Use of ozone for Legionella reduction in water systems

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Abstract

Ozone is reported to be effective against *Legionella* in water systems such cooling towers and tap water pipelines. Until now, most of the studies performed to asses the efficiency of disinfectants against *Legionella* were made *in vitro* or in existing water systems. *In vitro* studies do not take into account the higher resistance of this bacterium to biocides due to the protection offered by biofilms. On the other hand, studies in existing water systems do not offer repeatability of the results. In order to evaluate the efficiency of ozone to eliminate *Legionella* strains in water pipelines, a pilot plant has been designed aiming at simulating the microbiological growth of *Legionella erythra* and the disinfection capacity of ozone under these conditions. The plant, consisting of 9 independent water pipelines, has been used to compare treatment efficiency under equivalent conditions of system design, materials (i.e. iron, PVC and polypropylene) and effect of biofilms. Performance evaluation of ozone is based on the ability to reduce not only Legionella, but also biofilms, which contribute to the establishment and dissemination of these bacteria in water systems, and their resistance to treatments.

Technical issues about the application of ozone for *Legionella* decontamination in water systems are discussed in this paper, including injection methods, automation system, liquid and gas phase on-line measurements and interaction of ozone with the structure of the facility. Safety considerations and the environmental impact of the ozonitation are also been considered.

Key-words: ozone, *Legionella sp*., disinfection

Legionella

Legionella is a Gram-negative bacterium, including species that cause legionellosis or Legionnaires disease, most notably *L. pneumophilia*. *Legionella* are common in many environments, with at least 50 species and 70 serogroups identified.
Legionella species are the causative agent of the human Legionnaires disease and the lesser form, Pontiac fever. *Legionella* transmission is via aerosols by the inhalation of mist droplets containing the bacteria. Common sources include cooling towers, domestic hot-water systems and fountains. Natural sources of *Legionella* include freshwater ponds and creeks. Outbreaks of legionnaires disease have occurred in or near large building complexes such as hotels, hospitals, offices and factories.
Initial symptoms are flu-like, including fever, chills, and dry cough. Advanced stages of the disease cause problems with the gastrointestinal tract and the nervous system and lead to diarrhoea and nausea. Other advanced symptoms of pneumonia may also present. Legionnaires disease can be very serious and can cause death in up to 5% to 30% of cases.

Application of ozone as biocide to control *Legionella*

Over the course of the past decade, the use of ozone as the sole method of treating recirculating cooling tower water has gained much attention. Currently there are several hundred of such systems operating in the United States, and their success and failures have widely been published (11).
The interest in ozone as an alternative to chlorine and other chemical disinfectants is based on its high biocidal efficacy, wide antimicrobial spectrum, absence of by-products that are detrimental to health and the ability to generate it on demand, ‘in situ’, without needing to store it for later use.

Mechanism of ozone as biocide

Ozone is a powerful broad-spectrum antimicrobial agent that is active against bacteria, fungi, viruses, protozoa, and bacterial and fungal spores (13). When molecular ozone dissolves in water, the molecule can remain as O_3 or decompose by a variety of mechanisms, ultimately producing the hydroxyl free radical (HO*) even stronger oxidizing agent than ozone (19).
Those agents oxidise all the molecules capable to be oxidized around them starting from the easiest. Biological cells are not an exception and the components of their membranes (proteins, lipids and polysaccharides) become also oxidized and structurally modified giving cell lysis as result.
Inactivation by ozone is a complex process that attacks various cell membrane and wall constituents (e.g. unsaturated fats) and cell content constituents (e.g. enzymes and nucleic acids). Both molecular ozone and the free radicals produced by its breakdown play a part in this inactivation mechanism but there is no consensus on which of them is more decisive. The micro-organism is killed by cell envelope disruption or disintegration leading to leakage of the cell contents. Disruption or lysis is a faster inactivation mechanism than other disinfectants which require the disinfectant agent to permeate through the cell membrane in order to be effective.
As regards the spectrum of action, each micro-organism has an inherent sensitivity to ozone. Bacteria are more sensitive than yeasts and fungi. Gram-positive bacteria are more sensitive to ozone than Gram-negative organisms and spores are more resistant than vegetative cells.
Due to the mechanism of the ozone action, which destroys the micro-organism through cell lysis, it cannot lead to micro-organism resistance.

