If they are not, it is not possible to accurately convert ng to/from EU. In the publications we have been able to acquire (with conversion factors) the range is 4–17 EU/ng. In an effort to avoid an extensive discussion on this issue and the risk of making unsubstantiated claims by converting data from one unit of measurement to another it is requested that readers refer to a paper by Anderson *et al.* (2002) and a thesis (Anderson 2004) which provide additional discussion and a list of available references in the area of endotoxin in water. Discussion will be primarily limited to data generated in this study and one by Huck *et al.* (1998) which has parallels to the work here.

While the importance of endotoxin in drinking water is not completely clear, there are some situations where what appear to be higher than typical distribution system levels may be detrimental to human health. These include aerosolization by humidifiers (Rylander & Haglind 1984; Anderson *et al.* 2007) or saunas (Metzger *et al.* 1976; Muittari *et al.* 1980*a*,*b*) which have resulted in episodes characterized by fever, chills, pain and breathing difficulties. In the case of failures with respect to water preparation for dialysis patients, fatalities have been attributed to endotoxin in the make-up (tap) water (Hindman *et al.* 1975). Similarly, fatalities have been attributed to endotoxin-contaminated distilled water used to dilute intravenous medications (e.g. Williams 2001).

In full- and pilot-scale drinking water treatment plants it has been reported that endotoxins are removed during coagulation, flocculation and sedimentation (Huck *et al.* 1998; Rapala *et al.* 2002, 2006) and that free chlorine and monochloramine can, albeit very slowly, inactivate endotoxin (1.4 and 1.0 EU/mL. h, respectively, for *E. coli* O55:B5-derived LPS) (Anderson *et al.* 2003*b*). From limited observations it would appear that endotoxin is typically found at low levels in drinking water treatment systems and that it decreases as water moves through the distribution system over time as the water 'ages' (e.g. Korsholm & Søgaard 1988; Rapala *et al.* 2002).

Biofilters containing Gram-negative bacteria can be a source of endotoxin. Huck *et al.* (1998) reported that endotoxin levels were higher after pilot-scale biofilters were shut down for 24 h and restarted without first backwashing or discharging effluent to waste. Two single tests compared a pair of anthracite/sand dual media filters to a pair of GAC contactors (all four of which were preceded by ozonation). The highest recorded endotoxin concentration in the dual-media filter effluent following shutdown and subsequent restart was 20 EU/mL and in the GAC contactor effluent it was 100 EU/mL. In follow-up tests with bench-scale biofilters containing glass beads, Huck *et al.* (1998) observed that, under certain conditions, endotoxin levels increased from 40 to 500 EU/mL (with an influent assimilable organic carbon [AOC] feed of 300 μg/L) and from 160 to 5,120 EU/mL (with an influent AOC feed of 1,500 μg/L) in 24 h shutdown tests. The highest concentrations appeared in the 0.5 pore volume sample. Those authors noted that it took about five volumes of pore water for endotoxin levels to return to pre-shutdown levels. It is clear from the bench-scale work with glass beads that high endotoxin releases are possible, yet with the two tests conducted at pilot scale, releases were far less. The Huck *et al.* (1998) work is the only research that has

been published in this area and studies that systematically investigate endotoxin accumulation during shutdowns of non pre-ozonated biofilters could not be found in the literature.

Biofilters are often the last step in a treatment process, usually followed only by the application of free chlorine and/or monochloramine or ultraviolet irradiation. While chlorine can, under some circumstances, reduce endotoxin concentration it is unlikely that the UV fluences (dosages) typically utilized for the treatment of drinking water (around 40 mJ/cm^2) are sufficient to inactivate substantial quantities of endotoxin (Anderson *et al.* 2003*a*,*b*). As such endotoxin discharges could, without operational intervention, enter a distribution system largely unchanged in concentration from post-shutdown conditions. For this reason it is important to verify observations from a previous study that elevated endotoxin discharges from biofilters are possible and to quantify maximum concentrations for various types of filter media, operating under different process conditions and configurations, in different water types, and with or without the application of oxidants prior to biofilters.

The objective of this research was to more fully quantify potential endotoxin releases from bench-scale drinking water anthracite/sand biofilters, to understand how they occur and to make recommendations on how drinking water treatment plant operators can avoid or respond to these releases. Unlike the single previous study for which data is available (Huck *et al.* 1998) the biofilter influent water was not pre-ozonated (and the filters had been in operation for a substantially longer period).

MATERIALS AND METHODS

Prefilters (GAC contactors) for bench-scale biofilters

Influent water to the bench-scale biofilters was chloraminated tap water obtained in the Douglas Wright Engineering Building at the University of Waterloo (Figure 1). The water was first passed, in upflow mode, through two parallel 5 cm internal diameter (ID) × 60 cm high glass columns (contactors) containing granular activated carbon (GAC) (Filtrasorb 300, Calgon Carbon Corp., Pittsburgh) to remove any free or combined chlorine present in the water. These first two may have also supported biofilms in the upper portions where chlorine was not present. Two additional columns followed (i.e. two sets of two parallel contactors in series). The purpose of passing the dechlorinated water through the second set of contactors (installed to encourage the development of biofilms) was to remove any easily biodegradable carbon remaining in the tap water and, as such, provide water that had a consistent, low level of biodegradable organic carbon. The second set of parallel contactors potentially served to supply a constant inoculum of indigenous bacteria to the three downstream bench-scale anthracite/sand filters. Flows through the contactors were kept constant at slightly more than what was required by the dual-media filters to allow for some overflow at the top of the filter columns to ensure a constant head (HPC measurements, chlorine residual monitoring data, and additional operating detail are available in Anderson 2004).

The GAC used in the contactors was initially acquired from Filter 1 (a biologically active GAC/sand filter) at the Mannheim Water Treatment Plant in Kitchener, Ontario, Canada. On a couple of occasions fresh GAC (Calgon Filtrasorb 300) was added when some GAC had to be removed to improve flow through the contactors. Flow to the insulated, covered, contactors was initiated on 29 July 2002 and continued virtually uninterrupted, 24 h/d, until 9 September 2004. Water from the contactors was discharged to waste when not flowing through the filters (typically, though, water always flowed through the entire system except during periods of maintenance or power or water shutdowns). Endotoxin formation/removal was not measured through these GAC prefilters. However, endotoxin was measured in the downstream biofilter column influent water just above the media.

