HYDROGEN PEROXIDE AND SILVER:
THEIR USES AS DISINFECTANTS IN
UNITED KINGDOM DOMESTIC WATER SYSTEMS
HYDROGEN PEROXIDE AND SILVER: THEIR USES AS DISINFECTANTS IN UNITED KINGDOM DOMESTIC WATER SYSTEMS

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ABSTRACT

The biofilm in water distribution systems within multioccupancy buildings hosts microorganisms including legionella and must be removed to ensure good water hygiene management. The BRE and BSRIA have shown that traditional methods of disinfection using sodium hypochlorite and chlorine dioxide are not always effective in dealing with biofilm. We have developed a 50% hydrogen peroxide formulation containing a silver salt. This formulation shows fast (5min) efficacy on Legionella pneumophila according to European norms. The mechanical action coupled with the chemical action of the formulation allows rapid cleaning and treatment of biofilm.

Our ready to use solution shows a very low level of toxicity towards mammals. This formulation is being used to resolve persistent legionella problems in the United Kingdom. Its ease of use and detection indicates that the formulation has an important future role in the control of legionella bacteria in water systems within multioccupancy buildings.

KEYWORDS

Water distribution systems /Biofilm Legionella pneumophila Hydrogen peroxide Silver salt/

INTRODUCTION

The formation of a biofilm in a water distribution system provides a feeding substrate for living species including Legionella pneumophila. The formation of the biofilm depends upon different parameters like the origin of the distributed water and the nature of the materials used in the distribution system. Biofilms need to be removed from the internal surfaces of water systems to ensure consistent water hygiene.
It is known that hydrogen peroxide, a natural disinfectant, which breaks down to environmentally acceptable products, is used by the biological cells of the immune system to kill antigens. The use of silver salts enhances the biocidal activity of hydrogen peroxide.

A hydrogen peroxide formulation containing a silver salt has been developed and shows efficacy on Legionella pneumophila according to European norms. The formulation is safe to use and also demonstrates biofilm treatment capability.

The formulation* described in this paper already has approval from the Secretary of State for the Environment as an emergency disinfectant for public water supplies in the United Kingdom.

**THE BIOFILM PROBLEM**

Biofilms form in most water systems and start as an adhesive thin layer, which allows a constantly changing ecosystem to develop, on internal surfaces of pipes and fittings. This biofilm obtains nutrients from the substrate, on which it grows and can comprise biodegradable organic compounds together with oligoelements like iron, manganese, water salts, and scale. This biofilm shown in figure 1 constitutes the feeding substrate of many living species like bacteria, fungi, algae, protozoa and even invertebrates like Asellus aquaticus.

**FIGURE 1**

A SEM of a developed biofilm in water distribution system.

From: ASM-Biofilms collection. McLaughlin-Borlace.
See reference 3

The Dutch organization KIWA has set up a method to monitor the formation of a biofilm in water distribution systems (1). The density of the biofilm can be determined by the measurement of the content of adenosinetriphosphate (ATP). This study shows that the biofilm formation potential expressed as pg. ATP/cm² is a parameter, which is influenced by different factors.
Table 1 shows that the origin of the water plays an important role. Ground water supplies lead to the formation of 1,000 to 10,000 pg ATP/cm² while surface water supplies are responsible for a potential of 100 to 1,000 pg ATP/cm². Filtered water is less of a concern as only few tens of pg ATP/cm² are formed. The nature of the materials used to install the water distribution system is also an important parameter. The table 1 indicates that the least supportive growth surface is glass with only 3 pg ATP/cm² while soft PVC enables 10,000 pg ATP/cm².

**TABLE 1**

Water sources and materials in contact with water are important factors for the Biofilm Formation Potential

From.
1995 KIWA N.V Research & Consultancy
P.O. Box 1072, 3430 BB, Nieuwegein, The Netherlands

<table>
<thead>
<tr>
<th>WATER</th>
<th>Biofilm Formation Potential Pg ATP/cm²</th>
<th>MATERIALS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground water supply</td>
<td>10000</td>
<td>Soft PVC</td>
</tr>
<tr>
<td>(highest value)</td>
<td></td>
<td>Rubber tubing</td>
</tr>
<tr>
<td>Ground water supplies</td>
<td></td>
<td>GFR Polyester</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PE materials</td>
</tr>
<tr>
<td>Surface water supplies</td>
<td>1000</td>
<td>Silicone tubing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GFR Epoxy</td>
</tr>
<tr>
<td>GAC-filrate (fresh carbon)</td>
<td>100</td>
<td>PVC materials</td>
</tr>
<tr>
<td>Aerobic ground water</td>
<td></td>
<td>Teflon</td>
</tr>
<tr>
<td>Slow sand filtrate</td>
<td>10</td>
<td>Stainless steel</td>
</tr>
</tbody>
</table>

GAC = Granulated Activated Carbon
GFR = Glass Fiber Reinforced

Among the bacteria living in the biofilm, Legionella pneumophila (serotype 1) is of prime importance as this microorganism caused the death of people in many countries (2, 3). The problem for Legionella pneumophila (shown in figure 2) is even more complex as the biofilm does not only offer a feeding substrate but also harbours host cells, like Protozoa in which the bacteria can grow and proliferate. (fig3)
Classical disinfection with 0.5 mg/l of chlorine would kill the bacteria but more than 50 mg/l of chlorine would be needed to kill the protozoa (4). The latter concentration cannot be used routinely in practice. The literature stipulates that a complex of hydrogen peroxide together
with silver salts does present high stability, broad spectrum microbiocidal action and long lasting activity with significant power on biofilm (5).
WHY ARE HYDROGEN PEROXIDE AND SILVER EFFECTIVE

The literature refers to possible mechanisms of actions of both hydrogen peroxide and silver salts and potential activation of the former by the latter.