Legionella colonization and biofilm

Legionella species are aquatic bacteria widespread in nature which have been found in water over a wide temperature range but growing the best between 30°C and 40°C (24, 1, 17). Their tolerance to relatively high temperatures, probably help them to colonise some artificial water systems that are often above ambient temperatures. *Legionella* are prevalent in artificial water systems, and then is transmitted via aerosols and occasionally by direct aspiration.
Several strains of *Legionella* have been known for some time to live within amoebae in the natural environment (11, 20). In addition, *Legionella pneumophila* developed into amoebas are more resistant to chemical and other biocides than *in-vitro* Legionella (2, 3, 4).
The biofilm formed by *Legionella* and other microorganims is capable to fix on the surface of the water systems. This phenomenon has been observed in PVC, stainless steel, rubber, wood and softly in copper (20). The biofilm formation can especially be found on the surface of water pipes, tower basins and heat exchangers.

Background of water contamination

As the cooling effect works by evaporation of circulation water itself, an increase of total dissolved solids (TDS) is registered on water samples; reaching levels of 1500mg/L of TDS after 3 cycles of circulation (11) due to the concentration of the dust of the circuits in the water. The gas washing effect leads also to a permanent admixing of all kinds of biological germs from the operation air into the water, so that a constant recontamination of the water can not be avoided during the operation of the systems. The growth of biomass, especially bacteria, in the cooling system is supported by the increase of TDS, due to the increase of available nutrients. In addition, temperatures around 30 – 36°C, especially in Mediterranean countries, offers ideal growing conditions for micro-organisms living in water suspensions or biofilm.
Cooling towers, which are wet or evaporative condensers that form part of an air conditioning system, can present a particular hazard because they readily generate fine water droplets and there is an air current to transport them. As they are usually located on rooftops there is a potential for infecting large numbers of people. The bacteria may also colonise hot and cold water systems. For instance, showers and spa baths have been associated with infection.
In summary cooling towers and evaporative condensers have been implicated as the most common source of *Legionella spp*. and linked with most of the outbreaks of legionellosis (7). Hence, the HSE (Health and Safety Executive) recommends, at least quarterly, sampling of cooling towers and routine sampling of hot and cold water systems if the recommended temperature regime is not followed or has failed or if the local risk assessment suggests it is needed.

Description of real systems for *Legionella* inactivation based in ozone treatment

Ozone is one of the most powerful oxidizing agents available for treatment on industrial wastewater and legionary disease prevention. The main problem of the using ozone, is the instability of the compound (must be produced *in situ*) and the difficulty to solubilise it in water (19) in acceptable concentrations enough to kill bacteria.

Ozone has been applied on several water systems in industries and hospitals however the scientific conclusions regarding the effectiveness of those treatments are still not clear.

Cooling towers

Cooling towers are industrial devices for lowering the temperature of water by evaporative cooling in which atmospheric air is in contact with falling water, thereby exchanging heat. The term also includes those devices which incorporate a water-refrigerant or water-water heat exchanger.

The lack of maintenance, the accumulation of COD (Chemical Oxigen Demand), dust and micro-organisms create ideal conditions for the development of biological aerobic growth (including *Legionella*) in industrial cooling towers.

Besides several biocides as chlorine, electro-chlorination, chlorine dioxide, monochloramine, copper/silver copper and chlorine; ozone has been found as a suitable biocide to reduce the numbers of presumptive *Legionella pneumophila* in test cooling towers, offering several environmental benefits.

There are numerous developed systems using ozone, applied to keep cooling towers free of dust and microbiological growth. For example a system called Coolzon which applies ozone in a separate treatment loop. It consists in a filtration step followed by ozonation of the cleaned water. The first step aims to reduce the dust and COD content giving a reduction of available nutrients for biological growth. The second one oxidizes the developed biomass and the rest of dust attached to the circuit walls (12).