Bench-scale filters

Bench-scale experiments were conducted using a set of three parallel biologically active dual media filters (anthracite over sand) that were located downstream of the GAC prefilters discussed above. The filter media was housed in three custom-fabricated 5.1 cm ID × 2.1 m high glass columns (Namdar Custom Glass Blowing Production,

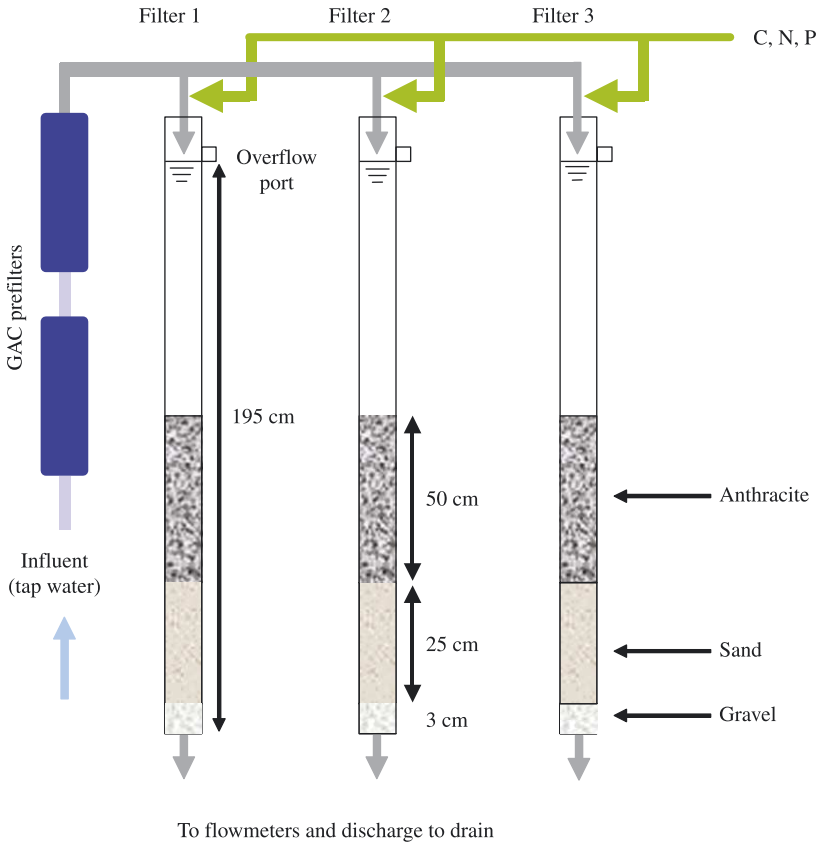

Figure 1 | Diagrammatic representation of bench-scale filter column set-up.

Mississauga, Ontario). Each column had one sample port located approximately 5 cm above the maximum level of the combined media and one overflow port approximately 5 cm from the top of the column to provide for overflow during backwashing and to maintain a constant head during regular filter operation (Figure 1). The port located 5 cm above the filter media was used to sample filter 'influent' water. Filter effluent samples were collected by redirecting filter effluent flow at the base of each filter with a stainless steel directional valve.

The filter media depth was 75 cm in total and consisted of anthracite over sand for all experiments. The anthracite and sand layers were 50 cm and 25 cm in depth, respectively. The anthracite had an effective size of 1.0 mm and a uniformity coefficient of 1.6. The sand had an effective size of 0.5 mm and a uniformity coefficient of 1.5 (Anthrafilter Media and Coal Ltd., Brantford, Ontario). Hydraulic loading in the columns was 6.0 m/h, providing approximately 7.5 min of empty bed contact time.

The filters were operated in a constant head, constant rate mode, and were backwashed once per week. Each filter was backwashed with water collected from that filter. Water was collected in 20 L polycarbonate carboys for about 1.5 h prior to using it for backwashing. Backwashing was initiated by shutting off the influent water and allowing the water to drop to about 10 cm above the top of the media. Air was pumped into the base of the filter at 2.0–2.5 L/min (no water flow) for about 20 s and then shut off. A valve to allow backwash water to enter the filter was opened, and backwash water was pumped at sub-fluidization velocity for 1.5 min. The air was turned back on as soon as the backwash pump was started, creating collapse pulsing conditions. The air was turned off a few seconds prior to the fluidized media reaching the column overflow port and backwash water flow was then increased to achieve 25% bed expansion for 30 s to 1 min to ensure that no plugs of media were carried upward in the filter. When it was confirmed that the media was fluidized and that no plugs

were present (or had broken up) water flow was increased to achieve a 50% bed expansion and held for about 4.5 min. Water flow was then slowly shut off, allowing the filter media to settle and appropriately stratify (1–2 min).

Filter 1 was used as a control (carbon, nitrogen and phosphorus were added as was the case in Filters 2 and 3) and was kept running when experiments were designed to investigate shutdown conditions (except for the last experiment run). As the dechlorinated tap water was low in biodegradable organic matter when it was discharged from the GAC prefilters (because most of the biodegradable organic matter was consumed in the distribution system and in the GAC prefilters) it was supplemented with a controlled level of organic carbon. The average TOC concentration in the chlorinated tap water was 1.10 ± 0.31 mg/L ($n = 44$) while the TOC in the dechlorinated tap water following the GAC prefilters was 1.08 ± 0.28 mg/L ($n = 44$). Organic carbon (C) sources utilized included the aldehydes, formaldehyde and glyoxal, and the carboxylic acids, formate and acetate. The influent water was also supplemented with nitrogen (sodium nitrate, $NaNO_3$) and phosphorus (potassium phosphate-dibasic; K_2HPO_4). The C:N:P ratio utilized was 15:5:1 (w/w/w) in order to ensure that sufficient nutrients were present to support biofilm growth. Camper (1994) indicated that, as a general rule of thumb, a ratio of 100:10:1 is required for balanced growth. The ratio of carbon to nitrogen/phosphorus was reduced somewhat from Camper's suggested ratio to ensure that carbon, and not nitrogen or phosphorus, was limiting. The 15:5:1 ratio is also very similar to the percentage of dry mass of each of the three elements in *Escherichia coli* cells (50:14:3) (Ingraham *et al.* 1983). In this work the 15:5:1 (C:N:P) ratio corresponds to target concentrations of 0.28 mg C/L, 9.33×10^{-2} mg N/L and 1.87×10^{-2} mg P/L in biofilter influent. All chemicals were manufactured by EM Scientific (Merck KGaA, Darmstadt, Germany) and acquired from VWR-Canlab (Mississauga, Ontario) except formaldehyde and glyoxal which were acquired from Sigma-Aldrich Canada (Oakville, Ontario).

