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Effects of magnetically treated water on the survival of bacteria in biofilms

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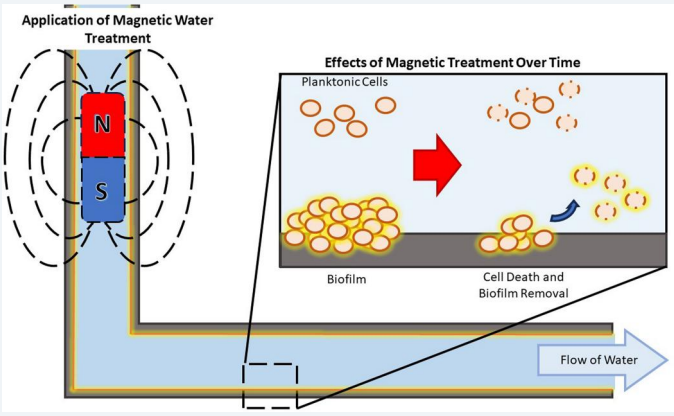
ABSTRACT

The goal of this study was to evaluate if a magnetic water treatment device could be used to mitigate biofilms in water systems. Magnetic treatment was applied to water upstream of a modified Robbins device in which *Pseudomonas fluorescence* biofilms were formed. Duration of magnetic treatment, system flow rate, and field strength were varied to assess the impacts on the biofilm. A control system was concurrently established in which no magnetic treatment was applied. After treatment, the number of viable cells in the biofilm was reduced by up to 2.46 $\log_{10}$ CFU cm^{-2} depending on the operational conditions. Increased cell stress, and ultimately death, was observed during treatment as indicated by an elevated AMPi stress index. These results indicate that magnetic water treatment may be an effective technology to decrease the extent of biofilms in water systems and a reduced need for chemical treatment. A mechanism is proposed in which metabolic processes are hindered due to the magnetic field effects on ions in the water. However, a mechanistic investigation remains outside the scope of this study. Future studies should aim to characterize both the impacts of treatment on the matrix and cellular processes to determine a mechanism for the observed effects.

GRAPHICAL ABSTRACT



HIGHLIGHTS

- Magnetic water treatment reduced the number of viable bacterial cells present in biofilms by 2.46 $\log_{10}$ CFU/cm^2 after 15 days of treatment.
- Magnetically treated water results in increased cellular stress as indicated by the ratio of AMP/ATP in bacteria.
- Removal of sessile cells from biofilms occurred without the direct interaction between the magnetic field and the biofilms.

ARTICLE HISTORY

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KEYWORDS

Magnetic water treatment; biofilm removal; *Pseudomonas fluorescence*; water treatment; disinfection

Introduction

The presence of microbial communities in an aqueous environment frequently results in the formation of biofilms, which pose operational and public health challenges in both process water and potable water. Biofilms are three-dimensional structures composed of extracellular polymeric substances (EPS) including lipids, proteins, and nucleic acids, and

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microorganisms (Dufour et al. 2010). These structures provide protection to biofilm associated microorganisms from environmental stressors such as desiccation (Flemming and Wingender 2010), pH (Hoštacká et al. 2010), salinity (Kim and Chong 2017), and disinfectants. The increased resistance to many common disinfectants including chlorine (Goeres et al. 2004), UV disinfection (Ahmad 2017; Yin et al. 2019), and chloramines (Herath and Sathasivan 2020) makes the removal of biofilms difficult without the aid of additional physical processes or enhanced chemical treatment. Unwanted biofilms represent a large economic cost to a multitude of industrial sectors and removal may be too impractical or costly to regularly implement (Highmore et al. 2022). Biofilm mitigation and removal *via* novel technologies (i.e. magnetic water treatment) may therefore provide a cost effective tool to supplement conventional practices.

Biofilms commonly form on the surfaces of pipes and along the surface-water interface in the built and natural environment (Springston and Yocavitch 2017). Biofilm formation occurs in four stages: reversible attachment; irreversible attachment; maturation; and detachment (Stoodley et al. 2002). Bacterial attachment can be facilitated by conditioning films (Donlan 2002) signaling molecules such as Ca^{2+} in the water (Geesey et al. 2000; Tischler et al. 2018), and quorum sensing (Liaqat 2019). During maturation, EPSs are secreted, and complex three-dimensional structures are produced that facilitate the flow of nutrients to the base layers of the biofilm (Okabe et al. 1996). These structures create diverse niches for the cohabitation of many environmental and potentially pathogenic bacterial species (Berry et al. 2006).

Biofilms act as a protective barrier from many environmental stressors and can provide a haven for organisms, including pathogens, that would not otherwise survive garnering concern from a public health perspective. Environmental biofilms may harbor bacterial pathogens such as *Pseudomonas aeruginosa* or *Legionella pneumophila* (Abdel-Nour et al. 2013). Although not all *Pseudomonas* spp. are pathogenic, their prolific production of EPS and abundance in environmental biofilms make them organisms of interest (Mann and Wozniak 2012). Infections caused by the aerosolization of bacteria from cooling towers (Walser et al. 2014), premise plumbing (Leslie et al. 2021), and hospitals (Craun et al. 2010) are of major concern as biofilm-associated *Legionella*, *Pseudomonas aeruginosa*, or other pathogens may be present in these systems (Pruckler et al. 2001; Pereira et al. 2017). Mismanagement of biofilms in cooling towers has been associated with outbreaks of legionnaires disease (Mouchtouri et al. 2010; Springston and Yocavitch 2017; Fitzhenry et al. 2017). Thus, a need for adequate biofilm control and prevention is necessary to ensure public safety.

Magnetic water treatment is commonly used in industrial settings to manage pipe scale, but the impacts on biofilm removal have been sparsely studied. These technologies facilitate the removal of pipe scale by altering the ratio of $CaCO_3$ polymorphs to favor aragonite instead of calcite (Kobe et al. 2001). In wastewater treatment, magnetic fields appear to enhance the activity in bioreactors for the removal of nitrogen (Tomska and Wolny 2008; Ji et al. 2010; Wang et al. 2012). However, many authors have also noted magnetic fields can be inhibitory, sometimes irreversibly, to many of these same process depending on both the organism and characteristics of the field (Gerencser et al. 1962; Jung et al. 1993; Liu et al. 2008). These magnetic field effects may be caused by influencing chemical reactions in which the efficiency of processes are either increased or decreased, ultimately impacting the metabolic activity of the cells (Timmel et al. 1998; Rodgers 2009). The results of these previous studies indicate that it may be possible to leverage magnetic fields of certain intensities or field characteristics for use as a biofilm control in water systems.

