Bacteriological water quality changes in parallel pilot distribution systems

R. Fabris, K. Braun, L. Ho, J. Q. J. C. Verberk and M. Drikas

ABSTRACT

In a component of an extensive pilot distribution system (PDS) study, the effects of four different water qualities on biological stability in distributed water were investigated through identical (parallel) single-pass pipe arrangements. Through 24 months of monitoring, a number of key observations were made. Incorporation of a biological treatment step reduced the overall dissolved organic carbon (DOC) loss through the PDS by reducing biodegradable DOC (BDOC) within the water prior to distribution. In the absence of chlorine residuals, the proliferation of culturable organisms was favoured with considerably higher heterotrophic plate counts in samples at the outlet of the PDS. Despite different bacterial cell counts (measured by flow cytometry) entering each PDS from the four treatment streams, equivalent outlet cell numbers were achieved in all systems after 8 months' operation; however multi-step treatment streams took longer to reach equilibrium.

Key words | BDOC, biofilm, flow cytometry, pilot distribution system

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INTRODUCTION

Water quality deterioration in drinking water distribution systems (DWDS) has become a key focus in recent years for many water utilities and service providers. There is agreement amongst water utilities that the aim should be to provide high quality water at the customer tap, while in reality the goal is commonly rationalised to a more achievable target of providing high quality water leaving the water treatment plant (WTP) (Liu *et al.* 2013). Unfortunately water quality deterioration can occur with increasing water age following detention within a distribution system. This perception is expanding rapidly with a better understanding and recognition that the DWDS has previously been largely considered a chemical and microbiological 'black box'. The availability of more sophisticated instrumentation has allowed greater insight into DWDS, with a greater focus on the distribution systems as a dynamic rather than static infrastructure component (Kerneïs *et al.* 1995). Recent investigations into DWDS particulate characterisation (Vreeburg *et al.* 2008) and microbial ecology (Douterelo *et al.* 2013) are yielding greater knowledge into the nature of the DWDS as bioreactors, and propagators of sediment accumulation and release. Despite this, standard water quality analyses in the laboratory such as colour and turbidity are still the predominant mode of monitoring DWDS quality, with bacteriological quality based upon chlorine residual maintenance and minimising heterotrophic plate counts (HPCs). Rapid bacteriological assessment tools such as flow cytometry (FCM) (Hoefel *et al.* 2003; Berney *et al.* 2008; Hammes *et al.* 2008) and adenosine triphosphate (ATP) measurement (Van der Kooij *et al.* 1995; Delahaye *et al.* 2003; Hammes *et al.* 2010) are being more widely applied in treatment and distribution system research. These rapid assessments have not yet been implemented into routine monitoring regimes by water utilities.

Due to risks involved in performing bacteriological experimentation on real DWDS, a considerable number of investigations have been reported using pilot facilities to study distribution system behaviour. The most commonly referenced is the Thames Water-developed 'TORUS' continuous looped pilot distribution facility (Holt *et al.* 1994;

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Smith *et al.* 1999; Maier *et al.* 2000; Boxall & Saul 2005). In addition a number of other investigations have examined the effect of water quality, nutrients and flow on the bacteriological stability of pilot distribution systems (PDS) (Piriou *et al.* 1998; Volk & LeChevallier 1999; Frias *et al.* 2001; Lehtola *et al.* 2006; Liu *et al.* 2013).

While there have been numerous past pilot plant investigations considering biofilms, few have simultaneously studied the effects of multiple different water qualities on biological stability in distributed water through realistic pipe dimensions and materials using identical (parallel) distribution systems over long term operation (24 months). This paper evaluates the change in microbiological enumeration using traditional and next generation techniques before and after passage through the distribution systems and the relationship to treatment methodology.

MATERIALS AND METHODS

The source water for the study was River Murray water taken from the Mannum to Adelaide pipeline at Mt. Pleasant WTP, located in the Adelaide Hills approximately 60 km from Adelaide. The feed waters for the four distribution systems were of increasing product water quality resulting from four different treatment streams in line with current advanced processes utilised within Australia. Source and feed water quality has been described extensively in previous publications (Fabris *et al.* 2013, 2015).

Treatment streams

S1 – Conventional (Conv) comprised alum coagulation followed by flocculation, sedimentation and dual media (sand/anthracite) filtration. Aluminium sulphate was used as the primary coagulant at dose rates dependent on water quality with pH controlled between 6.2 and 6.5 using either sodium hydroxide or sodium bicarbonate, depending on the source water alkalinity. A cationic poly-acrylamide LT22 (BASF Chemicals, Australia) was dosed as a flocculant aid.