Biofilm monitoring during steady state operation

The biological anthracite/sand filters were monitored during steady state operation through a filter maturation period that was almost two years in duration prior to conducting tests described herein (15 August 2002–24 July 2004). Routinely monitored parameters included: chlorine residual, total organic carbon (TOC) and dissolved oxygen (DO). In addition, endotoxin and potassium were measured during experiments. Potassium was monitored to test a theory that it may be shed in measurable quantities during filter shutdowns. Potassium is actively transported across bacterial cell walls and is found at higher levels inside the cell than in its outside environment. It is involved in several important functions including enzyme activation (e.g. those associated with protein synthesis) and regulating osmotic pressure within the cell (Ingraham *et al.* 1983).

Chlorine concentrations were determined as per Standard Method 4500-Cl D (*Standard Methods* 1998). Dissolved oxygen was measured using an ATI Orion Model 810 or 835 Dissolved Oxygen Meter (Orion Research Inc., Boston, MA). Total organic carbon (TOC) was measured with a Model 1010 Wet Oxidation TOC Analyzer (OI Analytical, College Station, TX) by Standard Method 5310 D (*Standard Methods* 1998). Aqueous dissolved potassium (as K^+) was measured using the tetraphenylborate method (Method 8049, Hach Company, Loveland, CO). Heterotrophic plate count (HPC) bacteria were determined by the Spread Plate Method 9215C (*Standard Methods* 1998) using Difco R2A agar (Becton, Dickson and Company, Sparks, MD) incubated at 20°C for 5 d. TOC samples were routinely analyzed in triplicate, HPC bacteria in duplicate, and dissolved oxygen, chlorine residual and potassium were single measurements. As chorine residual was monitored to ensure that it was absent in filter influent, single analyses only were conducted ($n = 80$). To confirm the precision of the potassium method, 5 replicate samples were collected from the influent and effluent of Filter 3 during regular filter operation and it was found that the average values were 1.4 ± 0.1 mg K^+/L and 1.5 ± 0.1 mg K^+/L, respectively.

Endotoxin concentrations were determined using the QCL-1000® Chromogenic Limulus Amebocyte Lysate Tube Method (BioWhittaker Inc., Walkersville, MD). The absorbance of endotoxin samples and standards were measured at 410 nm using a 1.0 cm quartz cell with a Hewlett-Packard 8453 UV-Visible Spectrophotometer. Every endotoxin sample run included a blank and four standards (run in

duplicate or triplicate). The mean absorbance of the blank was first subtracted from the mean absorbance value of the standards and samples to calculate mean Δ absorbance. After recording the Δ absorbance for the four standards a calibration curve was generated using Microsoft EXCEL®. An equation was generated to characterize the linear calibration and the unknown values were calculated by substituting the absorbances of the unknowns and solving the equation. Under normal conditions, the absorbance at 410 nm is linear in the concentration range 0.1 – 1.0 EU/mL. As this range is so small, most samples must be diluted multiple times in an attempt to get a dilution that falls in this range. This is time-consuming and introduces potential error associated with large dilutions (up to 1,000 times). This, in addition to costs associated with the testing, limits the potential for replicate analyses as it may take 4 or 5 dilutions (along with the large numbers of controls and standards) to get a single reliable endotoxin value. Most endotoxin values are single determinations, with some being the average of values if they overlapped in two dilution ranges.

All endotoxin work was conducted in a Class II NuAire biological safety cabinet model NU-425-400 (Plymouth, MN). All test tubes were heated using a VWRbrand Standard Heatblock (VWR-Canlab, Mississauga, Ontario) with three modular heating blocks, each with 24 – 10 mm holes.

Glassware preparation

All glassware was washed in a glassware washer (Lancer UP 1400, Lancer USA, Inc., Winter Springs, FL) using standard operating conditions (hot water prerinse, hot water wash, hot deionized water rinse and drying period). The hot and cold acetic acid rinses were skipped as acetic acid is used as the stop reagent in the endotoxin test. None of the glassware was autoclaved. Instead, the glassware was rendered pyrogen-free by heating at 350 – 400°C for at least 30 min (250°C would have been sufficient but the minimum temperature setting on the muffle oven was around 350°C).

Some products, such as tips for the micropipetters and deionized water, were purchased 'pyrogen-free'. A fresh bottle of pyrogen-free water was used for each experimental run to avoid potential contamination problems.

Experimental strategy

The main focus of these investigations involved shutting down biological filters for 2, 4 and 18 h and measuring endotoxin concentrations in the filter effluent before and after shutdown. The 2 and 4 h shutdowns were combined in a preliminary unreplicated experiment which was followed by six 18 h replicate shutdown experiments. Filter influent samples were taken immediately prior to shutdown. Filter influent samples were also taken in a parallel, similarly operated filter at the time of filter restart as a control to confirm the influent levels hadn't changed substantially during the shutdown period. Under shutdown conditions, water remains in the filter but does not flow through it. Potassium, dissolved oxygen and heterotrophic bacteria were also monitored. Samples were taken at calculated pore volumes of 0.5, 1, 2 and 5 to determine how quickly levels of monitored parameters returned to pre-shutdown levels. The sample times for these pore volumes corresponded to 1, 2, 8 and 21 min, respectively. A pore (void) volume corresponds to the volume of water contained within the depth of the entire filter media (in this case 0.82 L). The pre-shutdown samples ($t = -2$, -4 or -18 h) were typically referred to as '0' pore volumes on figures for graphing simplicity.