There are also in the market other, brands which provide compact equipment for controlling legionnaires disease in cooling towers and evaporating condensers using ozone. Those systems are prepared to operate in continue ozone dosage but the efficiency of occasional shock treatments is not reported.

Evaporative condensers

This kind of systems has shown high risk of *Legionella* infections especially during maintenance operations. Inadequacies exist in current biocidal treatment practices for cooling system waters.

Several organic biocides in use today have been shown to be ineffective in controlling *Legionella* densities (9, 10), but periodic discharges of cooling system water into local rivers or streams have begun to restrict the amount of chlorine residual (one of the most used biocides) present in such discharges (23). Thereby ozone treatments appear as very promising as environmental friendly alternative.

Several brands (14) already sale customized evaporative condensers coupled with ozone systems designed to control microbiology and corrosion in the condenser water; however not scientific results are reported on the efficacy.

Hospital plumbing water (water pipelines)

In vitro studies developed on a 38 L plumbing system using ozone, among other biocides was evaluated by Maruca et coauthors (15). Those studies showed effectively control of *L pneumophila* by a residual concentration of 1 to 2 mg/liter of ozone and the data suggested that ozone could remove *L. pneumophila* in a large water distribution system. However, because of the rapid decomposition of the ozone residual in water, its main utility may be limited as a supplemental disinfectant (15).

Lack of knowledge

The successful applications of ozone in water systems have been characterized by low corrosion rates of the tubing materials, very low biological counts (*Legionella* and other mesophilic micro-organisms), and scale free achievement of relatively high dissolved ozone concentration (11). The efficiency of ozone has also been proved in water pipelines and in-vitro models (14).

Nevertheless, several problems have been reported in real installations. Numerous operational shortcomings have been found as contributing factors. Those factors include failures to achieve the desired residual ozone (due to insufficient system sizing), inadequate or unmaintained equipment, chemical contamination of the system, high interaction of ozone with the materials or poor mixing of ozone into the recirculation water. As a result excessive biological growth and/or corrosion, depending on the particular application and water conditions have been reported.
As summery, no reliable data are available regarding the efficiency of ozone to kill *Legionella* in different water *in vitro* systems (8) as well as at real scale (5). The limitations of this technology and the key factors responsible in the decay of efficiency observed in some experiences described on the literature are also under discussion.
Therefore, more information on the subject is needed and the construction of a plant able to simulate real scale water facilities for *Legionella* inactivation by ozone treatment has been considered a significant challenge to overcome.

Pilot plant for *Legionella* controlling in water pipelines using ozone (OZOLEG project)

Concept

In order to evaluate the efficiency of ozone to eliminate *Legionella* strains in water pipelines, a pilot plant has been designed aiming at simulating the microbiological growth of *Legionella* in biofilms and the disinfection capacity of ozone under these conditions.
Three materials have been used for the construction of the plant in order to evaluate the behaviour of biofilms and *Legionella* for each material under different possible treatments with ozone.

General description of the pilot plant

The plant is composed by 9 independent circuits of water pipelines fixed on vertical panels and constructed in iron, PVC and polypropylene. Each material is used in 3 of the circuits. All circuits have identical components and measurement equipments, changing only the material used.
The dimensions of each circuit pipeline are: 14,6 m of length and 2,5 cm of internal diameter (excepting a 59 cm portion with internal diameter of 5 cm). Each circuit has 10 removable portions or coupons (8 of 2,5 cm internal diameter and 2 of 5 cm internal diameter) designed to evaluate the adherence capacity of *Legionella* in biofilms. Five of those coupons have artificially been modified making them rough in order compare the capacity of adherence of biofilms to irregular surfaces.
The circuits have been filled with tap water re-circulated by independent pumps which take the liquid from a 200 L deposit made of polypropylene. The pumps have been fixed on the top of each deposit where suck the liquid from a vertical pipe with a one-way valve at the bottom, avoiding the discharge of the pumps when stopped. Five valves are distributed on the circuits in order to take liquid samples from different points.
The distribution of the components of the circuits is shown in Fig. 1 and the description of them in Table 1.
The whole pilot plant is controlled by an automaton system and the undertaken on-line measurements recorded by it.