S2 – MIEX plus Coagulation (MIEX/Coag) consisted of pre-treatment using a magnetic ion-exchange resin (MIEX) for dissolved organic carbon (DOC) removal, coupled with coagulation/sedimentation/filtration treatment as a clarification step for turbidity reduction. The MIEX–DOC® removal process (IXOM, Australia) implemented at Mt. Pleasant WTP has been described in detail previously by Drikas *et al.* (2011).

S3 – MIEX plus Coagulation plus GAC (MIEX/Coag/GAC) comprised the product water from MIEX/Coag with further polishing by granular activated carbon (GAC). Two gravity fed filter columns filled with F400, a coal based steam-activated GAC (Calgon Corporation, USA), were used to achieve an empty bed contact time of approximately 14 minutes with the product streams combined.

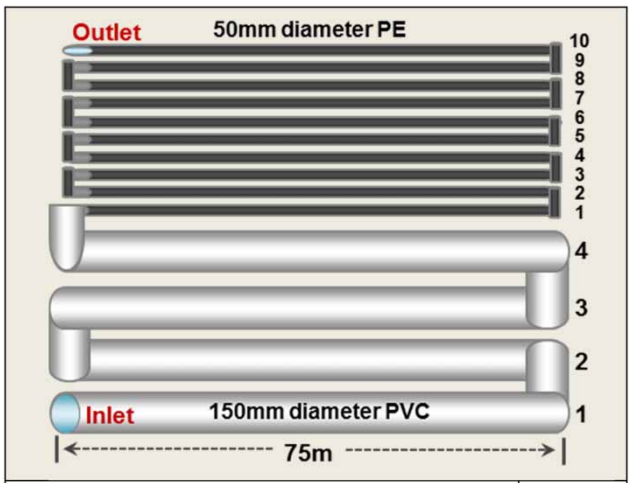
S4 – Nanofiltration with Microfiltration pre-treatment (MF/NF) incorporated dual stage membrane filtration with a Siemens–Memcor submerged microfiltration (MF) pre-treatment for particulate removal followed by a DOW–Filmtec NF270 nanofiltration (NF) membrane for organics removal and hardness reduction. This stream represented the most advanced treatment technology and consistently achieved the highest treated water quality.

All treated water streams were disinfected to meet a minimum 'Chlorine concentration x contact time' factor – Ct (Baumann & Ludwig 1962; White 1975) of 30 mg.min/L, according to demand but deliberately controlled to retain no residual at the inlet to the PDS following 4 hours contact in the treated water storage tank. This strategy was chosen to replicate low-flow ends of distribution systems, where disinfectant residual is often lost and to encourage more rapid establishment of any potential biofilms within the operational duration (24 months). Treated water storage tanks were modified 1,000 L HDPE intermediate bulk containers with treated and disinfected water entering through a ported lid and exiting to the distribution systems via the drain valve.

Pilot distribution systems

Four independent 1.05 km looped PDS using a combination of 150 mm OD polyvinyl chloride and 50 mm ID polyethylene pipe were studied. The use of large diameter pipework provided a realistic representation of bulk water and pipe wall interactions (volume to surface area) in relation to real distribution systems. Pipes were arranged in 75 m lengths with compact 180 degree bends allowing

the inlet and outlet to be collected at the same terminus (Figure 1). All pipe work was buried a minimum 600 mm depth to attenuate temperature extremes (Figure 2). Each PDS operated in single-pass mode with a hydraulic retention time of 78.5 hours, which included three overnight stagnation periods of 8 hours to mimic diurnal network flow rates. Outlet samples were collected 3 days after the inlet sampling to account for the retention time within the PDS and capture the same water, within practical WTP accessibility. Monitoring occurred between July 2010 and July 2012.



Length of 150mm diameter PVC	300m
Length of 50mm diameter PE	750m
Total PDS length	1050m
PDS volume	6,775L
PDS inner pipe surface area	248m^2
Residence time (with nightly 8hr stagnations)	78.5hrs

Figure 1 | PDS layout schematic and technical specifications.

Analyses

Grab samples for DOC analysis were filtered through 0.45-μm pre-rinsed membranes and measured using a Sievers 900 Total Organic Carbon Analyser (GE Analytical Instruments, USA).

BDOC

Biodegradable dissolved organic carbon (BDOC) was measured according to the method of Joret & Levi (1986). Briefly, the inoculum was biologically active sand (sand colonised by bacteria) originating from a local drinking WTP filter. A 900 mL water sample was inoculated with 300 g of sand and aerated for the duration of the experiment. DOC was measured at the beginning and then approximately every second day until a minimum value was reached (approx. 10–12 days). BDOC concentration is derived from the difference between the initial and minimum DOC values.