Biofilter effluent chlorination test

The final experiment discussed herein involved the collection of ten 225 mL samples of biofilter effluent following an 18 h shutdown and subsequent restart (0.5 and 1.0 pore volumes from parallel filters 1 and 2). Following restart, one 0.5 and one 1.0 pore volume sample from each of the two biofilters was analyzed immediately for endotoxin (nonchlorinated samples). Four 0.5 pore volume samples and four 1.0 pore volume samples were then dosed with sodium hypochlorite to achieve a free-chlorine residual of at least 1 mg/L after 24 h. The solutions were stored in 250 mL graduated Erlenmeyer flasks (Kimax®, VWR-Canlab, Mississauga, Ontario) which were sealed with black phenolic screw caps with rubber liners (all with aluminum foil between the cap and the flasks to inhibit oxidant demand from, and reactions with, the rubber liners). The flasks were continuously mixed on a Thermolyne

Model M49235 'Bigger Bill' orbital shaker (VWR-Canlab, Mississauga, Ontario) at 150 rpm at room temperature. The shaker and flasks were kept in the dark to avoid any potential photolysis-related reactions. Chlorinated samples from each biofilter at the two pore volumes were analyzed for endotoxin and free chlorine following 24 and 120 h of contact time with free chlorine.

RESULTS AND DISCUSSION

Background measurements and confirmation of bacterial activity

The biofilters were acclimated (continuously run) from 15 July 2002 to 9 September 2004. The experiments discussed herein were conducted only in the 6-week period from 29 July to 9 September 2004.

During the period from biofilter start-up through to the end of the experimental phase the average total chlorine residual was reduced from 0.47 ± 0.23 to 0.04 ± 0.02 mg Cl_2/L through the GAC prefilters ($n = 80$). Mean TOC levels dropped very slightly, if at all, from the influent (tap water) to post-GAC prefilter position (1.10 ± 0.31 to $1.08 \text{ mg} \pm 0.28$ C/L, $n = 44$). This would be expected, as the treated influent water was already low in biodegradable organic matter since it had already passed through a municipal drinking water distribution that already contained relatively low levels of TOC. Following the prefilter, carbon, nitrogen and phosphorus-containing compounds were added and the TOC increased from 1.08 ± 0.28 mg C/L to between 1.20 ± 0.25 and 1.23 ± 0.26 mg C/L at the interface between the filter influent water and the media. This is consistent with the calculated carbon input, which was 0.28 mg C/L, as some losses were incurred in the tubing and filter column surface biofilms before reaching the filter media. Following passage through the filter media, TOC concentrations were reduced to just slightly less than the concentration that was present before carbon addition (1.04 ± 0.26 to 1.07 ± 0.29 mg C/L). This supported evidence from other monitored parameters discussed below and visual observations during backwashing that the filters were biologically active.

Weekly dissolved oxygen (DO) measurements were initiated about three months prior to intensive testing and were carried on through the test period. Average DO dropped about 1 mg O_2/L, from 6.5 ± 1.1 to 5.6 ± 1.2 O_2 mg/L, following passage through the GAC prefilters ($n = 24$). This would be expected because the second prefilter was biologically active, as chlorine had been removed in the first prefilter (and GAC adsorbs oxygen). The DO then decreased, on average, to between 4.9 ± 1.2 and 5.3 ± 1.0 mg O_2/L following the biofilters, further supporting indications of biofilm development in the filters provided by the TOC data ($n = 24$).

Under normal operating conditions, the biofilters were typically net heterotrophic bacteria producers with higher heterotrophic bacteria levels being observed in the filter effluents on 26 of 30 occasions. The mean HPC level increased from 1.1×10^4 to 3.7×10^4 CFU/mL through the filters (a 0.5 log increase, significant at $\alpha = 0.001$, using a paired t-test). The samples were taken weekly during the shutdown experiments prior to shutting down any filters.

Similarly, under normal operating conditions, mean endotoxin levels increased through the biofilters from 6 ± 4 EU/mL to 16 ± 8 EU/mL ($n = 4$). While the concentration increased by a factor of about 2.5, these concentrations are relatively low and are unlikely to be cause for concern. In total, 9 biofilter effluent samples were analyzed (including the 4 discussed above for which there was corresponding biofilter influent data). The mean biofilter effluent endotoxin concentration for the 9 samples was 17 ± 10 EU/mL.

The water temperature during the filter shutdown experimental phase ranged from 20–22°C (measured weekly, $n = 5$).

Effect of filter shutdown on endotoxin and other monitored parameters

A preliminary, unreplicated experiment was conducted to investigate endotoxin accumulation following short shutdowns. Following start-up after a 2 h filter shutdown, effluent endotoxin levels increased from 13 EU/mL at the time of shutdown to 68 EU/mL at 0.5 pore volumes then dropping back to 29 EU/mL at the 1 pore volume sample time (Figure 2A). HPC bacteria concentrations increased from 2.9×10^4 CFU/mL at the time of shutdown to 4.3×10^5 CFU/mL and 1×10^6 CFU/mL in the 0.5 and 1

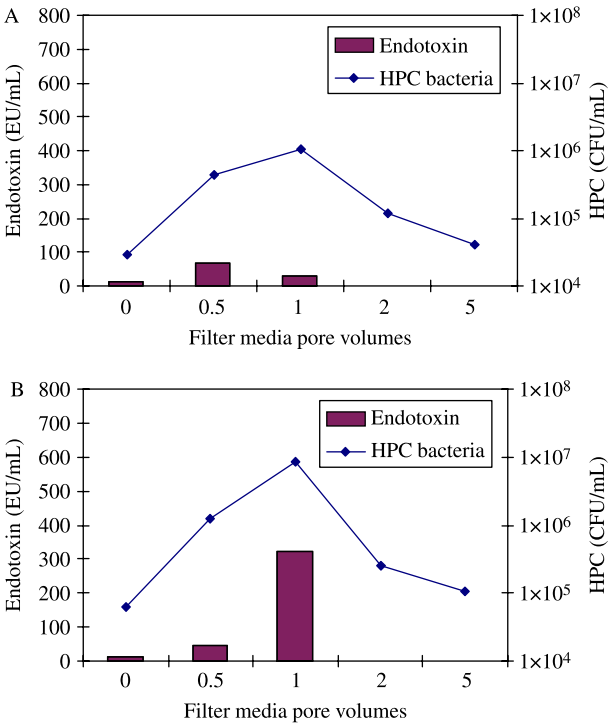

Figure 2 | Endotoxin concentrations and HPC bacteria levels in filter effluent following shutdown periods of 2 h (A: filter 2) and 4 h (B: filter 3) in parallel filters on the same date. Endotoxin concentrations are single measurements and HPC bacteria concentrations are the average of duplicate samples.

pore volume samples, respectively (a 1.6 log increase from pre-shutdown levels to highest effluent concentration).