The goal of the present study aimed to investigate if a commercial magnetic water treatment device had the potential to remove previously established *Pseudomonas fluorescence* biofilms. The efficacy of the magnetic treatment was determined through both microbial cultural viability (dilution and plating), and microbial activity (AMP/ATP) assessments to understand if there was any potential impact on the bacterial community. AMP and ATP analysis were chosen as a measure of microbial stress to determine if general metabolic activity was impacted by the treatment. Keasler et al. (2013) noted that shifts in the AMP to ATP ratio away from ~10:1 may be indicative of metabolic stress (Atkinson 1968; Walker-Simmons and Atkinson 1977). Duration of magnetic treatment, flow rate, and magnetic field strength were all evaluated over the course of this study to optimize the treatment conditions required for biofilm mitigation. A common critique of magnetic treatment is the localized nature of the magnetic field relative to the biofilms in many industrial systems (e.g. cooling towers). To incorporate this challenge, a system was designed such that the magnetic field was applied to only the recirculating water, with no direct exposure

of the biofilm within the modified Robbins device (MRD) to the magnetic field.

Materials and methods

Magnetic water treatment device

A magnetic water treatment device designed to fit around the outside of a 5 cm pipe was obtained from Vodaa Technologies (Chandler, AZ) (Figure 1). The strength of the electromagnet was controlled by a power supply unit operated at ~4 A per the manufacturer's instructions unless otherwise stated. The field strength through the center of the magnet was found to be 50, 74, and 20 gauss (top, middle, and bottom respectively) as measured by a DC Gaussmeter Model GM-1 HS (AlphaLab Inc, USA). The magnet dimensions were 35.56 cm x 13.97 cm x 5.78 cm (Height x Outer Diameter x Inner Diameter).

Preparation of Pseudomonas fluorescence culture

Pseudomonas fluorescence 15769 was obtained from ATCC. Bacterial cultures were grown at 26 ± 1 °C in either nutrient broth (HiMedia, Mumbai, India) or on nutrient agar plates. Bacterial cultures were prepared by streaking colonies to isolation on a nutrient agar plate and incubating for 48 h. A single colony was subsequently inoculated into a culture tube containing 20 mL of nutrient broth and incubated for 24 h. By performing a broth-to-broth transfer, 20–20 mL culture tubes of nutrient broth were inoculated with one loopful of broth and incubated overnight for 22 ± 2 h.

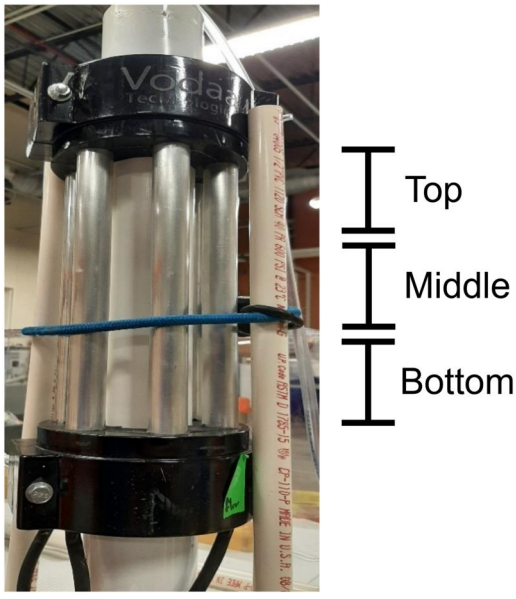

Figure 1. Magnetic water treatment device.
A static magnetic field with a maximum strength of 50, 75 and 20 gauss (top, middle, and bottom, respectively) when the magnetic device (14" x 5.5" x 2.25" (H x D x ID)) was operated at ~ 4 A.

Following overnight growth, the culture was collected with a pipette taking care to not transfer the pellicle. The cells were concentrated *via* centrifugation 3500 rpm for 15 min, and subsequently washed with 10 mL sterile tap H_2O three times. The cells were then resuspended in 175 mL of nutrient broth and the concentration was adjusted to an OD600 of 0.095–0.097 using a HACH DR-3900 spectrophotometer.

Generation of Pseudomonas fluorescence biofilms

Biofilms were generated by recirculating the prepared *Pseudomonas* culture across two polysulfone Modified Robbins Devices in parallel, both equipped with stainless-steel coupons, obtained from Tyler Research Corporation (Alberta, Canada) (Figure S1). Each MRD contained 25 studs outfitted with 50 mm^2 stainless steel coupons. The culture was recirculated over the coupons at room temperature for 24 h at 100 mL min^{-1} using a peristaltic pump. Flows across each MRD were equalized with valves if necessary. Excess air was removed from the tubing, valves, and MRDs to ensure the coupons had adequate contact with the culture. Brass fittings were used where possible due to their non-magnetic, limited antimicrobial and anticorrosive properties. Stainless steel was used where brass was not possible to limit corrosion. Tubing was either Tygon® Masterflex (Masterflex, Illinois) for the peristaltic tubing or poly-vinyl chloride (PVC) for the transfer tubing.

Description of the recirculating system for magnetic treatment

After the formation of biofilms, excess culture was removed from both MRDs. Each MRD was then transferred into a different recirculating system (Figure 2). Both recirculating systems contained a 2 L water reservoir, a peristaltic pump, a section of 5 cm x 43.2 cm (Diameter x Height) PVC pipe, and a MRD with the previously formed *Pseudomonas* biofilm. The section of PVC pipe served as the instillation location of the magnetic treatment device. Magnetic treatment was then applied to one reactor while the untreated system served as the control.

