

Comparison of Anolyte and Chlorine Dioxide for a Continuous Hot Water Disinfection in Nursing Home: A Two Years Legionnaires' Disease Prevention

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Abstract

Worldwide epidemiological reports assert that drinking water is a source for infections and Legionella control represents a critical issue in healthcare settings. Chemical disinfections of water networks are control measures that need to be fine-tuned to obtain satisfactory results in large buildings over prolonged time periods. Aim of study is the evaluation of the effect of anolyte and chlorine dioxide, applied in two different hot water networks of a nursing home to manage Legionella risk. Nursing home has two buildings (A and B), with the same point of aqueduct water entrance. From June 2016, following a shock chlorination, the continuous disinfections with chlorine dioxide and anolyte were applied in hot networks of building A and B, respectively. Hot water was sampled at the central heating system and at two points of use for Legionella research, while chemical tests of manganese (Mn), iron (Fe), zinc (Zn) and trihalomethanes compounds (THM) were implemented to evaluate the disinfection by-products presence. Before chlorination *Legionella pneumophila* sg1 was recovered with a mean count of 2.4×10^4 CFU/L, while chemical compounds concentrations were within the law limits (Directive 98/83/EC). Then the disinfections Legionella was not recovered in both hot water plants. After the disinfection with chlorine dioxide (from June 2016 to May 2018), a statistically significant increase of iron, zinc and THM concentrations was detected in building A ($p = 0.012$; $p = 0.004$; $p = 0.008$). Both disinfectants appear effective against *Legionella* spp. growth in water network, but anolyte ensures a lower disinfection by-products release.

Keywords

Legionella, Anolyte, Chlorine Dioxide, Hot Water Disinfection

1. Introduction

Legionella spp. is a waterborne pathogen frequently associated with nosocomial infections, particularly among immunosuppressed, transplanted patients and people undergoing aggressive chemotherapy [1]. Over the last years in Europe, some of the outbreaks of Legionnaires' disease reported by the European surveillance scheme have been linked to hospitals and healthcare facilities having a hot water system colonized by *Legionella* spp. [2]. Moreover, international and Italian guidelines for Legionnaires' disease prevention [3] [4] suggest that ancient water networks of healthcare facilities provide optimal conditions for *Legionella* colonization into corroded pipelines and dead leg branches.

Hot water network treatments may be applied with different compounds such as chlorine dioxide [5], sodium hypochlorite [6], monochloramine [7] [8] and hydrogen peroxide [9] ensuring a continuous disinfection procedure. All these treatments are useful to control *Legionella* spp. counts, but some disinfectants may get worse the chemical water quality after the pipelines corrosion [10]. In fact, worldwide studies reported that high concentrations of toxic disinfection by-products (DBPs) in drinking water favor the probability to be taken up through inhalation, ingestion and dermal absorption, increasing the potential health risks of exposure for the users [11] [12]. For this reason, a planned choice of appropriate pipelines materials and chemical disinfection methods must be done for each healthcare water network [13].

Water disinfection may be achieved by new strategies, such as the electrochemically activated solution generators, which produce two solutions during electrochemically activation of dilute salt solutions: an oxidant solution capable of penetrating biofilm termed anolyte and a catholyte with detergent properties.

In detail, the dilute saline solution is activated by passing through a cylindrical electrolytic cell in which the anodic and cathodic chambers are separated by a permeable membrane. Two separate streams of activated water are produced: anolyte (hypochlorous acid) with a pH range of 2 - 6 and an oxidation-reduction potential of +400 mV to +1000 mV. It is an oxidizing compound having an antimicrobial effect. Catholyte (sodium hydroxide, pH of 12 and an oxidation-reduction potential of -900 mV) has surfactant properties and is an anti-oxidant [14]. Several studies assert the efficacy of anolyte disinfection method in water [15], food [16] [17] and surface samples [18].

In Italian healthcare facilities, despite the increase of critical points linked to water disinfections, electrochemical methods are not frequently applied for hot water treatment. In fact, few published papers about water network disinfection have focused on the comparison between electrochemical and common chemical

disinfection.

In this study, we report a plan for Legionnaires' disease prevention, which was scheduled to compare two different water disinfection strategies, applied in an Italian nursing home by using anolyte and chlorine dioxide, for continuous hot water disinfection. Considering the increase of Italian Legionnaires' disease cases linked to hospitals and healthcare facilities, this research may assess the choice of new strategies aimed to improve the chemical disinfection activity in hot water networks.