Following start-up after a preliminary unreplicated 4 h filter shutdown on the same date as the 2 h experiment above, effluent endotoxin levels increased from 11 EU/mL at shutdown to 47 EU/mL at 0.5 pore volumes and 323 EU/mL at the 1 pore volume sample time (Figure 2B). HPC bacteria concentrations increased from 6.3×10^4 CFU/mL to 1.3×10^6 CFU/mL to 8.4×10^6 CFU/mL for the same time intervals (a 2.1 log increase from pre-shutdown levels to highest effluent concentration). Endotoxin samples were not taken at the 2 and 5 pore volume time intervals in this test but HPC bacteria were, and by 5 pore volumes the HPC bacteria levels had essentially returned to pre-shutdown levels.

The remaining experiments were conducted with 18 h shutdown periods and clear trends with respect to endotoxin, HPC bacteria, potassium and dissolved oxygen were observed. Table 1 provides a numerical summary of endotoxin concentrations and heterotrophic bacteria in six 18 h filter shutdown experiments. It can be seen that endotoxin concentrations in the filter effluent following restart exceeded 600 EU/mL in at least one sample on three of the sample dates, with a high concentration of 745 EU/mL.

Figure 3 shows average endotoxin concentrations and HPC vs. pore volumes for all experiments (including those run with 2, 4 and 18 h shutdowns). The observed trend was generally consistent for all experiments and was characterized by a large increase in effluent endotoxin and heterotrophic bacteria levels in samples collected at 0.5 pore volumes. Although levels decreased somewhat there was still considerable endotoxin and heterotrophic bacteria in the 1.0 pore volume sample but by the 2 and 5 pore volume samples both endotoxin and HPC levels had returned to close to pre-shutdown levels. The assessment of data using a

Table 1 | Summary of endotoxin and heterotrophic bacteria levels in filter effluent following 18 h shutdown and subsequent restart experiments. Note: all filters contained identical media and were run under identical conditions (with the exception of frequency between filter shutdowns). Values in bold are the highest recorded in each run. The 0 pore volume sample was taken immediately prior to the 18 h shut-down. Endotoxin concentrations are single measurements and HPC bacteria concentrations are the average of duplicate samples

Pore volumes	Filter 3 (5 Aug.)	Filter 3 (12 Aug.)	Filter 3 (19 Aug.)	Filter 2 (19 Aug.)	Filter 2 (26 Aug.)	Filter 1 (26 Aug.)	Average
	Endotoxin (EU/mL)						
0	10	9	4	7	15	11	9
0.5	**687**	**274**	**106**	**745**	**420**	108	**390**
1.0	286	113	21	106	238	**635**	233
	HPC bacteria (log CFU/mL)						
0	4.92	4.38	4.39	5.37	4.84	4.84	4.92
0.5	**5.62**	**5.16**	**5.30**	**6.09**	**6.71**	5.63	**6.10**
1.0	5.24	4.91	4.67	5.89	5.26	**6.73**	6.05

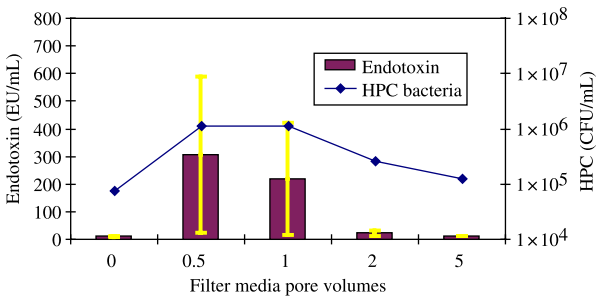

Figure 3 | Mean endotoxin concentrations and HPC bacteria in filter effluent ($n = 8$) (following 2, 4 and 18 h shutdowns and subsequent restarts); 0 pore volume is effluent concentration at time of filter shutdown.

paired t-test confirmed that the observed differences between the pre-shutdown and the 0.5 pore volume endotoxin concentrations (means of 10 and 307 EU/mL) are significant at the $\alpha = 0.05$ level. Using the log transformed data, the observed 1.2 log mean difference between the pre-shutdown and the 0.5 pore volume HPC bacteria levels is significant at the $\alpha = 0.001$ level.

To illustrate this point, and to show changes over time, Figure 4(A–C) shows three consecutive sample runs spaced at weekly intervals in filter 3. On every sample occasion the endotoxin peak corresponded to the peak concentration of heterotrophic bacteria. Over time, however, it can be seen that continuing shutdowns generally reduced peak heterotrophic bacteria levels and corresponding peak endotoxin concentrations. This, and the previous observation that the highest endotoxin concentrations corresponded with the peak HPCs, suggests that endotoxin may be being produced during periods of optimal growth and not only during periods of stress (recalling that the HPC method only detects viable, culturable bacteria).