Figure 2 | (a) Construction image of partially buried pipe connections (single PDS) highlighting arrangement necessary to achieve 1.05 km of pipe length in compact spacing; (b) buried PDS from far end (with air purge valves) looking towards entry/exit points.

Biofilm potential

Biofilm formation potential monitors (KWR, The Netherlands) based upon an upflow column filled with 12.4 cm^2 surface area glass coupons were employed (Van der Kooij *et al.* 1995). Biofilm coupons were sampled aseptically using a pre-flamed stainless steel wire hook into sterile 30 mL Eppendorf tubes containing 10 mL of autoclaved tap water. Sample tubes containing the glass coupon were ultra-sonicated in a water bath for 10 minutes, then decanted into another sterile tube. An additional 10 mL of autoclaved tap water was added and the procedure was repeated twice more with all solutions combined to obtain a composite biofilm solution. This was centrifuged at 4,500 relative centrifugal force (rcf) for 30 minutes then the supernatant was removed and the pellet was vortexed to resuspend. ATP concentrations were determined according to the method of Hammes *et al.* (2010). A commercial bacterial kit (BacTiter Glo, Perkin Elmer, USA) was applied with ATP calibration standards of 1×10^{-6}, 1×10^{-7}, 1×10^{-8}, 1×10^{-9} and 1×10^{-10} M. Due to the constraints of running alongside a full-scale WTP, only one continuous 140-day assessment could be made between February and June 2012.

HPCs and FCM

Bacterial enumeration was conducted using both traditional HPCs and FCM, an advanced laser optical technique. HPCs were performed in accordance with the Australian Standard AS/NZS 4276.3.1 (Australian Standard, 1995) using R2A solid media (Oxoid, Australia). Dilutions, when necessary, were performed in maximum recovery buffer (0.1% (w/v) neutralised bacteriological peptone (0.85% (w/v) NaCl, pH 7.0). Incubation was performed using standard conditions of 20 °C for 72 hours. Results for HPC were presented as colony forming units per mL (CFU/mL). HPC samples were taken fortnightly throughout the operational period. FCM analysis was conducted using a FACSCalibur flow cytometer (Becton Dickinson, USA), emitting at a fixed wavelength of 488 nm. Bacteria were enumerated following staining of the bacteria with SYTO-9 and propidium iodide (BacLight™ bacterial viability kit, Molecular Probes, USA) as described previously (Hoefel *et al.* 2003). Bacterial enumeration data were processed to determine similarity of inlets and outlets across all four streams using mathematical cluster analysis, visually presented as dendrograms, with separation expressed by Euclidean linking distances representing the dissimilarity of datasets if they were spatially positioned ('R' version 2.15.1, R Core Team 2012).

RESULTS AND DISCUSSION

Distribution system bacteriology

Microbiological stability of distributed water is traditionally evaluated using cell culturing techniques such as HPC. As a baseline for comparison, HPC on the water entering and exiting each PDS was conducted weekly within the regular sampling program. In the system inlets, there appeared to be no relationship between the increasing degree of treatment and numbers of culturable organisms for the Conv, MIEX/Coag and MIEX/Coag/GAC streams. For the Conv, MIEX/Coag and MIEX/Coag/GAC systems, HPC results at the distribution system inlets following Ct of 30 mg.min/L over 4 hours were low; with frequent zero detection (Figure 3(a)). On average, the Conv stream produced 74% less HPC response than the levels found in the source water, with MIEX/Coag and MIEX/Coag/GAC producing 90% and 70% less, respectively. The increased HPC

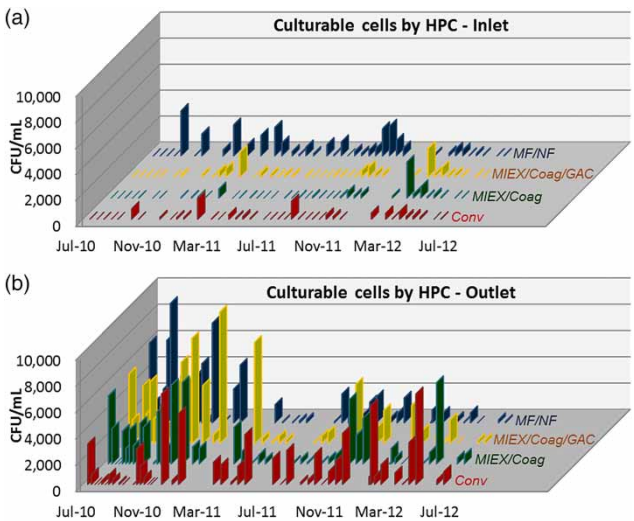


Figure 3 | HPC in the bulk water prior to entry and after detention within the PDS. (a) Inlet; (b) outlet.