<u>Ozone generation</u>

Each circuit hold an independent ozone generator able to produce 2 g O_3/h by a corona electrical discharge applied on ambient air. The generated ozone is injected into the liquid phase of the circuits by a PVC venturi. The driving force for the ozone injection is done by the liquid in motion passing through the venturi thereby ozone injection is only possible to be done when pumps are on but not always.
The level of dissolved ozone in each circuit is automatically controlled by the automaton system depending of the correspondent measurement on the liquid phase and the desired level to be achieved.

<u>Equipment for measurement and automation system</u>

Each circuit hold probes for on-line measurements of pH, conductivity temperature and redox potential automatically recorded each 15 minutes by the automation system.

Four electro-valves collect 2 samples of gas and 2 of liquid from each circuit every 45 minutes for the corresponding ozone measurements. All the measurements of ozone are undertaken by ANSEROS equipment; gas phase (Ozomat GM-6000-pro) and liquid phase (Ozomat MP coupled with ozone disrober Ozomat WP). The control of electro-valves, equipment and recording of data are automatically controlled by the automaton system.

Microbiological assays

From the pilot plant 2 type of samples, liquid samples and coupons, are investigated in different points of the applied treatments. In both samples *Legionella erythra* and total aerobic mesophilic bacteria have been determined based on plates counting. The method ISO 11731 has been used for *Legionella* detection in water samples using BYCE as culture media whereas total aerobic mesophilic bacteria have been determined at 30 ºC using PCA as culture media.
Counts from coupons require gently ultrasonic extraction (without cell destruction) prior to apply the method described above for bacteria counting.