As was observed with heterotrophic bacteria, potassium concentrations increased following periods of filter shutdown (Figure 5). The pattern over time was also similar, with the highest potassium levels being seen in the 0.5 pore volume samples. The difference in potassium concentration between $t = 0$ (pre-shutdown) and 0.5 pore volume samples (increasing from 1.6 ± 0.2 to 2.0 ± 0.3 mg/L ($n = 6$) was significant ($\alpha = 0.05$). This suggests that, at some point following shutdown, the environment in the biofilter changed and the growing cells were forced to transport potassium outward through the cell wall to maintain appropriate internal osmotic pressure. Cell lysis may also

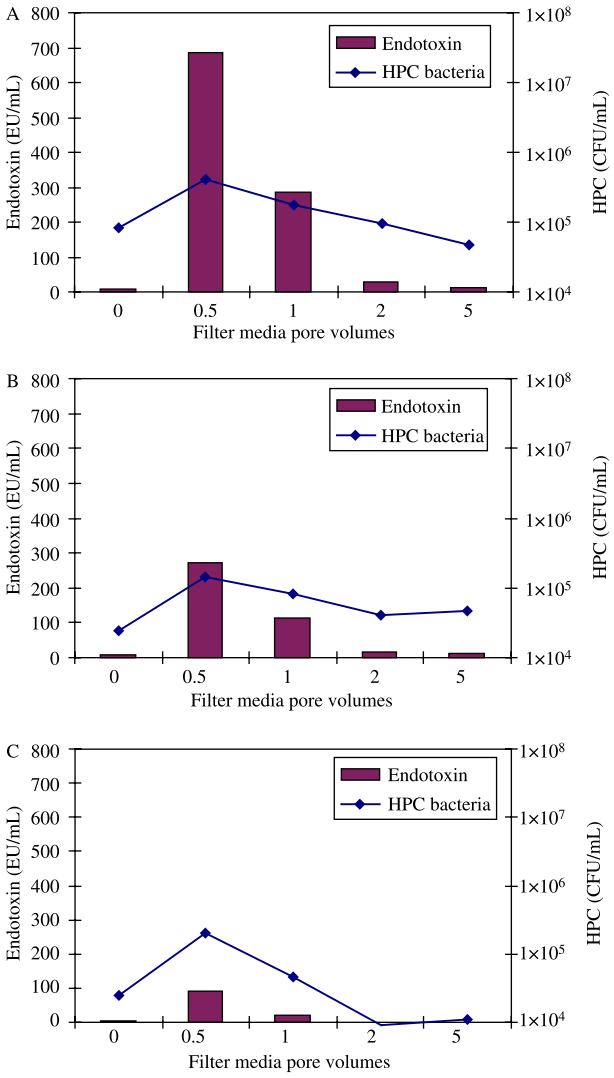

Figure 4 | Endotoxin and HPC vs. pore volumes for 18 h filter shutdown experiments conducted consecutively on 2 Aug. (A), 12 Aug. (B) and 19 Aug. (C) in filter 3.

have contributed to the observed increase. While it appeared that substantial increases in endotoxin concentration occurred in the first few hours following shutdown, there were insufficient data to confirm this observation with respect to potassium. In fact, in the one 4 h shutdown experiment for which data is available, potassium did not increase, suggesting that potassium concentrations may have increased/accumulated toward the end of experiments with 18 h shutdown periods (or be associated with increased cell concentrations as discussed below). There are very simple tests for potassium that can be done in minutes rather than hours for endotoxin and days for HPC

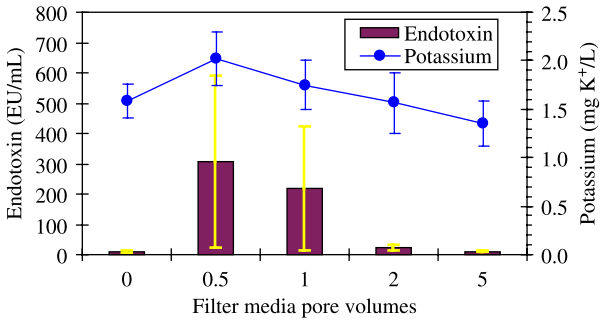

Figure 5 | Average endotoxin and potassium concentrations vs. pore volumes for 18 h filter shutdowns ($n = 6$).

bacteria. Potassium analysis could be utilized by plant operators as an early indication that endotoxin and/or HPC bacteria may be present in large quantities following filter shutdown and subsequent restart. The one caveat with this is that potassium levels prior to shutdown would have to have been measured, as the potassium concentration in and of itself provides no useful information at a single point in time. Follow-up studies with potassium as an indicator should include a pre-filtration step to remove bacteria in the event that cell stress/lysis is sufficiently induced by chemicals used in the analysis to release potassium.

Dissolved oxygen concentrations were also monitored as it was expected that oxygen levels would drop during the course of the shutdown (Figure 6). On average, dissolved oxygen dropped about 1.1 mg/L following shutdown, from 5.0 ± 1.1 mg/L to 3.9 ± 0.6 mg/L ($n = 8$). The difference in dissolved oxygen concentration between $t = 0$ (pre-shutdown) and 0.5 pore volume samples was significant ($\alpha = 0.01$) as had been observed in the case of HPC bacteria and potassium with the lowest dissolved oxygen levels being measured in the 0.5 and 1.0 pore volume samples.

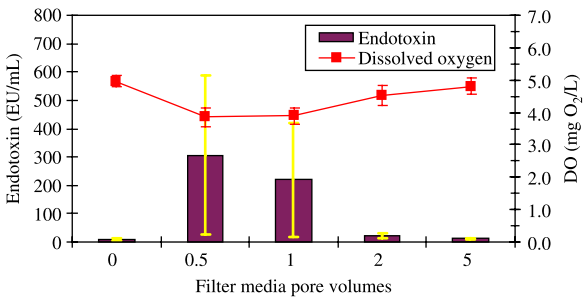

Figure 6 | Average endotoxin and dissolved oxygen concentrations vs. pore volumes for 18 h filter shutdowns ($n = 8$).

Effect of chlorinating endotoxin released from biofilms during filter shutdown

While the biofilter effluent following shutdown and subsequent restart contains elevated levels of endotoxin it also contains elevated levels of live heterotrophic bacteria. In theory, stress and cell death (and decomposition, if time and conditions were appropriate) should result in endotoxin release and it would be expected that endotoxin concentrations could increase substantially upon chlorination. An experiment was conducted to investigate this further. It involved chlorinating and holding biofilter effluent 0.5 and 1.0 pore volume samples for up to 120 h (5 d). Endotoxin concentrations in the effluent of biofilters 1 and 2 prior to the 18 h shutdown were 11 and 14 EU/mL, respectively. As can be seen in Figure 7(A, B), endotoxin levels increased in both filters following shutdown and subsequent restart as expected (at both pore volumes). However, endotoxin levels remained constant or decreased slightly in samples taken following 24 and 120 h contact with free chlorine.