2. Materials and Methods

2.1. Nursing Home Setting and Hot Water Disinfections

The healthcare setting is a nursing home of North-Western Tuscany (Italy), organized in two identical buildings (A and B) active since 1975. For both buildings, the architectural structure is a monoblock with a central plate on 3 levels, with 13 rooms and 39 beds. Nursing home hosts non-self-sufficient elderly patients with cognitive and behavioral disorders and chronic degenerative diseases, such as Parkinson-Alzheimer's diseases, cardiovascular diseases, diabetes, etc.

In June 2016, within the Water Safety Plan (WSP) implementation program, the hot water system disinfection, and a systematic monitoring program with samplings at the final points of use, began.

Following the WSP introduction, the municipal drinking water was pre-filtered and softened before entering the two nursing home hot water distribution systems. Despite the old and galvanized steel-made pipelines, hot water networks were treated with in continuous chlorination.

Following a shock chlorination (50 mg/L sodium hypochlorite; 1 h), a continuous disinfection with chlorine dioxide was applied in building A, while anolyte compound was introduced in building B.

2.2. Microbiological Hot Water Samplings and *Legionella* Spp. Research

Before and after the WSP introduction, each building's hot water distribution system was sampled at the recirculation point (R) and at the boiler (B) of the central heating system and at two points of use located at the first and second floors (P1; P2) for *Legionella* detection, as suggested by Italian Legionnaires' guidelines [3]. Microbiological hot water samplings were performed on a monthly basis. From January 2017 samplings were done on a quarterly basis.

Legionella research and physical-chemical parameters assessment (total chlorine concentration, pH and water temperature) were recorded during the sampling activity.

Chlorine concentration was determined by the colorimetric Visocolor HE® test (Macherey-Nagel, Germany).

The isolation of *Legionella* spp. in hot water samples was performed as suggested by the international standards procedure ISO11731 [19]. Briefly, one liter

of water was filtrated through a 0.2 µm diameter membrane (Millipore, Billerica, MA). The membrane was then immersed in 10 ml of the same water and sonicated for 5 minutes, allowing the detachment of cells. The suspension was thermal-inactivated at 50 °C for 30 minutes. Afterwards 0.1 ml of the suspension was plated on Legionella BMPA selective medium (Oxoid Ltd, Basingstoke, Hampshire, UK) and the plates were incubated at 37 °C for 7 - 10 days within jars with a modified atmosphere (2.5% CO₂). Finally, almost 10% of the Legionella colonies grown on the medium were subjected to species and serogroup identification analysis using a multi-purpose latex agglutination test (Legionella Latex Test, Oxoid Ltd, Basingstoke, Hampshire, UK) and the *Legionella pneumophila* group sera set (Biogenetics, Italy).

2.3. Chemical Hot Water Samplings and Analysis

From June 2016, samplings for chemical analysis were performed at the point of aqueduct water entrance (Aq) and at the same sampling points chosen for microbiological tests. Chemical analyses were performed in June and December 2016; January, May, September 2017; January, May 2018. Chemical parameters such as iron (Fe) ions, manganese (Mn) ions, zinc (Zn) ions and trihalomethanes compounds (THM) were researched as established by Council Directive 98/83/EC [20].

Metal ions were determined by ICP-MS (Inductively Coupled Plasma-Mass Spectrometry) according to EPA 6020B with an analytical error < 10% [21]. Samples for the determination of the solutes were filtered at 0.45 µm.

THM compounds were analyzed by GC-MS (Gas Chromatography-Mass Spectrometry) equipped with Purge & Trap device following EPA 5030C and the EPA 8260D [22] [23]. The detection limits were 0.1 µg/L for tetra-chloro-ethylene and tri-chloro-ethylene and 0.01 µg/L for chloroform, bromoform, dichloro-bromo-methane, chloro-bromo-methane and 1,2 dichloro-ethane. The analytical error was <10%.

2.4. Statistical Analysis

The Kolmogorov-Smirnov test was performed to verify the normality of distributions. For each building, Kruskal-Wallis and Dunn tests were used to compare the chemical parameters concentrations detected during the continuous disinfection with chlorine dioxide and anolyte. Power tests were performed to estimate the sample sizes. The 1-beta values of the significant variables were >0.8, proving that the sample sizes were acceptable. A SPSS Version 17.0.1 (IBM Corp., Armonk, NY, USA) was used for the statistical analysis.