Each reservoir consisted of three sets of brass ports for inlet tubing, outlet tubing, and a port fitted for a 0.2 µm air-vent to facilitate gas transfer into the system. For all experiments, 2 L of sterilized tap water

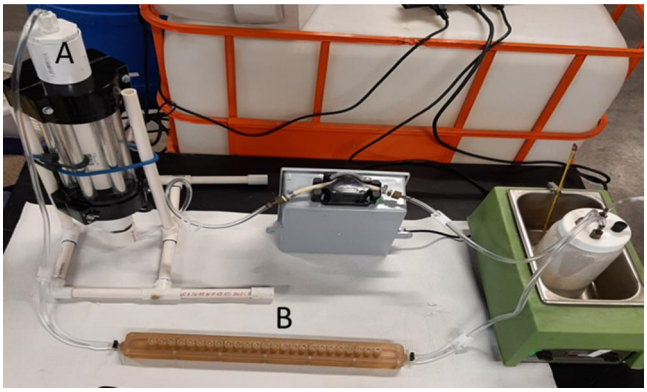

Figure 2. Magnetic water treatment reactor.
Water in the reactor was passed through the magnetic treatment device (a) followed by the modified Robbins device containing previously established *Pseudomonas* biofilm coupons (b).

were used in each system. Temperature was maintained by submerging the reservoirs in a 26–27 °C water bath. Flow rates were controlled by a peristaltic pump located immediately after the reservoir. The magnetic treatment device was installed around PVC pipe of the treated system, with the flow going from the north to south pole of the electromagnet. The PVC section was oriented vertically and remained filled to maximize treatment volume and prevent bubbles from entering the MRD. The MRD was located after the PVC section. The magnetic field produced by the device did not extend into the MRD and thus only treated water passing through the PVC section was magnetic treated rather than the biofilms directly.

Biofilm sampling

Coupon sampling locations were determined by subdividing the MRD, into sections based on the number of assays, replicates, and timepoints for the study. One coupon from each section was removed and used for either dilution and spread plating, or for the ATP/AMP analysis. This was subsequently repeated for each section. The sampling scheme for all experiments is presented in Table S1. To sample, pumps were turned off and transfer tubing was clamped to prevent leaks. Studs holding the stainless-steel coupons were carefully removed from the MRD. Coupons were then washed using 5 mL of phosphate buffered saline (PBS) (Sigma Aldrich, St. Louis) dropwise to remove any non-adhered cells. Excess liquid was removed using a KimWipe and capillary action to prevent damage the biofilm. The coupon was then utilized either for dilution and spread plating to assess viable cells, or ATP/AMP analysis for cellular activity. After completion of the assays, the coupons were submerged in 95% EtOH and flame sterilized. Once cooled, the coupons were replaced in their respective location in the MRD, and the system was turned back on to allow for subsequent treatment prior to the next sampling event. Samples of water were also collected from the reservoir and assessed for the number of viable planktonic cells.

Assessment of viable cells in the biofilm by dilution and spread plating

After sampling and washing, the coupons were placed into 5 mL snap capped tubes filled with 2 mL PBS. Biofilms were disaggregated from the coupon by vortexing the tube at high speed for 30 s, followed by 30 s of ultrasonication at 42 kHz, and then vortexing again for 30 s. The suspensions were transferred to sterile 1.5 mL Eppendorf tubes. Quantification of viable cells was determined by ten-fold serial dilutions and spread plating onto nutrient agar plates. Plates were incubated at 26 °C for 48 h and *Pseudomonas* colonies were subsequently counted.

ATP and AMP assessment

ATP and AMP levels for each biofilm were assessed using the LuminUltra DSA kit (New Brunswick Canada). In brief, the biofilm was sampled using a sterile swab wetted with LumiSolve™ reagent to swab the surface for 20 s. The swab was then placed in the UltraLyse™ solution, briefly vortexed, and incubated for 5 min. After incubation, 1 mL of the lysed sample was then transferred to the UltraLute™ solution and inverted to mix. ATP was analyzed by combining 100 µL of the Luminase™ enzyme and 100 µL of the UltraLute™ solution and the RLU tATP was then immediately measured using a luminometer. AMP was analyzed using the same procedure with the use of the LumiAMP™ enzyme and incubating for 1 min after the addition of the sample. The AMP stress index (AMPi) was calculated using the following equation:

$$AMPi = \frac{RLU\ tAMP\ per\ coupon}{RLU\ tATP\ per\ coupon}$$

Assessment of Pseudomonas fluorescence biofilm formation consistency along the MRD

The consistency of the parallel *Pseudomonas* biofilm seeding procedure was assessed to eliminate potential discrepancies across each MRD or caused by sampling location. 12 coupons were sampled across the length of each MRD and evaluated through dilution and

spread plating as described previously. Coupon locations were sampled as follows: the initial 3 coupons, final 3 coupons, and 6 equidistant coupons in the middle. This sampling scheme was chosen due to observations on seeding variation in MRD's noted by Kerr et al. (2000). The experiment was repeated three times.

Proof of concept: pilot study evaluating the influence of magnetic water treatment on biofilms

A pilot study evaluating the effects of 7 days of magnetic water treatment on previously established biofilms was performed to determine if the treatment impacted cell viability and cell stress in the biofilm. After the formation of the *Pseudomonas* biofilms, baseline samples ($t = 0$ days) from each MRD were evaluated prior to being transferred into the recirculating system. After installing the MRD, sterile tap water was circulated through the system at 100 mL min^{-1}, and excess air was removed from the MRD's and tubing. The magnetic water treatment device was powered on, and the power supply unit was adjusted to ~4 A to prior to treatment.

A 7-day trial was performed, and three sample locations were used for dilution and plating, while one was assayed for ATP/AMP assessment. Samples were collected after 0, 1, 3, 5 and 7 days of treatment.

Evaluation of the effects of flow rates on magnetic water treatment

The impacts of different flow rates (100 mL min^{-1}, 500 mL min^{-1}, or 1000 mL min^{-1}) on the effectiveness of magnetic water treatment of previously established biofilms was evaluated in separate 15-day studies. The extended duration of the experiment was chosen due to the results of previous experiments. After the formation of the *Pseudomonas* biofilms, background samples ($t = 0$ days) from each MRD were evaluated prior to transfer into the recirculating system. Sterile tap water was circulated through the system at 100 mL min^{-1}, 500 mL min^{-1}, or 1000 mL min^{-1}, and excess air was removed from the MRD's and tubing. The magnetic water treatment device was turned on following adjustment of the power supply unit to ~4 A.