reduction of the MIEX/Coag stream compared to the Conv stream demonstrated that with improved DOC removal by MIEX adsorption, the subsequent coagulation stage was more efficient for removal of bacteria (Ho *et al.* 2012). The lower average HPC reduction by the MIEX/Coag/GAC was likely due to bacteria being re-introduced via shedding from the biologically active GAC treatment stage (Velten *et al.* 2011). In the MF/NF stream PDS inlet, more than 1,000 CFU/mL were detected frequently throughout the monitoring (only average 34% reduction from source water). Although the high rejection capacity of the NF membrane would have partitioned almost all bacterial cells, it is postulated that several factors contributed to the resulting frequent detection of culturable bacteria. Firstly, the removal of over 99% of bacteria through the combined MF/NF process (Ho *et al.* 2012) would have removed much of the competitive natural biota within the water source. Secondly, following all treatment streams, the product waters were held in a series of vented storage tanks used for system automation and minimum disinfection contact time which allowed atmospheric exposure and potential for re-inoculation with native biota. Thirdly, due to the very low treated water DOC (average 0.5 mg/L C), the disinfection dose to meet the required Ct factor of 30 mg.min/L during 4 hours of tank residence time was also low (average 0.12 ± 0.16 mg/L Cl_2) which may not have been sufficient to effectively inactivate resistant bacteria species. Hallam *et al.* (2001) suggested that for water with DOC of between 1.5 and 3.9 mg/L a free chlorine residual of at least 0.2 mg/L is required to control biofilm activity. In contrast, the average chlorine dose for the other three treatment streams (Conv = 0.83 ± 0.38 mg/L Cl_2; MIEX/Coag = 0.30 ± 0.18 mg/L Cl_2; MIEX/Coag/GAC = 0.59 ± 0.70 mg/L Cl_2), coupled with a diverse and competitive natural biota may have successfully limited the ability of culturable bacteria species to proliferate. Other studies have proposed that low culturability is typical in young biofilms (months to several years) with bacteria potentially adopting a viable but not culturable state (Wingdenger & Flemming 2004). HPC data showed that in a distribution system with a deliberate lack of disinfectant residual, the conditions appeared to favour the proliferation of culturable organisms with considerably higher numbers in samples at the outlet of the PDS for all the four treated water streams (Figure 3(b)), irrespective of the water quality entering the PDS. The largest differences were seen within the first 3 months of operation (July–October 2010), presumably before the establishment of a stable biofilm.

FCM was applied as an alternative and more sensitive method of quantifying viable cell numbers. Conventional coagulation (Conv) was the least efficient treatment stream for the removal of bacteria (single treatment stage) and therefore introduced greater numbers to the PDS than the other multi-barrier treatments (Figure 4(a)). The MIEX/Coag/GAC stream was the most variable across the monitored duration due to the initial development and shedding of GAC biofilm, consistent with the findings of other pilot studies (Piriou *et al.* 1998; Volk & LeChevallier 1999) coupled with the eventual loss of adsorption capacity from November 2011 onwards. In the first 12 months of operation (Year 1) at the outlet of the pilot PDS, cell numbers within the distributed water varied weekly with changing water quality but generally increased in all streams, achieving stable outlet levels between 4 months in the Conv PDS and 8 months in the MF/NF PDS (Figure 4(b)). More advanced treatment streams took longer to reach similar equilibrium levels due to lower initial input counts. This equilibrium cell count was likely controlled by background water quality within the source water. The rate of increase of cell numbers exiting the PDS was shown to be first or pseudo-first order for streams 1 to 3 (Supplementary Table S1, available with the online version of this paper) with rate constants of less than 0.014 /day. The dual membrane stream (Stream 4) experienced short-term cell number variations confounding attempts to define a longer-term trend. The Conv stream (Stream 1) had the lowest rate (0.006 /day) due to a higher initial cell count at the exit of the PDS (outlet), reflecting the higher input cell counts. In July 2011, a PDS pipe failure necessitated a 49-day suspension of flow through the PDS for repairs. Following resumption of normal operation, active cell numbers exiting the PDS were initially depressed as a result of depletion of biodegradable organic carbon within the PDS during the extended stagnation but subsequently produced a second cycle of increasing cell discharge and equilibrium through the second year of operation (July 2011–June 2012). Unlike the initial cell count increase, which showed a relationship with the water quality, the second year appeared independent with all streams