3. Results

3.1. Legionella Spp. Results

Before WSP implementation, between April 2016 and May 2016, high *Legionella pneumophila* sg1 concentrations were detected in all the tested water points of

use. As shown in **Figure 1**, *Legionella* concentrations observed in hot water samples ranged from 1×10^4 to 3.5×10^4 CFU/L, with a mean value of $2.4 \times 10^4 \pm 7.1 \times 10^3$ CFU/L. Moreover, low temperature values were detected in all sampling points (mean value of $38.8^\circ\text{C} \pm 4.2^\circ\text{C}$).

Considering the absence of hot water disinfection, this data highlight a significant *Legionella* spp. colonization of both nursing home buildings water distribution systems.

After WSP plan introduction and the application of the two continuous disinfection methods, from June 2016 to June 2018 *Legionella* spp. was not recovered in both hot water networks.

3.2. Physical and Chemical Results

A good chemical quality was observed in all the hot water samples and all the values were within the limits recommended by Council Directive 98/83/EC (98/83/EC).

Table 1 shows iron, manganese, zinc ions and THM compounds detected on the point of aqueduct entrance (Aq) in the period between June 2016 and May 2018.

Iron ions values ranged from 22.8 to 28.4 $\mu\text{g/L}$ (mean $24.9 \pm 1.9 \mu\text{g/L}$) (Council Directive 98/83/EC law limit = 200 $\mu\text{g/L}$).

Zinc ions values ranged from 31 to 39 $\mu\text{g/L}$ (mean $36.2 \pm 3 \mu\text{g/L}$) (Council Directive 98/83/EC law limit not provided).

Manganese ions values ranged from 1.3 to 1.5 $\mu\text{g/L}$ (mean $1.4 \pm 0.08 \mu\text{g/L}$) (Council Directive 98/83/EC law limit = 50 $\mu\text{g/L}$).

THM values ranged from 1.1 to 1.4 $\mu\text{g/L}$ (mean $1.3 \pm 0.1 \mu\text{g/L}$) (Council Directive 98/83/EC law limit = 30 $\mu\text{g/L}$).

Table 2 shows physical-chemical results detected from the hot water network

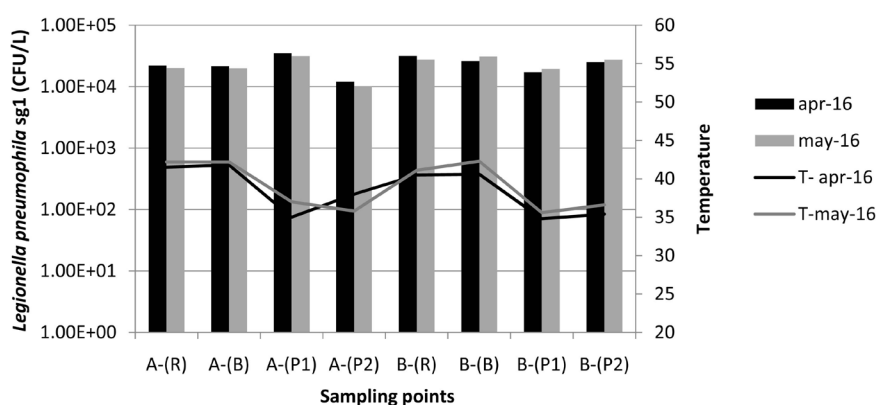


Figure 1. *Legionella pneumophila* sg1 counts and temperature values detected in all sampling points of building A and B before the application in hot water disinfections (April and May 2016). A-(R) = Building A-Recirculation; A-(B) = Building A-Boiler; A-(P1) = Building A-Point of use 1; A-(P2) = Building A-Point of use 2; B-(R) = Building B-Recirculation; B-(B) = Building B-Boiler; B-(P1) = Building B-Point of use 1; B-(P2) = Building B-Point of use 2.

Table 1. Results of chemical analysis (iron ions, zinc ions, manganese ions, trihalomethanes compounds) performed on the point of aqueduct entrance in the period between June 2016 and May 2018.