Due to the increased number of timepoints in the 15-day trials, 2 sample coupons were used for dilution and plating and 2 more for ATP/AMP for each timepoint. Samples were collected on days 0, 3, 6, 9, 12, and 15.

Evaluation of reduced magnetic field strength on the effectiveness of magnetic water treatment

In a subsequent experiment, the power supply of the magnetic treatment device was reduced to ~3 A to investigate the effects of a weaker magnetic field on the bacteria in the biofilms. A flow rate of 500 mL min^{-1} was chosen for this 15-day study based on results from previous experiments. After the formation of the *Pseudomonas* biofilms, baseline samples ($t = 0$ days) from each MRD were evaluated prior to transfer into the recirculating system. Sterile tap water was circulated through the system at 500 mL min^{-1} and excess air was removed from the MRD's and tubing. Once the system was operating, the magnetic water treatment device was turned on, and the power supply unit adjusted to ~3 A. The resulting magnetic field was reduced to 40, 60, and 20 gauss (top, middle, and bottom, respectively).

This 15-day experiment assessed two sample locations per timepoint for viable cells by dilution and plating, while 2 more were assayed for ATP/AMP assessment. Samples were collected on days 0, 3, 6, 9, 12 and 15.

Data analysis

Statistical significance was determined using a Students T-test. Log reduction of the treatment was determined by the following equation, with the number of viable cells in the biofilm at day 0 as the 'initial' for given system:

$$Log\ \ Reduction = log_{10}Initial - log_{10}Final$$

Standard error was calculated where sufficient replicates allowed.

Results

Assessment of Pseudomonas fluorescence biofilm formation consistency along the MRD

The *Pseudomonas* biofilm formation protocol was evaluated to verify that consistent initial biofilm formation occurred across the different coupon locations within each MRD and between MRDs. The log_{10} average of viable cells cm^{-2} was 6.78 and 6.86 in MRD-1 and MRD-2, respectively (Figure 3). Some variation in biofilm concentration occurred in the middle of both MRDs however, no significant difference ($p < 0.05$) across MRD-1 and MRD 2 was observed ($p = 0.18$). No significant difference was observed in the number of viable cells recovered from the initial 3 coupons ($p = 0.18$), final 3 coupons

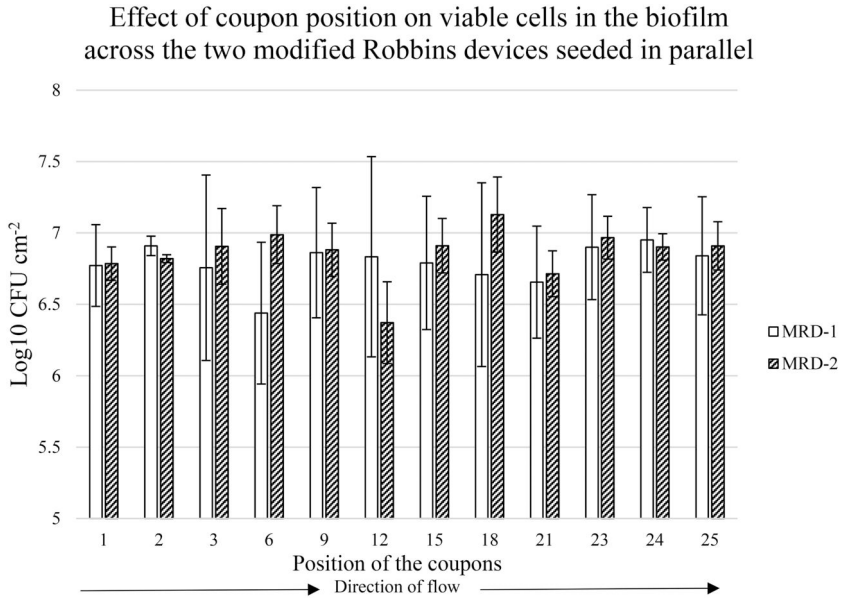

Figure 3. Evaluation of biofilm consistency by location across MRD's seeded in parallel.
The quantity of viable cells of the biofilm was evaluated for each MRD seeded in parallel. Numbers indicate the position of the coupon in the MRD. No significant ($p < 0.05$) difference was observed between MRD-1 and MRD-2 ($p = 0.18$). No significant ($p < 0.05$) differences were observed across the initial, middle, or final coupons of MRD-1 when compared to MRD-2 ($p = 0.18$, 0.17, and 0.27 respectively). Error bars indicate the standard deviation of each coupon position ($n = 3$).

($p = 0.17$), or middle 6 coupons ($p = 0.27$) between the MRDs.

Proof of concept: pilot study evaluating the influence of magnetic water treatment on biofilms

The influence of magnetic water treatment on previously established *Pseudomonas* biofilms was evaluated *via* cellular stress and cell viability assays over a 7 day period. With respect to cellular stress, an elevated AMPi was often observed prior to transferring the biofilms into the recirculating loop. Due to this, day 0 is omitted when discussing general trends in the AMP, but the data are presented in the corresponding figures (Figures 4 and 5). This issue is addressed in 'Discussion' section.

The AMPi of the untreated system remained constant throughout the course of the experiment with a range of 0.73 (min 2.16–max 2.89) (Figure 4). More variation in the AMPi was observed in the treated system with a range of 8.06 (min 2.37–max 10.43) with the largest increase in cell stress occurring between day 3 and day 5. The number of viable sessile cells in the untreated system remained constant throughout the experiment. However, the number of viable sessile cells in the treated system was reduced by 1.02 and 1.34 $\log_{10}$ CFU cm^{-2} at day 5 and day 7, respectively, compared to day 0 (Figure 5).