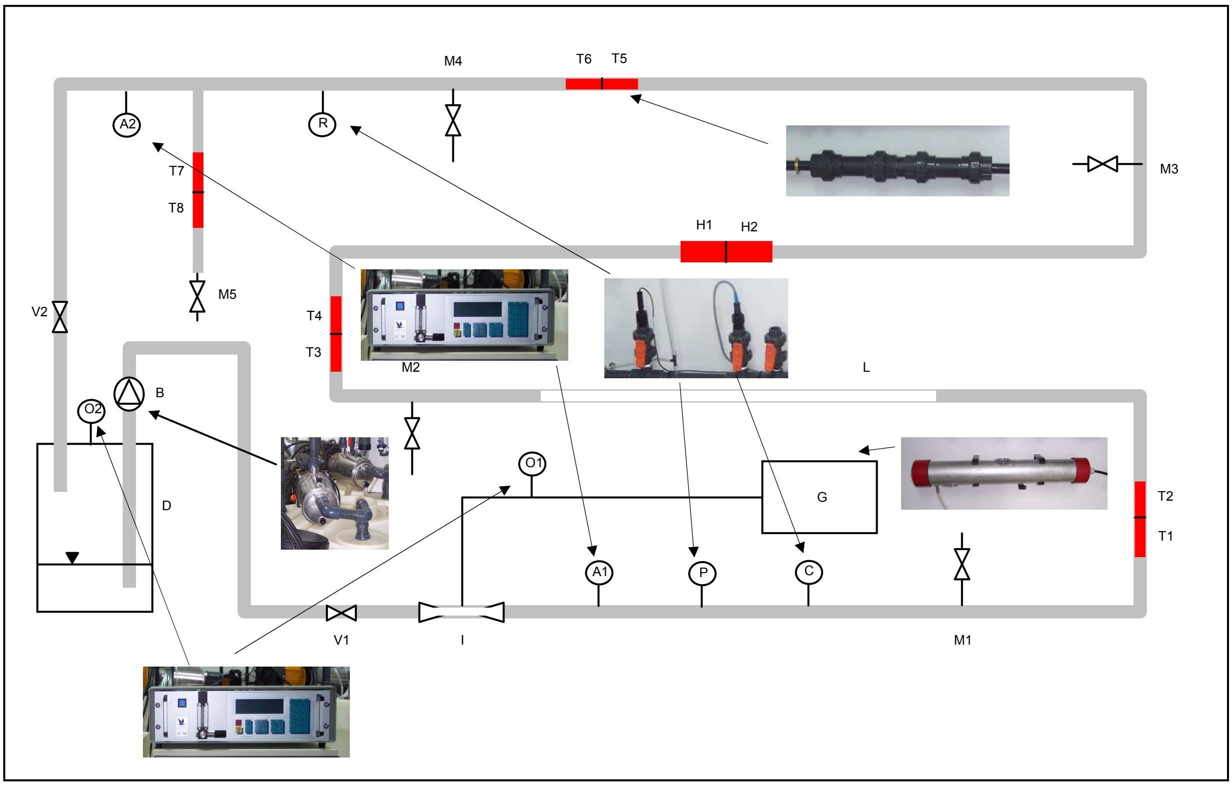

Fig. 1. Scheme of the plant for studies of Legionella growth and disinfection using ozone

Safety

In order to reduce the risk of contamination in the pilot plant, a non-pathogenic strain of *Legionella* (*L. erythra*), with a similar behaviour than *L. pneumophila* regarding inclusion in biofilms, has been chosen for the inoculation of the circuits.
The whole pilot plant is physically isolated into a restricted area where the air is filtered by an absolute filter HEPA (mod. PE11ST00) coupled with an air extractor (THLZ-200, 1 CV) giving a negative pressure in the area. The pilot plant area is also equipped with three UV lamps (36W) aimed to inactivate any possible generated aerosol of *Legionella.*
Water samples are automatically collected for ozone detection, the measurements are recorded by the automaton system and the samples disinfected by chlorination prior to be eliminated. The chlorination is controlled by an independent system for detection of volume and dosage, calibrated to reach 50 mg/L of free chlorine on the water samples.
The pilot plant area is equipped with an ambient ozone detector (ANSEROS, Sen 6060-S) coupled with a remote monitor.

The operators authorised to access in the restricted area, are equipped with integral clothes (chemical and microbiological protection), goggles, mask filter for microbiological protection, rubber boots, gloves and a portable ozone detector (ANSEROS, Ozomat SEN-P).

Table 1. Circuit components and the corresponding short form

ref. code		details
D		200 L deposit
B		pump
I		venturi
O	1	sampling point ozone gas phase 1
G		ozone generator
A	1	sampling point ozone liquid phase 1
P		pH probe
C		conductivity probe
M	1	water sampling point 1
T	1	coupon 1
T	2	coupon 2
L		transparent pipeline
M	2	water sampling point 2
T	3	coupon 3
T	4	coupon 4
H	1	coupon (big) 1
H	2	coupon (big) 2
M	3	water sampling point 3
T	5	coupon 5
T	6	coupon 6
M	4	water sampling point 4
R		redox probe
T	7	coupon 7
T	8	coupon 8
M	5	water sampling point 5
A	2	sampling point ozone liquid phase 2
O	2	sampling point ozone gas phase 2

Operational conditions during the pilot plant starting up

Each circuit has been filled with at least 58 L of tap water which has been re-recirculated for about 2 weeks without any external inoculation or ozone application in order to stimulate a natural installation of aerobic biofilms.
After this period, a suspension of *L. erythra* has been inoculated in each circuit, reaching a concentration of ca. $1x10^5$ cfu/mL in the water of the circuits.

Conclusions

An automated pilot plant has been developed at ainia to operate under safe microbiological conditions. The plant is automatically recording on-line ozone measurements (liquid and gas), pH, conductivity, temperature and redox potential without the intervention of the operators.

This plant will allow the investigation of *Legionella* survival after applying different ozone treatments. Effect of biofilm formation will also be studied.

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