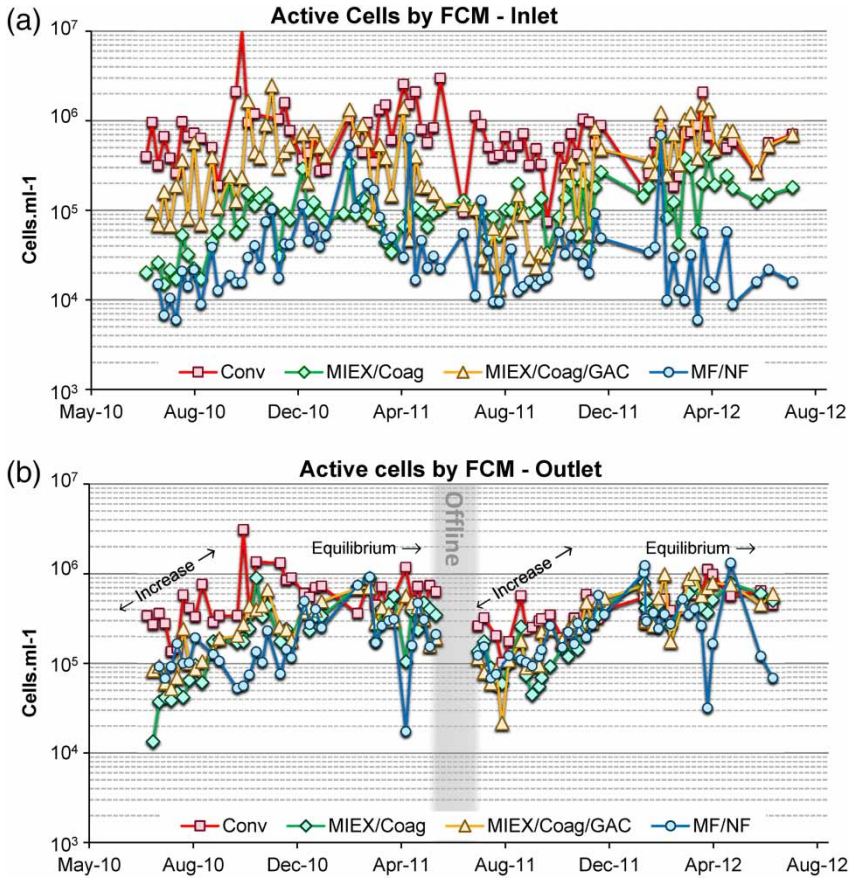


Figure 4 | Comparison of active (membrane intact) cells by FCM of bulk water at (a) inlet and (b) outlet of PDS following 78 hour detention. Offline period represents an unplanned maintenance event during which the PDS were quiescent.

stabilising after approximately 4 months at similar levels. The implication was that in the distribution system where disinfectant residuals were zero, the biological activity became stabilised such that improvements to water quality through the four different pre-treatments did not have any effect on the control of PDS microbiology. Once again, cell increase rates were statistically either first or pseudo-first order with rate constants between 0.009 and 0.014/day (Supplementary Table S1). In the second year increase, no significant relationship was established with the Conv stream data due to greater variability and higher initial active cell numbers at operational re-start. It is hypothesized that the higher DOC of this stream provided greater support for the survival of biofilm within the PDS during the extended offline period, such that less recovery was required in the Conv stream compared to the other streams before stable biofilm-bulk water interaction was restored.

Bacterial enumeration data from both FCM and HPC over the full 26 month monitoring period was processed via cluster analysis to determine the relative similarity of the four PDS inlets and outlets (Figure 5). For FCM total and active cell counts, Conv inlet was shown to be the most disparate to all other inlets and outlets. This is an indication of the different effectiveness between traditional coagulation-based, single-stage treatments and multi-stage treatments for the reduction of overall bacterial numbers. Despite the fact that passage through the PDS attenuated cell numbers in all streams, the impact of the considerably higher Conv inlet values was shown to carry through the distribution system, as the Conv stream outlet was also significantly different to all other streams. The relationship of the culturable cell numbers from HPC (Figure 5(c)) was very different to the FCM results highlighting the fact that these two tests account for separate and unrelatable