POINT OF AQUEDUCT ENTRANCE (Aq)				
MONTHS	IRON IONS ($\mu\text{g/L}$)	ZINC IONS ($\mu\text{g/L}$)	MANGANESE IONS ($\mu\text{g/L}$)	THMs ($\mu\text{g/L}$)
June 2016	25.4	38.5	1.5	1.1
December 2016	28.4	32.2	1.5	1.3
January 2017	22.8	35.9	1.4	1.4
May 2017	23.8	32	1.4	1.4
September 2017	25.7	39	1.4	1.3
January 2018	23.3	37.2	1.3	1.4
May 2018	25	38.3	1.5	1.3

Table 2. Mean values of total chlorine, temperature, pH, iron ions, zinc ions, manganese ions, and trihalomethanes compounds detected from April 2016 to May 2018 in the hot water systems treated with chlorine dioxide (building A). (NP = Not Performed).

Building A-(Chlorine Dioxide)							
Months	Total chlorine (mean \pm SD mg/l)	Temperature (mean \pm SD $^{\circ}\text{C}$)	Mean pH \pm SD	Iron Ions (Mean \pm SD $\mu\text{G/L}$)	Zinc Ions (Mean \pm SD $\mu\text{G/L}$)	Manganese Ions (Mean \pm SD $\mu\text{G/L}$)	THMs (Mean \pm SD $\mu\text{G/L}$)
April 2016	0	41.5 \pm 2.1	6.5 \pm 0.01	NP	NP	NP	NP
May 2016	0	41.5 \pm 2	6.5 \pm 0.01	NP	NP	NP	NP
June 2016	0.3 \pm 0.04	50.6 \pm 1.9	6.5 \pm 0.01	8.9 \pm 4.9	54.7 \pm 10.3	1.5 \pm 0.3	2.1 \pm 0.2
July 2016	0.26 \pm 0.05	49.9 \pm 2.3	7 \pm 0.03	NP	NP	NP	NP
August 2016	0.34 \pm 0.11	50.6 \pm 2.4	6.5 \pm 0.02	NP	NP	NP	NP
September 2016	0.37 \pm 0.10	50.7 \pm 2	6.5 \pm 0.02	NP	NP	NP	NP
October 2016	0.42 \pm 0.08	50.6 \pm 2,3	6.5 \pm 0.02	NP	NP	NP	NP
November 2016	0.30 \pm 0.06	43.8 \pm 3,1	6.4 \pm 0.03	NP	NP	NP	NP
December 2016	0.32 \pm 0.05	48.8 \pm 4,7	6.7 \pm 0.04	22.2 \pm 5.2	86.2 \pm 9.5	1.5 \pm 0.4	9.7 \pm 2
January 2017	0.31 \pm 0.06	50.1 \pm 3.5	6.7 \pm 0.02	22.8 \pm 7.4	85.7 \pm 9.3	1.7 \pm 0.3	13.2 \pm 3.4
May 2017	0.36 \pm 0.09	50.9 \pm .2,9	6.8 \pm 0.03	22.9 \pm 7.5	88.6 \pm 9.8	1.9 \pm 0.5	13.8 \pm 5.1
September 2017	0.41 \pm 0.06	52.4 \pm 3.2	6.5 \pm 0.01	22.4 \pm 8.8	86.1 \pm 9.2	1.7 \pm 0.5	13.8 \pm 5
January 2018	0.38 \pm 0.08	53 \pm 3.2	6.5 \pm 0.03	23.4 \pm 8.3	86.1 \pm 9.2	1.7 \pm 0.5	14.8 \pm 5.2
May 2018	0.39 \pm 0.05	52.2 \pm 2.8	6.4 \pm 0.01	24.7 \pm 8.5	93 \pm 11.8	1.7 \pm 0.1	14.8 \pm 4.7

treated with chlorine dioxide (building A) in the period between June 2016 and May 2018.

Mean temperature and values were between 43.8 $^{\circ}\text{C}$ \pm 3.1 $^{\circ}\text{C}$ (November 2016) and 53 $^{\circ}\text{C}$ \pm 3.2 $^{\circ}\text{C}$ (January 2018) while mean pH values were between 6.5 \pm 0.01 to 7 \pm 0.03.

From June 2016 mean total chlorine concentration were between 0.26 \pm 0.05 mg/L (July 2016) to 0.42 \pm 0.08 mg/L (October 2016).