Free-chlorine residuals were roughly 1 mg/L after the 24 h hold time in filter 1 and 2 effluent samples. At the end of

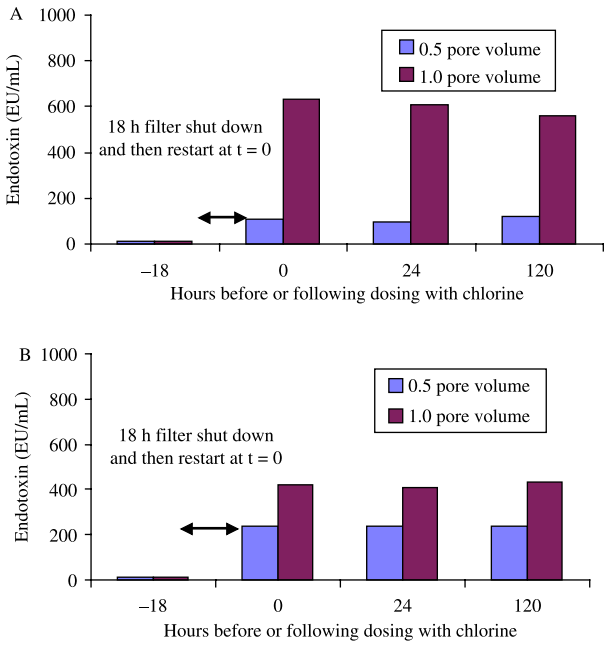

Figure 7 | Endotoxin concentrations in the effluent of filter 1 (A) and filter 2 (B) 18 h prior to filter shutdown, following shutdown and subsequent restart (hold time [t] = 0, chlorine not yet added), and 24 and 120 h effluent hold time after chlorination. Endotoxin concentrations are single measurements.

the 120 h test period, free-chlorine residuals were 0.11 mg/L in filter 1 (0.5 pore volume sample) and 0.02 mg/L (1.0 pore volume). In filter 2, free-chlorine residuals were 0.20 mg/L (0.5 pore volumes) and 0.02 mg/L (1.0 pore volume).

It is likely that some endotoxin was released by stressed or dead cells because, if not, then levels should have decreased in the presence of chlorine. Previous work has demonstrated endotoxin is inactivated by free chlorine at a rate of 1.4 EU/mL.h (Anderson *et al.* 2003*b*), at least for the type of endotoxin used in these experiments. Over 120 h this would have corresponded to a decrease of approximately 170 EU/mL. It would appear, therefore, that endotoxin was being released at roughly the same rate as it was being inactivated by chlorine (or that some bacteria had survived and began dividing as chlorine levels dropped to near detection). From an experimental design point of view, it could be argued that the chlorine could have been quenched following a suitable chlorine residual contact time to remove its presence as a variable, but this would not be the case in a typical distribution system where some form of chlorine residual is typically maintained. The absence of chlorine would allow heterotrophic bacteria to grow, thereby further confounding the test. In any event, order-of-magnitude increases in endotoxin concentration were not observed, indicating that, from a drinking water treatment perspective, the most important source of endotoxin associated with biofilter operation or at downstream points is following a filter shutdown without immediate backwashing or running a filter to waste.

Summary

Biofilters are now integral parts of many drinking water treatment plants (including ozonated and non-ozonated systems) and play a valuable role in stabilizing the quality of water in distribution systems. However, due to potential health concerns with respect to dialysis patients and users of impellor or ultrasonic humidifiers, it is recommended that, following a filter shutdown and subsequent restart, filters should always be immediately backwashed, or the first five pore volumes should be discharged to waste prior to reconnecting to drinking water distribution networks. Alternatively, it may be preferable to keep all biological filters operating at a reduced loading where possible, rather than shutting some down and restarting them. In any event, sudden changes of filter flowrate should be avoided to reduce the possible release of deposited particles.

It may be possible to dilute contaminated filter effluent with product water from other filter streams but it has yet to be determined what a safe level for endotoxin in drinking water is, so while this might be helpful it does not guarantee safe water.

While heterotrophic bacteria are not typically in and of themselves a health threat they are sometimes used as indicators of the general state of treated water or distribution systems and, as such, large discharges following filter shutdowns may confound the interpretation of results (may have regulatory implications in some jurisdictions).

The findings of this study confirm general observations made by Huck *et al.* (1998) in that endotoxin concentrations spiked following periods of filter shutdown (even in filters without a pre-ozonation step vs. those with a pre-ozonation step in the Huck *et al.* study). The important difference in this study with respect to filter operation and potential human health effects was the magnitude of the increase (maximum of 745 EU/mL vs. 20 EU/mL) in dual media anthracite over sand filters. The higher endotoxin concentrations observed in this study were more consistent with those recorded from the non-backwashed bench-scale columns containing glass beads in the Huck *et al.* study (40 to 500 EU/mL and 160 to 5,120 EU/mL for AOC feeds of 300 and 1,500 μg/L, respectively). Differences that might account for this are biofilter acclimation time (1 year vs. 2 years in this study), temperature at the time of experiments (<10°C in the Huck *et al.* study, 20°C in this study), nutrient conditions (nothing fed in Huck *et al.* pilot filters vs. amendment with carbon, nitrogen and phosphorus in this study) and backwash frequency (daily in Huck *et al.* study vs. weekly in this study). This study also confirms that the highest endotoxin concentrations were typically found in the 0.5 pore volume sample and that endotoxin concentrations drop back to prefilter shutdown levels with five pore volumes. This study extends the Huck *et al.* study by noting that potassium may be an indicator of the presence of increased heterotrophic bacteria and/or endotoxin as well as by demonstrating that chlorination of the filter effluent

following shutdown and subsequent restart does not dramatically increase endotoxin concentrations in the plant or (simulated) distribution system. Drinking water treatment plant operators can continue with normal chlorination procedures even if it is anticipated that water could contain high concentrations of heterotrophic bacteria.