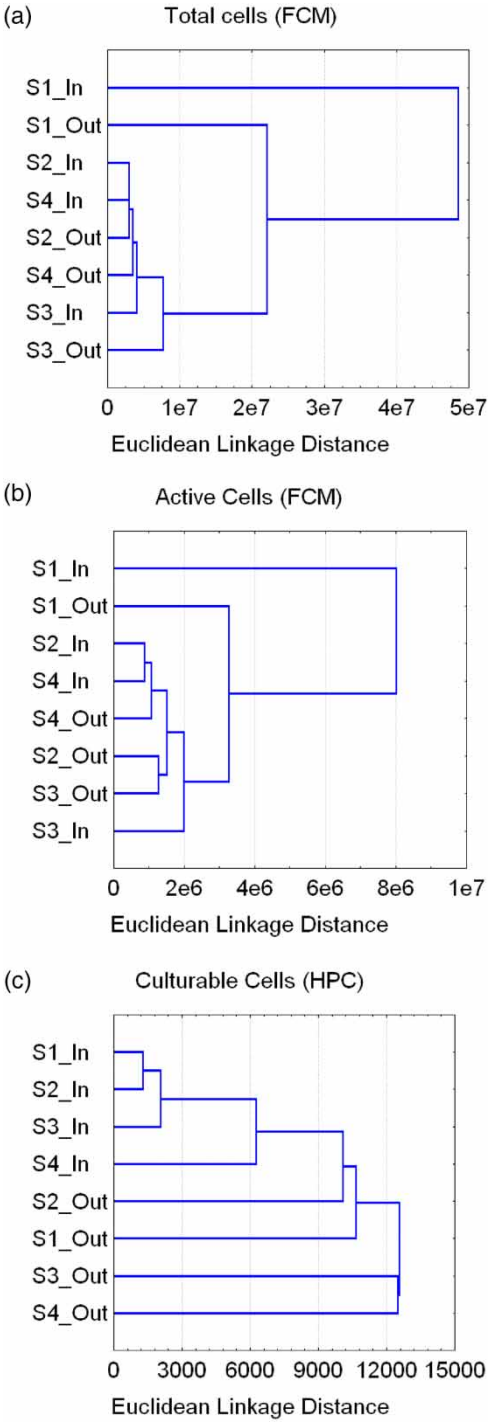


Figure 5 | Dendrogram for relationship of PDS inlets and outlets over 26 month operation by FCM and HPC: (a) total cells; (b) active cells; and (c) culturable cells. S1 = Conv; S2 = MIEX/Coag; S3 = MIEX/Coag/GAC; S4 = MF/NF.

characteristics of the bacterial community (Ho *et al.* 2012). Conv, MIEX/Coag and MIEX/Coag/GAC inlets were very similar for trends of culturable cells numbers and were considerably different to the MF/NF inlet. However, once the water had passed each respective PDS all outlets were both very different to each other and also all inlets (>10,000 Euclidean linkage distance). Given the identical pipe infrastructure in each stream, this suggests that the water quality of each treatment stream was instrumental in determining the capacity to support culturable cell (HPC) proliferation within the PDS, even when the inlet numbers were similarly low, or undetectable (Figure 3(a)).

As the systems were newly installed at the start of the project and then operated for 2 years, a change in the bacteriological character of the distribution systems was demonstrated over time. This is demonstrated in Figure 6, where the relationship of the active cell number in the

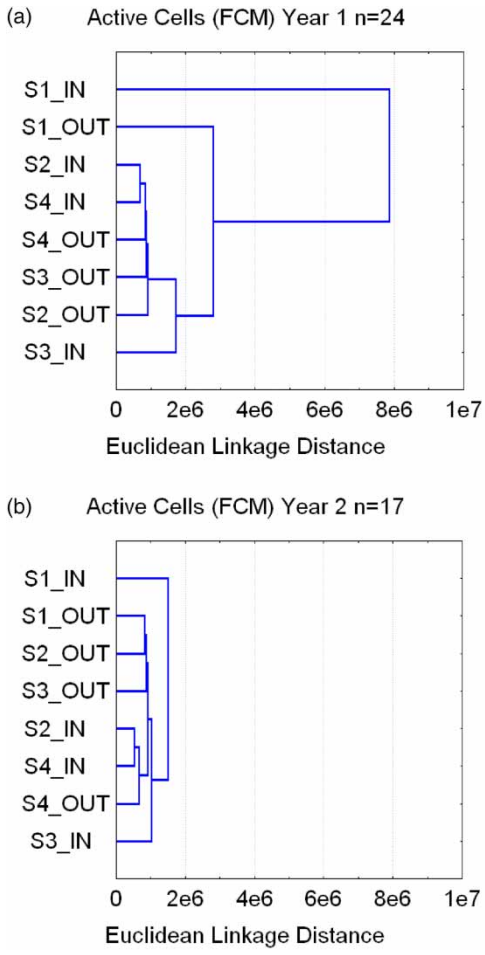


Figure 6 | Dendrogram for relationship of PDS inlets and outlets by FCM active cell counts in: (a) Year 1 (July 2010–June 2011) and (b) Year 2 (August 2011–July 2012). S1 = Conv; S2 = MIEX/Coag; S3 = MIEX/Coag/GAC; S4 = MF/NF.