Mean iron ions values ranged from 8.9 ± 4.9 $\mu\text{g/L}$ (June 2016) to 24.7 ± 8.5 $\mu\text{g/L}$ (May 2018) (Council Directive 98/83/EC law limit = 200 $\mu\text{g/L}$).

Mean zinc ions values ranged from 54.7 ± 10.3 $\mu\text{g/L}$ (June 2016) to 93 ± 11.8 $\mu\text{g/L}$ (May 2018) (Council Directive 98/83/EC law limit not provided).

Mean manganese ions values ranged from 1.5 ± 0.3 $\mu\text{g/L}$ (June 2016) to 1.9 ± 0.5 $\mu\text{g/L}$ (May 2017) (Council Directive 98/83/EC law limit = 50 $\mu\text{g/L}$).

Mean THM values ranged from 2.1 ± 0.2 $\mu\text{g/L}$ (June 2016) to 14.8 ± 5.2 $\mu\text{g/L}$ (January 2018) (Council Directive 98/83/EC law limit = 30 $\mu\text{g/L}$).

Statistically significant increases of iron, zinc and THM concentrations were detected after the disinfection with chlorine dioxide (from June 2016 to May 2018) ($p = 0.012$; $p = 0.004$; $p = 0.008$).

Table 3 shows physical-chemical results detected from the hot water network treated with anolyte (building B) in the period between June 2016 and May 2018.

Mean temperature and values were between $39.8^\circ\text{C} \pm 3.3^\circ\text{C}$ (April 2016) and $43.1^\circ\text{C} \pm 2.9^\circ\text{C}$ (October 2016) while mean pH values were between 6.4 ± 0.02 to 6.7 ± 0.03 .

From June 2016 mean total chlorine concentration were between 0.2 ± 1.4 mg/L (September 2016) to 0.35 ± 0.2 mg/L (September 2017).

Mean iron ions values ranged from 32.2 ± 8.6 $\mu\text{g/L}$ (June 2016) to 36.4 ± 9 $\mu\text{g/L}$ (September 2017) (Council Directive 98/83/EC law limit = 200 $\mu\text{g/L}$).

Table 3. Mean values of total chlorine, temperature, pH, iron ions, zinc ions, manganese ions, and trihalomethanes compounds detected from April 2016 to May 2018 in the hot water systems treated with anolyte (building B). (NP = Not Performed).

Building B-(Anolyte)							
Months	Total Chlorine (mean \pm SD mg/L)	Temperature (mean \pm SD $^\circ\text{C}$)	Mean pH \pm SD	Iron Ions (mean \pm SD $\mu\text{g/L}$)	Zinc Ions (mean \pm SD $\mu\text{g/L}$)	Manganese Ions (mean \pm SD $\mu\text{g/L}$)	THMs (mean \pm SD $\mu\text{g/L}$)
April 2016	0	39.8 ± 3.3	6.5 ± 0.01	NP	NP	NP	NP
May 2016	0	42.1 ± 1.9	6.5 ± 0.02	NP	NP	NP	NP
June 2016	0.27 ± 0.7	43 ± 2	6.5 ± 0.01	32.2 ± 8.6	41.8 ± 7.3	1.4 ± 0.2	1.2 ± 0.2
July 2016	0.22 ± 0.8	43 ± 2.2	6.5 ± 0.02	NP	NP	NP	NP
August 2016	0.21 ± 1.1	42.8 ± 2.7	6.6 ± 0.03	NP	NP	NP	NP
September 2016	0.2 ± 1.4	42.6 ± 2.5	6.4 ± 0.02	NP	NP	NP	NP
October 2016	0.23 ± 1.7	43.1 ± 2.9	6.5 ± 0.01	NP	NP	NP	NP
November 2016	0.22 ± 1.5	39.2 ± 3	6.5 ± 0.01	NP	NP	NP	NP
December 2016	0.25 ± 1	40.5 ± 2.8	6.5 ± 0.01	33.2 ± 6.4	44.4 ± 8.2	1.5 ± 0.5	1.9 ± 0.9
January 2017	0.29 ± 1.2	42 ± 3.1	6.5 ± 0.02	34 ± 9.9	45.5 ± 10.6	1.6 ± 0.5	2.2 ± 0.3
May 2017	0.3 ± 0.9	41.1 ± 1.7	6.4 ± 0.02	36.1 ± 8.8	42.5 ± 6.5	1.4 ± 0.3	2 ± 0.4
September 2017	0.35 ± 0.2	40.9 ± 1.4	6.6 ± 0.02	36.4 ± 9	43.1 ± 8.4	1.4 ± 0.4	1.8 ± 0.8
January 2018	0.3 ± 0.4	41.2 ± 2.4	6.7 ± 0.03	35.5 ± 5.8	42.5 ± 5.3	1.6 ± 0.5	2 ± 0.6
May 2018	0.32 ± 0.4	41.6 ± 2.5	6.5 ± 0.03	34.3 ± 10.8	42.2 ± 6.7	1.6 ± 0.5	2.2 ± 0.6