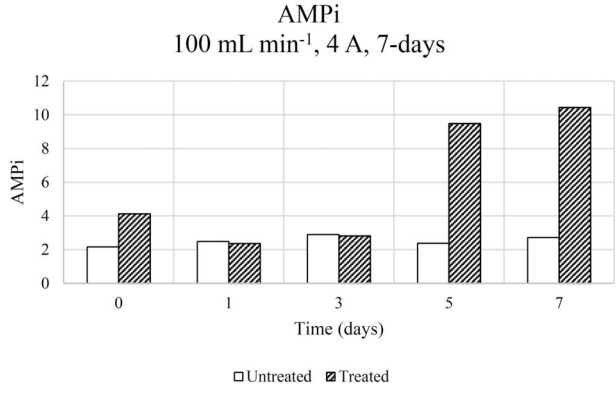

Figure 4. Cell stress after 7-days of magnetic water treatment.
Average AMPi of biofilms after 7-days at a flow rate of 100 mL min^{-1}. Magnetic device was operated at ~ 4 A.

Evaluation of the effects of flow rates on magnetic water treatment of biofilms

The impact of different flow rates (100, 500 and 1000 mL min^{-1}) on magnetic water treatment was evaluated in three separate 15-day experiments. As previously noted, elevated AMPi was observed prior to transferring the established biofilms into the recirculating system. Due to this, day 0 is omitted when discussing trends in the AMPi but the data are presented in the corresponding figures (Figures 6 and 7). This issue is addressed in the 'Discussion' section.

When the recirculating system was operated at 100 mL min^{-1} the AMPi of the untreated system remained consistent throughout the experiment

(range 0.96, min 1.07–max 2.03) (Figure 6a). The AMPi in the treated system magnetic showed a trend of elevated stress starting from day 6 onward (range 14.7, min 1.42–max 16.1). The cell viability data showed an increase of 1.20 $\log_{10}$ CFU cm^{-2} in the untreated biofilm, whereas a decrease of 1.06 $\log_{10}$ CFU cm^{-2} occurred in the treated system by the end of 15 days (Figure 7a).

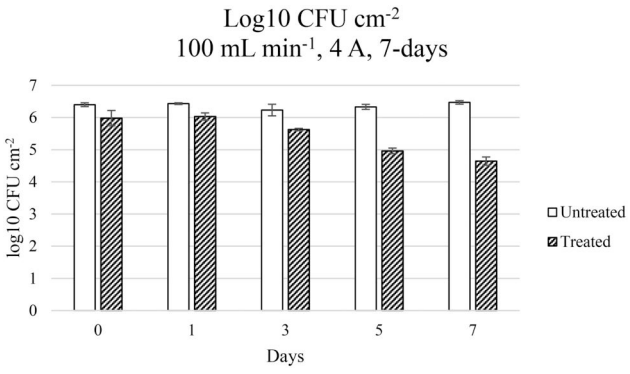

Figure 5. Cell viability after 7-days of magnetic water treatment.
Viable cells in biofilms after 7-days at a flow rate of 100 mL min^{-1}. Magnetic device was operated at ~4 A. Cell viability in the biofilm was consistent throughout the 7-days in the untreated system. Reduction of 1.02 and 1.34 $\log_{10}$ CFU cm^{-2} were observed after 5 and 7 days of magnetic treatment. Error bars indicate the standard error between coupons on each day (n = 3).

At a flow rate of 500 mL min^{-1}, the range of the AMPi in the untreated system was 1.82 (min 1.27–3.08 max), whereas there was approximately a 3-fold greater range of 5.66 (min 1.65–max 7.32) observed in the treated system (Figure 6b). The AMPi of the treated system was elevated from day 6 onward and remained higher than the untreated system throughout the remainder of the experiment. The number of viable sessile cells in the untreated system increased by 0.56 $\log_{10}$ CFU cm^{-2}, whereas a decrease of 2.46 $\log_{10}$ CFU cm^{-2} was observed in the treated system by day 15 (Figure 7b).

When the flow rate was increased to 1000 mL min^{-1} the range of the AMPi was 3.59 (min 1.36–max 4.95) and 2.24 (min 1.66–max 3.90) for the untreated and treated systems respectively (Figure 6c).

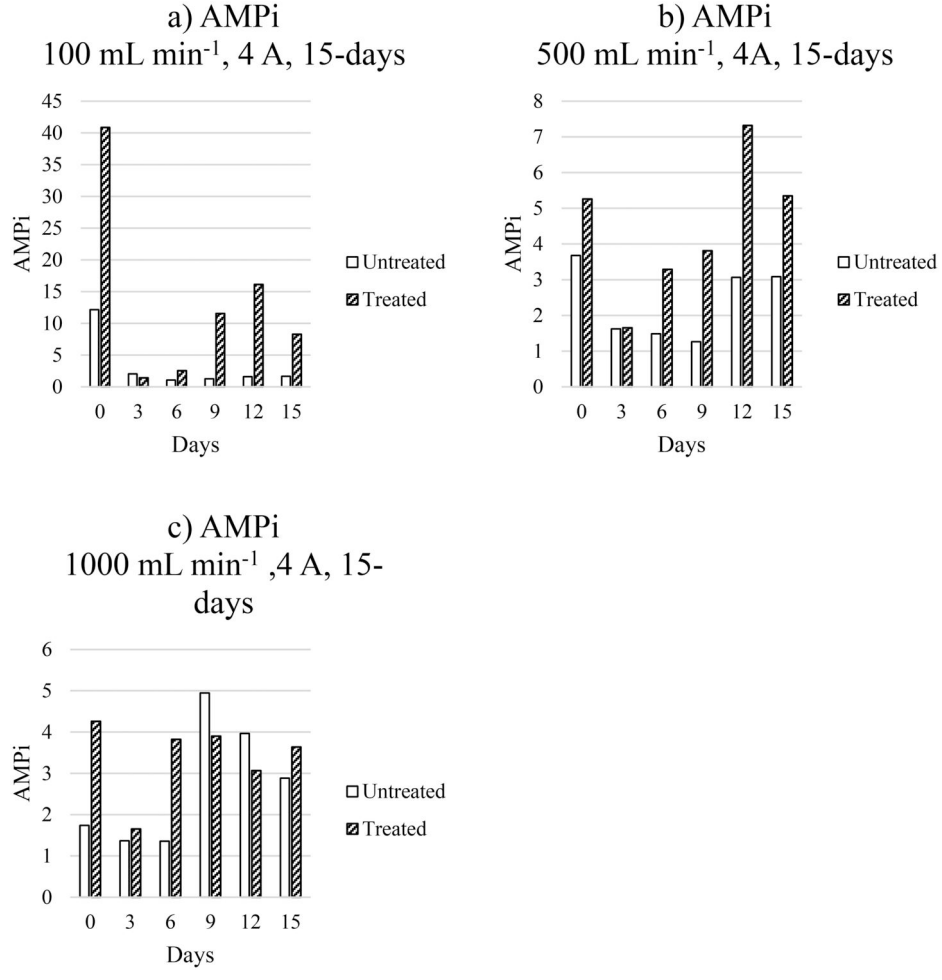

Figure 6. Influence of flow rate on cell stress of biofilms under magnetic water treatment.
AMPi of biofilms over 15-days of magnetic water treatment (~4 A) at three different flow rates 100 mL min^{-1} (a), 500 mL min^{-1} (b), and 1000 mL min^{-1} (c).