PDS inlets and outlets from the first year of operation were compared for similarity to data from the second year. In Year 1 (Figure 6(a)), the MIEX/Coag and MF/NF inlets and outlets and the MIEX/Coag/GAC outlet were the most closely related in terms of active cell numbers. The MIEX/Coag/GAC treatment PDS inlet was less related due to the impact of the developing biological activity on the GAC columns. The conventional coagulation stream was notably different in active cell numbers to the three more advanced multi-step processes, especially with regard to the numbers of bacteria introduced at the inlet to the PDS. Within the second year of operation (Figure 6(b)), active cell numbers entering and exiting the PDS were much more closely related, even for the previously disparate Conv and MIEX/Coag/GAC streams, demonstrating a statistical convergence of bacterial numbers in the PDS outlets. This similarity suggests that as the PDS approached 24 months' operational life without a disinfectant residual, incorporation and shedding of certain levels of bacterial numbers by the PDS biofilm had stabilised and once established, was relatively constant regardless of what water quality was supplied at the inlet. As a result, the bacterial water quality exiting the PDS had become independent of the degree of treatment in the absence of adequate disinfection. The consequence is that any future attempts to 'recondition' the PDS through improved treatment performance, while not impossible, would be difficult to achieve once the biofilm to bulk water equilibrium had been established. It is important to note however that the objective should not be to attempt to prevent biofilm formation entirely but to ensure that biofilms do not impair water quality or harbour opportunistic pathogens through adequate controls.

Treatment efficiency link to biofilm potential

Two different measures of bacterial activity were applied in the latter half of the monitoring period to evaluate the potential of the treated waters to support bacterial growth. BDOC represents the fraction of DOC that can be mineralised by bacteria and is designed to simulate the degradation of organic matter due to biological 'pipe wall' interactions. The reduction of BDOC in drinking water is an important part of the water treatment process as even low concentrations are sufficient to support bacterial growth in the distribution system (Volk & LeChevallier 1999; Van der Kooij 2000). Figure 7(a) shows the average of BDOC values over 10 months of monitoring during Year 2 (July 2011 – June 2012) and demonstrates that the Conv stream contained higher amounts of biodegradable organic matter than the other three streams. Although averages from the other three sources suggest greater reduction with increasing level of treatment, differences were not statistically significant, with individual results often below quantifiable limits (<0.1 mg/L DOC). Literature sources (Huck 1990; Escobar & Randall 2001) suggest that it is the reduction or transformation of assimilable organic carbon (AOC) that limits bacterial regrowth, rather than absolute DOC removal. The MIEX/Coag stream removed an average 15% more DOC than the Conv stream (51%) (Fabris *et al.* 2013) accounting for 94% of source water BDOC, compared

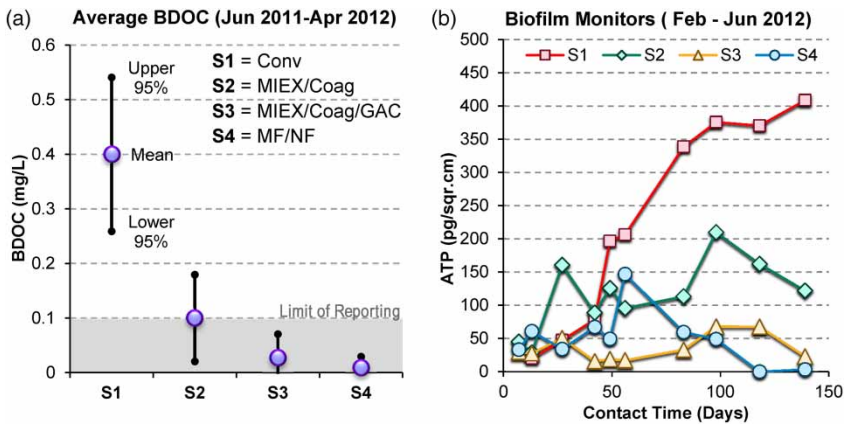


Figure 7 | Biological growth potential measures for PDS inlet water: (a) average BDOC and (b) biofilm monitor activity by ATP.

to 77% for Conv. This demonstrates the removal of additional low molecular weight organic material by the MIEX treatment (Braun *et al.* 2014) was more effective in limiting the primary energy source, yet it did not improve bacterial stability in the PDS over longer term operation (>8 months).