Mean zinc ions values ranged from 41.8 ± 7.3 µg/L (June 2016) to 45.5 ± 10.6 µg/L (January 2017) (Council Directive 98/83/EC law limit not provided).

Mean manganese ions values ranged from 1.4 ± 0.2 µg/L (June 2016) to 1.6 ± 0.5 µg/L (May 2018) (Council Directive 98/83/EC law limit = 50 µg/L).

Mean THM values ranged from 1.2 ± 0.2 µg/L (June 2016) to 2.2 ± 0.6 µg/L (May 2018) (Council Directive 98/83/EC law limit = 30 µg/L).

No Statistically significant increases of ions and THM concentrations were detected after the disinfection with chlorine dioxide (from June 2016 to May 2018) ($p > 0.05$).

4. Discussion

According to literature, the application of a plan for Legionella prevention and control is needed in healthcare facilities, mostly in hospitals hosting immunosuppressed patients [24]. Considering some occurrences of Legionnaires' disease outbreaks in nursing homes [25] [26] [27], the implementation of the Water Safety Plan may also be extended to these kinds of healthcare settings.

For this reason, Italian guidelines for Legionnaires' disease prevention and control [3] recommend the application of control measures ensuring a good microbiological quality in water networks. In fact, the choice of an adequate and continuous chemical disinfection system may prevent the occurrence of waterborne pathogens colonization in water pipelines, mostly in high and ancient buildings with large and complex water network having critical points [28] [29].

Despite literature data assert that chlorine dioxide enhances the corrosion process of metal ions, which may be released in drinking water, this is the most common chemical compound disinfectant used for hot water disinfection. In fact, treatment with chlorine dioxide is effective against bacteria, virus and protozoa. If not controlled carefully, chlorine dioxide can corrode pipelines releasing metal ions and disinfection by-products in drinking water, getting worse the organoleptic properties of water [30].

Moreover, alternative techniques using electrochemically-activated solutions are less used, and only few studies support this method for the prevention of microbiological water risk.

Anolyte, applied for drinking water disinfection, can act directly on the biofilm inside the plumbing, eliminating it in few days. It is highly effective in disinfection processes against bacteria (including spores), virus and protozoa. Treatment has low operating costs and the neutral pH is fully compatible with the materials of water networks. Moreover, anolyte does not cause excessive corrosion of pipework [31].

Our comparative study shows how chlorine dioxide and anolyte are able to avoid *Legionella* spp. growth in both water networks in a long-term period. Although in two years study we always observed a good chemical quality of drinking waters, some statistically significant differences were detected between building A and B. In details, from hot water network treated with chlorine dioxide we observed an increase of metal ions (iron and zinc) and THM concentra-

tions in the period between June 2016 and May 2018. This trend was not detected in hot water samplings collected from building B and disinfected with anolyte. However, during the whole period of study chemical results of water sampling collected from the point of aqueduct entrance remained unchanged. Despite this comparison may confirm the advantage and disadvantage declared in literature, the choice of an appropriate disinfection method for hot drinking water may be continuously controlled as described by the Water Safety Plan [28] [30] [31].

5. Conclusion

In conclusion, our study is one of the few assessing a technical comparison of two different incontinuous disinfections, which were applied in two separate, similar and pre-contaminated hot water networks. After two years, it is possible to assert that both disinfectants appear effective against *Legionella pneumophila* sg1 growth in water pipelines, but anolyte ensures a lower metal ions and disinfection by-products (THM) release.

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Conflicts of Interest

All authors have no conflict of interest to declare.

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