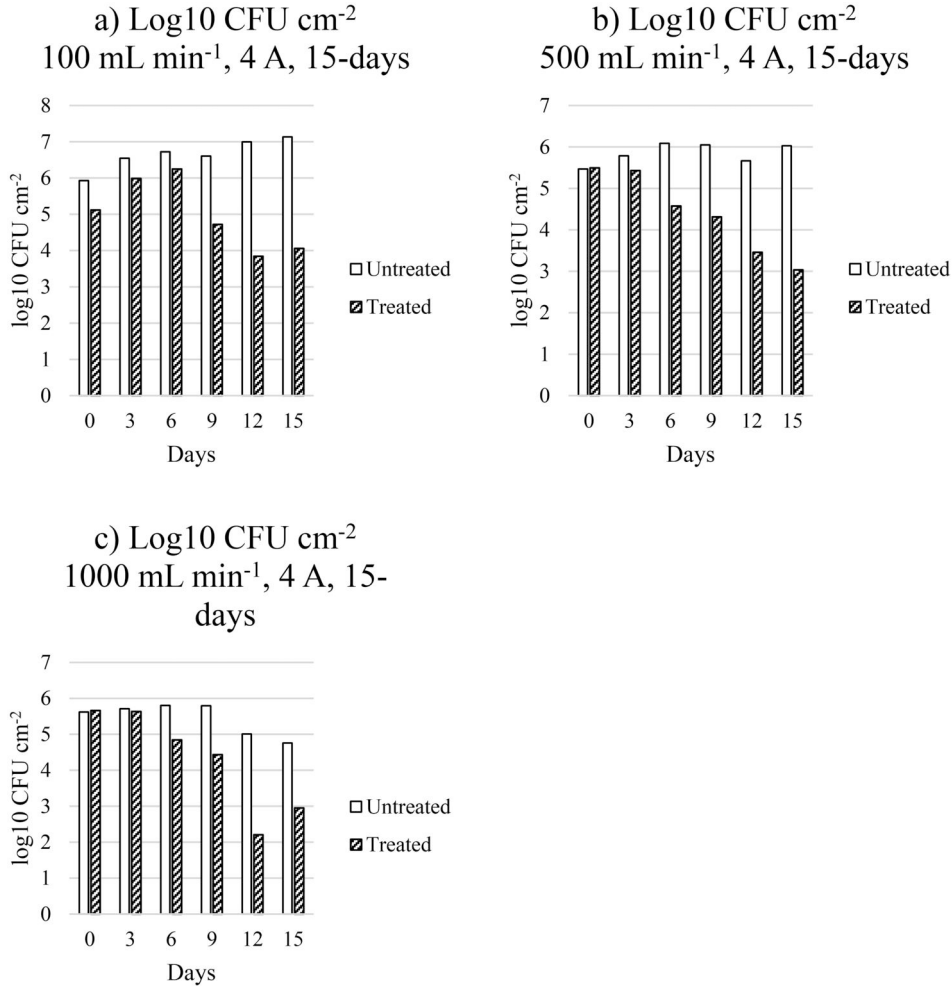

Figure 7. Influence of flow rate on cell viability in biofilms under magnetic water treatment.
Viability of cells biofilms over 15-days magnetic water treatment (~4 A) at three different flow rates 100 mL min^{-1} (a), 500 mL min^{-1} (b), and 1000 mL min^{-1} (c).

Although elevated AMPi occurred in the treated system at day 6, a similar magnitude of increase was observed at day 9 in the untreated system. The number of viable sessile cells in the untreated system remained consistent until day 12, where upon a reduction of viable cells began, resulting in a decrease of 0.86 log_{10} CFU cm^{-2} by day 15 (Figure 7c). A more rapid decrease of viable cells in the biofilm began on day 6 in the treated system, and resulted in a final removal of 2.71 log_{10} CFU cm^{-2} at day 15.

Evaluation of reduced magnetic field strength on the effectiveness of magnetic water treatment

The impact of magnetic field strength was evaluated by decreasing the power supply to ~3 A to produce a weaker magnetic field. Magnetic water treatment at this weaker magnetic field was then conducted for 15 days at 500 mL $min^{-1.}$ Prior to transferring the MRD to the recirculating system an elevated AMPi was observed. Due to this, day 0 is omitted when discussing trends in the AMPi but the data are presented in the corresponding figures (Figures 8 and 9). This issue is addressed in the 'Discussion' section.

The AMPi of both systems increased over the duration of the study with the range of the untreated system found to be 2.24 (min 1.41–max 3.65) whereas the treated system had a range of 2.87 (min 1.69–max 4.56) (Figure 8). Although the AMPi in both systems increased the number of viable cells in the biofilm increased as well. A 0.68 and 0.84 log_{10} CFU cm^{-2} increase was observed in the untreated and treated systems respectively at the end of 15 days (Figure 9).

Discussion

The inadequate removal of biofilms may pose a potential public health risk *via* the ingestion or inhalation of biofilm associated pathogens (e.g. *Pseudomonas* and *Legionella*) (Huq et al. 2008).