In addition, biofilm potential monitors were installed towards the end of the investigation period to evaluate the capacity for new biofilm establishment in each of the four treated waters prior to disinfection and entry to the PDS. After a 45-day lag period, biofilm activity in the Conv stream biofilm potential monitor increased, while the other three streams exhibited a lack of biofilm growth, expressed through low metabolic activity from the removed glass coupons (low/zero ATP). This suggests that at the time of the application of the biofilm monitors, multi-stage and advanced treatments were creating more restricted and selective conditions for biofilm proliferation (Figure 7(b)). This is consistent with the findings of Liu *et al.* (2013), who found that a tight membrane treatment (NF) was more effective at controlling biofilm formation than ion exchange treatment and loose membrane treatment (ultrafiltration). Since all treatment technologies applied except for the NF were ineffective for reduction of inorganic nutrients, this implies that they were not growth limiting. In a pilot study, Frias *et al.* (2001) showed that addition of nitrogen, phosphorous and sulphur did not contribute to greater growth of bacteria; however the organic nutrients were critical to the proliferation or lack of bacterial growth. The relationship of biological activity to organic carbon was also seen in this water source where the treatments that reduced bulk DOC more effectively were more successful in slowing the development of biological activity in the biofilm potential monitors. In addition, the MIEX/Coag/GAC stream, which incorporated a biological treatment through the GAC filter, showed the lowest level of biological activity, suggesting that AOC was also reduced and more efficiently than even the lower DOC MF/NF stream.

PDS bacteriology link to water quality parameters

In most literature sources it has been shown that there is poor correlation between bacterial enumeration in distribution systems and traditionally monitored water quality parameters (Power & Nagy 1999; Carter *et al.* 2000). In most cases this is because water quality parameters are often present at orders of magnitude greater than what is required to limit or control the rate of growth; therefore the relationships rely on a degree of covariance rather than true correlation (varies together with a parameter, not because of the parameter). In addition, for many water quality parameters that represent growth factors for biological activity, the relationship is causal, with the change producing a growth response only after a lag period during which the composition of the natural biota may shift to favour the species which are best adapted to the new water quality. Regardless, the use of easily measured surrogate parameters is still very appealing. One of the major growth factors controlling bacterial growth is organic carbon. While analyses like BDOC and AOC may be more directly relevant to bacterial regrowth and can help explain changes that occurred in past operation (Huck 1990; Escobar & Randall 2001; Volk & LeChevallier 2002), the lengthy period required to undertake these analyses limits their operational usefulness. Although DOC can be viewed as a bulk parameter containing both biodegradable and refractory organic matter, the change in DOC through the PDS may still be relatable to the potential for the water to support biological activity. All PDS experienced both losses and increases at different times throughout monitoring. DOC measurement before and after passage through the PDS, averaged over 26 months, showed that 0.29 mg/L (Conv); 0.22 mg/L (MIEX/Coag); 0.06 mg/L (MIEX/Coag/GAC) and 0.03 mg/L (MF/NF) of the average DOC that was input was lost, likely through incorporation into cell biomass and/or mineralisation. This represents 7.5%, 9.4%, 3.7% and 5.9% of the inlet DOC for Conv, MIEX/Coag, MIEX/Coag/GAC and MF/NF, respectively. The smallest normalised loss through the distribution system was in the MIEX/Coag/GAC stream, due to the fact that it was the only treatment train that incorporated a biological filtration process (GAC) in which a portion of the biodegradable DOC was already removed. Escobar & Randall (2001) showed that although NF removed up to 97% of the BDOC, no significant AOC was removed and that aligned with the findings of this study. Despite the high level of DOC removal through the MF/NF treatment stream,

subsequent loss through the PDS was comparable to other streams (in percentage terms).

CONCLUSIONS

- In a distribution system with a deliberate lack of disinfectant residual, conditions appeared to favour the proliferation of culturable organisms with considerably higher cell counts in samples at the outlet of the PDS.
- Despite different bacterial cell counts entering each PDS from the four treatment streams, equivalent outlet cell numbers were achieved in new systems after 8 months' operation. Superior treatment streams took longer to reach equilibrium due to lower initial input counts of active bacteria and less biodegradable organic matter.
- In the first year of operation, active bacterial numbers from the conventional coagulation stream (Conv) were significantly different to the other three treatment streams (MIEX/Coag; MIEX/Coag/GAC and MF/NF) but achieved a higher degree of similarity within the second year as biological stability established in all PDS.
- Incorporation of a biological treatment reduced the overall DOC loss through the PDS by reducing BDOC within the treatment prior to distribution.
- Overall, the data suggests that although improving treatment to produce better water quality delayed bacterial deterioration of drinking water through the PDS, maintenance of disinfection residuals would still be imperative to manage long term bacterial water quality.

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