CONCLUSIONS

This work was conducted using bench-scale dual-media biological filters containing anthracite over sand. The filters had been acclimated for over two years prior to initiation of this work and were fed with low TOC, dechlorinated tap water amended with organic carbon (formaldehyde, glyoxal, formate and acetate) at 0.28 mg C/L, nitrogen ($NaNO_3$) at 0.09 mg N/L and phosphate (K_2HPO_4) at 0.02 mg P/L. The filters were backwashed once per week. Based on this configuration and operating characteristics the following conclusions can be drawn:

- substantial concentrations of endotoxin (up to 745 EU/mL) and heterotrophic bacteria (up to 6.7 log CFU/mL) were found in biofilter effluent when a filter was restarted without immediately backwashing or discharging to waste;
- filter shutdowns of even 2–4 h resulted in substantial increases in effluent endotoxin concentrations and heterotrophic bacteria levels;
- biofilter effluent potassium concentrations immediately following filter restart were statistically higher than effluent concentrations prior to filter shutdown;
- effluent heterotrophic bacteria, potassium and endotoxin concentrations were positively correlated following filter shutdown and subsequent restarts;
- as expected, dissolved oxygen concentrations dropped when filters were shut down and were negatively correlated with endotoxin following filter shutdown and subsequent restarts;
- while stress and cell death theoretically could result in endotoxin release, the magnitude of that which was observed following chlorination of biofilter effluents following restart after an 18 h shutdown was far less remarkable than during periods of filter shutdown.

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REFERENCES

Anderson, W. B. 2004 *Endotoxins in Drinking Water: Mechanisms of Release from Biofilms and Response to Treatment Technologies*. PhD Thesis. University of Waterloo, Waterloo, Ontario, Canada.

Anderson, W. B., Dixon, D. G. & Mayfield, C. I. 2007 Estimation of endotoxin inhalation from shower and humidifier exposure reveals potential risk to human health. *J. Water Health* **5**(4), 553–572.

Anderson, W. B., Huck, P. M., Dixon, D. G. & Mayfield, C. I. 2003*a* Endotoxin inactivation in water by using medium pressure UV lamps. *Appl. Environ. Microbiol.* **69**(5), 3002–3004.

Anderson, W. B., Mayfield, C. I., Dixon, D. G. & Huck, P. M. 2003*b* Endotoxin inactivation by selected drinking water treatment oxidants. *Water Res.* **37**(19), 4553–4560.

Anderson, W. B., Slawson, R. M. & Mayfield, C. I. 2002 A review of drinking-water-associated endotoxin, including potential routes of human exposure. *Can. J. Microbiol.* **48**(7), 567–587.

Beveridge, T. J. 1999 Structures of Gram-negative cell walls and their derived membrane vesicles (minireview). *J. Bacteriol.* **181**(16), 4725–4733.

Brewer, W. S. & Carmichael, W. W. 1979 Microbiological characterization of granular activated carbon filter systems. *J. AWWA* **71**(12), 738–740.

Burlingame, G. A., Suffet, I. H. & Pipes, W. O. 1986 Predominant bacterial genera in granular activated carbon water treatment systems. *Can. J. Microbiol.* **32**, 226–230.

Camper, A. K. 1994 Coliform regrowth and biofilm accumulation in drinking water systems: a review. In: Geesey, G. G., Lewandowski, Z. & Flemming, H.-C. (eds) *Biofouling and Biocorrosion in Industrial Water Systems*. CRC Press, Boca Raton, FL, Ch 6.

Dawson, M. E. 1994 Endotoxin standards and CSE potency. *LAL Update (Associates of Cape Cod Incorporated, Falmouth, MA)* **11**(3), 2–5.

Hindman, S. H., Favero, M. S., Carson, L. A., Peterson, N. J., Schonberger, L. B. & Solano, J. T. 1975 Pyrogenic reactions during haemodialysis caused by extramural endotoxins. *Lancet II* **7938**, 732–734.

Huck, P. M., Finch, G. R., Hrudey, S. E., Peppler, M. S., Amirtharajah, A., Bouwer, E. J., Albritton, W. L. & Gammie, L. 1998 *Design of Biological Processes for Organics Control*. Report No. 90722. American Water Works Association Research Foundation, Denver, CO.

Ingraham, J. L., Maaløe, O. & Neidhardt, F. C. 1983 *Growth of the Bacterial Cell*. Sinauer Associates, Sunderland, MA.

Korsholm, E. & Søgaard, H. 1988 An evaluation of direct microscopical counts and endotoxin measurements as alternatives for total plate counts. *Water Res.* **22**, 783–788.

Metzger, J. W., Patterson, R., Fink, J., Smerdijan, R. & Roberts, M. 1976 Sauna-takers' disease: hypersensitivity pneumonitis due to contaminated water in a home sauna. *J. Am. Med. Assoc.* **236**, 2209.

Muittari, A., Kuusisto, P., Virtanen, P., Sovijärvi, A., Grönroos, P., Harmoinen, A., Antila, P. & Kellomäki, L. 1980*a* An epidemic of intrinsic allergic alveolitus caused by tap water. *Clin. Allergy* **10**, 77–90.

Muittari, A., Rylander, R. & Salkinoja-Salonen, M. 1980*b* Endotoxin and bath-water fever. *Lancet II* **8185**, 89.

Prescott, L. M., Harley, J. P. & Klein, D. A. 2002 *Microbiology*, 5th edition. McGraw-Hill, New York, pp. 799–801.

Rapala, J., Lahti, K., Räsänen, L. A., Esala, A.-L., Niemelä, S. I. & Sivonen, K. 2002 Endotoxins associated with cyanobacteria and their removal during drinking water treatment. *Water Res.* **36**(10), 2627–2635.

Rapala, J., Niemelä, M., Berg, K. A., Lepistö, L. & Lahti, K. 2006 Removal of cyanobacteria, cyanotoxins, heterotrophic bacteria and endotoxins at an operating surface water treatment plant. *Water Sci. Technol.* **54**(3), 23–28.

Rylander, R. & Haglind, P. 1984 Airborne endotoxins and humidifier disease. *Clin. Allergy* **14**, 109–112.

Standard Methods for the Examination of Water and Wastewater 1998 20th edition. American Public Health Association/ American Water Works Association/Water Environment Federation, Washington, DC.

Williams, K. L. 2001 *Endotoxins, Pyrogens, LAL Testing, and Depyrogenation*, 2nd edition. Marcel Dekker, New York, pp. 15–16.

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