



Is nitrification the only cause of microbiologically induced chloramine decay?



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ABSTRACT

Ammonia degradation was investigated in three batch reactors with differing initial concentrations of bacteria present in the same filtered water source based on pre-treatment filtration techniques. The potential for the bacterial community to degrade the ammonia present was determined in the absence of monochloramine, simulating a distribution system where a loss of disinfectant residual has occurred. Nitrification was observed in only one of the three batch reactors, whereas rapid microbiologically induced chloramine decay was present in two reactors. Results suggest that the microbial decay factor is not a valid tool for indication of nitrification, but may be used as an indicator of the occurrence of rapid monochloramine decay. Intact bacterial cell numbers did not correlate with changes in ammonia, nitrite or nitrate concentrations and hence did not correlate with the nitrification observed. Neither use of the microbial decay factor or monitoring of ammonia oxidising prokaryotes provided an early indication for the occurrence of nitrification. Hence, monitoring of ammonia and nitrite would still be the most suitable tool for indicating nitrification.

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1. Introduction

Chloramination is the chemical disinfection of drinking water achieved by the addition of chlorine and ammonia. While offering greater stability over chlorine, monochloramine will still degrade over time. Monochloramine reacts with natural organic matter (NOM) or dissolved organic carbon (DOC) present in treated drinking waters resulting in a reduction of residual (Vikesland et al., 1998; Wilczak et al., 2003). The auto-decomposition rate of monochloramine is known to increase with decreasing pH, inorganic carbon content and temperature, as well as initial chloramine concentration (Vikesland et al., 2001).

The main cause of chloramine loss is nitrification, where ammonia produced by the decomposition of chloramine is oxidised to nitrite by ammonia-oxidising prokaryotes (AOP), including ammonia oxidising archaea (AOA) and ammonia oxidising bacteria (AOB), and nitrite to nitrate by nitrifying bacteria (van der Wielen et al., 2009). Nitrifying bacteria derive energy from the oxidation of ammonia to nitrite or nitrite to nitrate. The oxidation of ammonia can be undertaken by several genera of nitrifying bacteria

“including *Nitrosomonas*, *Nitrosolobus*, *Nitrosococcus*, *Nitrosovibrio*, and *Nitrospira*, while oxidation of nitrite in water is undertaken predominantly by members of the genera *Nitrobacter* and *Nitrococcus*” (Cunliffe, 1991). The process of nitrification can reduce the pH and alkalinity of the water, resulting in the corrosion of water pipes and storage vessels (Zhang et al., 2008) while the nitrite produced can increase chloramine decay and affect the disinfectant residual therefore affecting the ability of water authorities to ensure a safe drinking water supply (Hoefel et al., 2005a). Nitrification has been observed at low chloramine residuals (Odell et al., 1996; Skadsen, 1993; Wilczak et al., 1996) and nitrification events have been shown to coincide with rapid degradation of monochloramine residual (Cunliffe, 1991; Wolfe et al., 1988). However the mechanism by which this occurs has not been established (Wolfe et al., 1988). Remedial action required to recover affected areas of the distribution system from nitrification often involves chlorination and mains flushing as well as additional water quality monitoring. The management of chloramine decay and the prevention of nitrification are critical for water utilities managing chloraminated drinking water distribution systems. However, no single nitrification control method has been found that is effective across all water utilities, and there are cases where selected control strategies are not consistently effective for the same distribution system.

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Wolfe et al. (1988) suggested that AOB may play a role in shifting the equilibrium reaction of monochloramine, whereby monochloramine is hydrolysed while free ammonia is metabolised. Wooschlagher et al. (2001) supported this finding when they found that the rate of reduction of chloramine that occurred in a nitrifying distribution system was unable to be attributed to the reaction of nitrite increasing monochloramine decay alone and suggested AOB co-metabolism of chloramines. A recent study by Maestre et al. (2013) found in batch experiments, using a pure culture of AOB *Nitrosomonas europaea*, that monochloramine co-metabolism and biomass reactions accounted for 30–60% of the monochloramine decay observed and found that the rate of metabolisation increased with increasing concentrations of free ammonia. Krishna and Sathasivan (2010) and Krishna et al. (2012) demonstrated that soluble microbial products (SMP) originating from microbial activities may play a role in accelerating monochloramine decay by catalysing auto-decomposition and nitrite oxidation.

In this work batch experiments were utilised to determine the potential of the bacterial community present in filtered water from a water treatment plant (WTP) to degrade and/or metabolise the ammonia present, as well as investigate the resulting changes in bacterial populations in the absence of monochloramine. This simulated the planktonic bacteria present in water at the end of a distribution system where the chloramine residual has been lost and nitrification may then occur. Rapid microbiological monochloramine decay, measured as microbiological decay factor (F_m) (Sathasivan et al., 2005) has been observed in WTP filtered water previously (Cook et al., 2014). By the analysis of F_m , discrimination between chemical and microbiological factors impacting on monochloramine decay can be determined (Sathasivan et al., 2005). Monochloramine decay tests were completed in waters from each batch to determine if the onset of nitrification could be predicted by the onset of microbiologically-assisted monochloramine decay or F_m analysis.