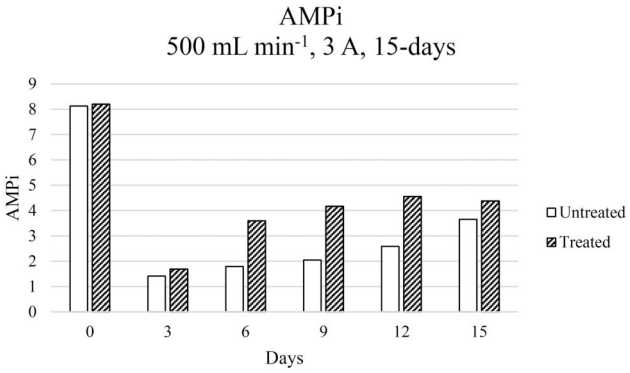

Figure 8. Cell stress after magnetic water treatment with reduced magnetic field strength.
Cell stress induced by a weaker magnetic field (~3 A) at over 15-days at a flow rate of 500 mL min^{-1}.

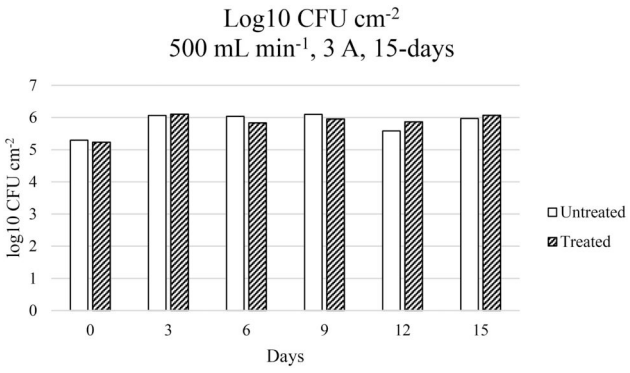

Figure 9. Cell viability after magnetic water treatment with reduced magnetic field strength.
Changes in cell viability induced by a weaker magnetic field (~3 A) over 15-days at a flow rate of 500 mL min^{-1}.

Biofilm associated bacteria are often more resistant to chemical disinfectants, when compared to planktonic cells, requiring enhanced disinfectant procedures to be employed to address these challenges (LeChevallier et al. 1988; Buckingham-Meyer et al. 2007). Additionally, a multitude of factors may influence the efficacy of chemical water treatment practices such as dissipation of disinfectant, stagnation of water, and biofilms (Carducci et al. 2010). Due to the limitations of chemical disinfection the implementation of novel water treatment technologies, including magnetic treatment, should be considered to augment existing biofilm management strategies.

The initial pilot study (Proof of concept: pilot study evaluating the influence of magnetic water treatment on biofilms' section) was conducted to determine if magnetic field effects could adversely affect *Pseudomonas fluorescence* biofilms. A low flow rate (100 mL $min^{-1)}$ was selected to limit the detachment of biofilm caused by shear forces. The magnetic field was produced using the current indicated by the manufacturer (~4 A). The pilot study revealed an elevated ratio of AMP to ATP in the magnetically treated system after both 5 and 7 days (Figure 4). Keasler et al. (2013) utilized the LuminUltra test kit to evaluate the AMP Stress Indices (AMPi) during a study on microbial communities within wastewater. Based on this study the authors proposed that the AMPi could be used to quantify the level of stress on the microbial communities. Specifically they suggested, AMPi ranges of <1 to be indicative of low stress conditions and an active microbial community; between 1 and 10 as increasing stress conditions; >10 to indicate extreme stressed conditions or a dormant microbial community. The number of viable bacteria in the biofilm was reduced by 1.02 and 1.34 log_{10} CFU cm^{-2} at days 5 and 7 after magnetic treatment, respectively, when compared to day 0 (Figure 5). Little change was observed in either the number of viable cells or the AMPi of the biofilm in the untreated system.

In subsequent experiments the duration of magnetic exposure was increased to 15 days and the rate of flow was maintained at 100, 500, or 1000 mL min^{-1}. As the flow rate was increased, the number of viable cells in the biofilm decreased. A 1.06, 2.46 and 2.71 log_{10} CFU cm^{-2} reduction occurred at 100, 500, and 1000 mL min^{-1} respectively after 15 days of treatment at ~4 A (Figure 7). The final concentration of cells increased in the untreated systems at both 100 mL min^{-1} and 500 mL min^{-1} but not at 1000 mL $min^{-1.}$ A trend of elevated AMPi, which was greater than the corresponding control, appeared to occur from day 6 onwards in the treated systems, regardless of flow rate again illustrating increased stress in the biofilm due to magnetic treatment (Figure 6). However, an elevated AMPi of comparable magnitude appeared to also occur in the untreated system at 1000 mL min^{-1} after 9 days. The discrepancies observed at 1000 mL min^{-1} were likely the result of increased shear forces increasing detachment of unhealthy biofilms in both the treated and control systems. A flow rate of 1000 mL min^{-1} is likely too rapid for reliable results to be obtained using this system and was therefore not chosen for subsequent experiments although the greatest overall reduction in sessile cells occurred (Figure 7c).

The properties of a magnetic field (strength, shape, directionality, changes in flux, etc.) can influence how particles will interact with the field (Kronenberg 1985; Timmel et al. 1998; Fujimoto 2007). As an electromagnet was used, a change in the current also represents a change in the strength of the magnetic field. At a reduced field strength, no change in number of

Table 1. Log_{10} viable planktonic cells found in the recirculating water for each experiment.

Experimental description	100 mL min^{-1} 4 A 7-days		100 mL min^{-1} 4 A 15-days		500 mL min^{-1} 4 A 15-days		1000 mL min^{-1} 4 A 15-days		500 mL min^{-1} 3 A 15-days	
	Viable cells (Log10 CFU mL^{-1})									
Day	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
0	5.05	4.81	6.22	6.35	6.03	5.94	6.75	6.79	6.15	6.24
1	4.79	5.69	–	–	–	–	–	–	–	–
3	5.31	4.82	6.42	6.69	5.53	5.86	7.35	7.41	6.26	5.36
5	5.17	4.65	–	–	–	–	–	–	–	–
6	–	–	6.79	6.73	5.69	5.12	7.65	7.15	6.59	5.72
7	5.18	4.57	–	–	–	–	–	–	–	–
9	–	–	6.76	5.37	5.51	4.80	6.92	6.15	7.50	5.95
12	–	–	6.88	4.85	5.55	4.53	6.48	6.17	6.07	6.03
15	–	–	6.75	4.50	5.38	4.34	6.23	4.86	6.24	6.26
Final log_{10} removal	−0.12	0.24	−0.52	1.85	0.65	1.59	0.52	1.94	−0.09	−0.02