2. Methodology

2.1. Batch details

Three 20 L batch reactors were used to investigate the metabolism of ammonia by indigenous bacteria present in filtered water from a WTP. Water for the reactors was sourced from the Tailem Bend WTP, South Australia, Australia. Samples were collected post filtration but prior to disinfection, denoted as Tailem Bend Filtered Water (TBFW). The Tailem Bend WTP is located along the River Murray in South Australia, approximately 100 km east of Adelaide and sources water directly from the River Murray through an off-take pump station. It utilises conventional treatment (coagulation, flocculation, sedimentation and filtration) prior to disinfection by chloramination and distribution. The water quality challenge of the WTP results from the variable nature of the River Murray raw water, over the past 12 years the turbidity has ranged from 8 to 240 NTU (average 45 NTU, $n = 643$), dissolved organic carbon (DOC) has ranged from 2.5 to 7.8 mg/L (average 5.1 mg/L, $n = 620$), total dissolved solids has ranged from 93 to 1200 mg/L (average 391 mg/L, $n = 642$), true colour (456 nm) has ranged from <1 to 147 HU (average 16 HU, $n = 547$). Historically the distribution system supplied from this WTP has been subject to episodes of rapid monochloramine decay leading to nitrification.

The first batch reactor contained 20 L of the TBFW taken from the plant; the second reactor contained 20 L of the TBFW supplemented with particulate material obtained from filtration of 40 L of sample water through a 0.2 μm mixed cellulose ester (MCE) filter paper (Advantec MFS, Inc. California) to increase the number of bacteria present, while the third reactor contained 20 L of 0.2 μm

TBFW. TBFW was filtered directly through a 0.2 μm MCE filter paper and the filter paper was collected and placed in a 50 mL centrifuge tube with a small volume of TBFW. The tube was vortexed to release the captured material from the membrane into solution. Captured material in solution was added to batch reactor 2, ensuring a total volume of 20 L. Each batch reactor was then dosed with 0.8 mg/L free ammonia. The amount of ammonia added was selected to promote nitrification and was chosen to be similar to that observed in the early stages of nitrification following loss of chloramine residual in a chloraminated water supply. All reactors were covered to exclude light to simulate conditions experienced in distribution system pipes and agitated only when samples were taken. Batch reactor details are shown in Fig. 1. The chemical water quality was uniform across the three batch reactors. However, there was a potential for a higher abundance of particulate material as well as the bacterial population in Batch 2 due to the addition of captured material, and a lower abundance in Batch 3 due to filtration through 0.2 μm .

Samples were collected regularly from each reactor and analysed for ammonia, nitrate and nitrite. Microbial abundance was determined by flow cytometry for bacterial counts, quantitative polymerase chain reaction (PCR) for AOA and AOB and by next generation sequencing of 16S rRNA PCR amplicons for diversity profiling. Chloramine decay tests and calculation of F_m were conducted weekly to fortnightly.

2.2. Analyses

2.2.1. Ammonia analysis

Analytical grade liquid ammonia (1000 mg/L as N) was used as the source of ammonia made from ammonium chloride AR (Chem-Supply Pty Ltd. Australia). Ammonia concentrations were determined using ammonia-selective electrode Standard Method 4500-NH₃ (D) (APHA et al., 1998). Ammonia measurements were recorded routinely for each of the three batch reactors to monitor for degradation over time.

2.2.2. Nitrate and nitrite measurements

The Hach DR890 Colorimeter was used for both nitrate and nitrite methods. Nitrate (NO₃⁻-N) was measured using the cadmium reduction method (Method 8039) (Hach Company, 2013). Briefly, 10 mL of sample is vigorously mixed for 1 min with NitriVer 5 Nitrate Reagent Powder Pillow and then the amber colour allowed to develop over a 5 min reaction period before reading on the colorimeter. The range for the method used is 0.0–30.0 mg/L. Nitrite (NO₂⁻-N) was measured using the diazotization method (Method 8507) (Hach Company, 2013). Briefly, 10 mL of sample is reacted over 15 min with NitriVer 3 Nitrite Reagent Powder Pillow and read using the colorimeter. A pink colour develops in the presence of nitrite, range 0.0–0.350 mg/L.

2.2.3. Chloramine decay tests

Chloramine decay tests were conducted to determine the relative contribution of chemical and microbiological factors on monochloramine decay with batch reactor samples assessed weekly to fortnightly; parameters are shown in Table 1. A target dose of 2 mg/L monochloramine was used as that level is typically observed at the end of the TB distribution system (Cook et al. (2014)). It was hypothesised that the presence of microbiological monochloramine decay could be used as an early indicator of nitrification. Combined chlorine residuals, pH, temperature and ammonia were analysed at times 0hr, 4hr, 3d and 7d.

Free chlorine stock water was prepared by the addition of gaseous chlorine (99.8%) to ultra-pure water to produce a saturated stock of 2000–4000 mg/L. Free and combined chlorine was

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