viable sessile cells were observed (Figure 9); however, there was an increase in the AMPi from day 6 onwards (Figure 8). This appears to indicate that although stress can be induced in the cells at a lower magnetic field strength, it is not sufficient to result in cell death. Reductions to the number of viable cells in the biofilm could indicate either bacterial detachment from the surface or cell death. The number of viable planktonic cells in the bulk water was also assessed in an attempt to decouple this relationship (Table 1). Analysis of planktonic cells in the system resulted in a final 1.85, 1.59 and 1.94 log_{10} reduction in the treated system compared to a −0.52, 0.65 and 0.52 log_{10} for the untreated system at 100, 500 and 1000 mL min^{-1}, respectively (Table 1). As with the sessile cells, the decrease in viable cells in the bulk water began at day 6 and continued through the remainder of the experiment. These data indicates that magnetic treatment contributed to a reduction of viable bacteria and not simply detachment of these cells into the bulk water. It would be expected that if direct interaction with the magnetic field were required for cell death, then a greater reduction would occur in the bulk water passing through the field than the biofilm located outside the field. This appears to indicate that interaction of the magnetic field on the water matrix, not the bacteria, results in cell death.

This study demonstrated that magnetic water treatment can be used as a tool to reduce *Pseudomonas fluorescence* biofilms within a recirculating water system (Figure 2). Removal of the biofilm was characterized by the removal of viable cells in the biofilm and through a measure of general metabolic stress (AMPi). The impact of water treatment for biofilm removal was enhanced as both treatment time and flow rate increased (Figures 4–7). However, the biocidal properties of the treatment were not present when the field strength was decreased, although elevated cell stress was observed (Figures 8 and 9). This indicates that the characteristics of the magnetic field and the deployment of the technology likely play a pivotal role in the efficacy of magnetic treatment.

Changes in cellular AMP and ATP can be an effective tool for monitoring the general metabolic status of the cells (Keasler et al. 2013; Hardie 2018). However, it is important to note that these changes in AMPi do not indicate how or which processes were impacted by the magnetic treatment. Elevated levels of AMPi occurred concurrently with cell death in the biofilm, whereas a low AMPi tended to be indicative of steady state or cell growth. An elevated AMPi was occasionally observed immediately after the biofilms were established and prior to any magnetic treatment (day 0). This increased stress may be an artifact of the cells reaching stationary phase during biofilm formation. Thus, the incidence of elevated stress on day 0 is omitted when discussing general trends throughout the experiments however, it is necessary to acknowledge this phenomenon.

This study demonstrated cell stress was impacted using a general measure of metabolic activity (e.g. AMPi). The underlying mechanism and the specific processes impacted by the treatment remains unknown but several possible mechanisms have been identified in other studies. Activity of bacterial systems have been shown to be affected both positively and negatively by magnetic fields (Gerencser et al. 1962; Chen and Li 2008; Łebkowska et al. 2013; Zaidi et al. 2014). Some of these effects can be attributable to the influence of magnetic fields on conformational changes within enzymes or cofactors (Blanchard and Blackman 1994). Additionally, magnetic field effects on the substrate or charged intermediates of these enzymatic reactions may also positively or negatively influence the rates of reactions. Black et al. (1995)

showed that ATPase activity was altered in a magnetic field by influencing the enzyme itself or the availability of the requisite ions. However, many of these studies apply a magnetic field directly to the cells themselves, which appears to be unnecessary for the outcomes observed in this study.

Literature investigating the influence of magnetic fields on ionic solutions may hold a potential explanation to the localization challenge. Higashitani et al. (1993) noted that for up to 120 h after $NaCO_3$ and $CaCl_2$ solutions had been exposed to a 0.3 T uniform magnetic field, the crystal structure and rate of $CaCO_3$ formation outside the magnetic field using these solutions was altered. A memory effect of magnetic fields on ions, specifically Na^+ and Ca^{2+}, may explain how a localized effect may influence bacterial activity outside of the field. Both Na^+ and Ca^{2+} are of interest for their roles in cell signaling, efflux pumps, and general metabolic functions. Given the potential for magnetic water treatment to influence a variety of metabolic pathways, tools such as nucleic acid content, metabolomics, or metagenomics could be used to better understand magnetic field effects (Dunn et al. 2005; Gérard et al. 2015; Xiao et al. 2020; Liu et al. 2022). Thus, future studies should consider changes to the matrix characteristics caused by magnetic treatment when assessing the impact on cell activity. Evaluating changes to Na^+ and Ca^{2+} as a driver for the observed effects may also provide agreement between literature on the impact of magnetic treatment on biological function and those assessing crystal formation. Although this question cannot be directly addressed by this work, future studies should aim to determine if metabolic processes relying on Na^+ and Ca^{2+} are altered after magnetic water treatment.

Conclusion

This study demonstrated that magnetic water treatment can decrease the number of viable bacterial cells in previously established biofilms in the absence of chemical bactericides. Notably, the biofilms were not directly influenced by the magnetic field and thus treatment of the water alone was sufficient for removal of biofilms. The reduction in viable *Pseudomonas* cells appeared to be the result of the magnetically treated water inducing increased cellular stress (as measured by the AMPi), ultimately resulting in cell death. These effects were more pronounced at higher flow rates likely due to increased shear forces on the biofilms and the rate at which the water was exposed to the magnetic field. When the magnetic field strength was reduced, the bacterial cells experienced elevated stress; however, there was no resulting cell death. This study paves the way for further investigation into the real world application of these magnetic technologies to supplement existing water treatment practices. Although a full understanding of the nuances of how this treatment works on a cellular level remains elusive, a mechanism in which magnetic treatment influences ions in the water which in turn impacts the enzymatic activity of the cells is proposed.

Author's contributions

Aidan Foster – investigation, writing-original draft, project administration. Erika R. Stark – investigation. Luisa A. Ikner – resources, supervision. Ian Pepper – supervision, writing-review & editing, funding acquisition.

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Vodaa Technologies was not involved in collection, analysis, and interpretation of data, in the writing of the report, or in the decision to submit the article for publication. The authors report there are no competing interests to declare.

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