The production of hydrogen peroxide occurs naturally in the body as part of our defence against antigens.

Hydrogen peroxide is formed in the cells of the immune system and is used in the killing of the antigen. The hydrogen peroxide is formed by the reduction of molecular oxygen passing through the superoxide anion intermediate. Hydrogen peroxide’s reduction goes further to water passing through the hydroxyl radical intermediate. Substances like lipids, proteins and nucleic acids are able to be attacked by all these oxidative agents. (6)

In the cell the following sequence of reactions takes place.

\[
\begin{align*}
O_2 + e^- & \rightarrow O_2^- \\
H^+ + O_2^- & \rightarrow HO_2^- \\
HO_2^- + O_2^- + H^+ & \rightarrow H_2O_2 + \text{singlet } O_2 \\
H_2O_2 + O_2^- + H^+ & \rightarrow H_2O + OH^- + \text{singlet } O_2 \\
OH^- + O_2^- + H^+ & \rightarrow H_2O + \text{singlet } O_2
\end{align*}
\]

The antigen produces enzymes like superoxide dismutase which transform the superoxide anion into hydrogen peroxide and catalase or peroxidase which remove hydrogen peroxide. These reactions (shown below) can be considered as a primary defence against this Redox scheme (7).

\[
\begin{align*}
2O_2^- + 2H^+ & \rightarrow H_2O_2 + O_2 \quad \text{SOD (superoxide dismutase)} \\
H_2O_2 & \rightarrow H_2O + \frac{1}{2} O_2 \quad \text{Catalase} \\
H_2O_2 + RH_2 & \rightarrow 2H_2O + R \quad \text{Peroxidase}
\end{align*}
\]

Although there is not a complete understanding of how hydrogen peroxide works it is proposed that ferrous iron present in the microorganisms facilitates the germicidal effect by reacting with hydrogen peroxide to form hydroxyl radicals according to the Fenton reaction (8).

\[
Fe^{++} + H_2O_2 \rightarrow Fe^{+++} + 2 OH^-
\]

While the primary Hydrogen peroxide targets are the lipids, proteins and nucleic acids it is suggested that silver mainly acts on —SH protein groups. The enhancement of the hydrogen peroxide’s activity may be associated with the damage to the enzymes involved in the hydrogen peroxide deactivation (9).

POSSIBLE CHEMICAL AND MECHANICAL ANSWERS TO THE BIOFILM PROBLEM

In view of these facts, formulations based on hydrogen peroxide and silver salts might constitute a chemical answer to the biofilm problem. Hydrogen peroxide, now the subject of a European norm EN 902, needs to be of high purity. The silver used is subject to a registration eligibility decision (RED) in USA. Discontinuous treatment of water distribution systems using hydrogen peroxide and silver salts is now allowed in many countries. For example, in
France, the Ministere de la Santé Publique considers this combination for the treatment of Legionella pneumophila (10). The Secretary of State for the Environment has allowed our formulation* to be used in potable water as an emergency disinfectant.

Apart from a possible chemical answer, an additional advantage of the use of hydrogen peroxide is that its degradation leads to the formation of gaseous molecular oxygen. This formation results in small amounts of turbulence (effervescence) at the vicinity of the biofilm which causes the biofilm to fragment. The small pieces are then better chemically attacked by the disinfectant (11). It is well known that the efficacy of biocidal cleaning is a function of several parameters like temperature, the time of action, the nature of the chemicals which are used and the amount of mechanical energy which is given to the system. Soluble chemicals like chlorine or sodium hypochlorite have almost no mechanical action and are therefore ineffective against biofilm. Chlorine Dioxide does not produce the same effervescence or micro-turbulence and is therefore less effective as a biofilm-removing agent.

**FIGURE 4**

The components (%) of an efficient disinfective cleaning: temperature, time of contact, chemicals, mechanical energy.

**HYDROGEN PEROXIDE FORMULATIONS CONTAINING A SILVER SALT AND EFFICACY ON LEGIONELLA PNEUMOPHILA**

We* have developed formulations based on 50 % hydrogen peroxide and containing a silver salt. These formulations have different niches in the area of disinfection. Formulations with a low silver concentration are suitable for bulk treatments like water in swimming pools for example (12). Solutions with a higher silver concentration are used in surface treatments and more generally in applications where short contact times are needed.

Colorimetric titration of the different formulations with potassium permanganate showed that they are all stable upon aging. The decrease of hydrogen peroxide level at 96ºC during 16 hours always fell in the range of 1-4 % maximum. These conditions are equivalent to a one year aging at room temperature.
The formulation* recommended for water distribution system disinfection has been tested against Legionella pneumophila by the Dutch institute TNO and by the INSTITUT PASTEUR in France respectively. Results are shown in tables 2 and 3.

At the TNO, tests were performed at 30º C against Legionella pneumophila serotype 1 only. From the results of table 2, it can be concluded that at concentrations of 100 ppm and 200 ppm, the formulation meets the requirements of the water treatment products with a slow bactericidal effect. Indeed, a delta log N> 5.1 is obtained after 24 hours of contact. N is the colony forming units.

At a concentration of 2%, the formulation* meets the same requirements but with a fast bactericidal action as at this concentration, a delta log N> 6.6 is obtained after 5 minutes. These results were obtained according to the draft of the European standard EN 13 623 (1999) called “Bactericidal activity of products against Legionella pneumophila- Test method and requirements (phase 2/ step 1)”.

### TABLE 2

Microbiocidal effect (ME) against Legionella pneumophila serogroup 1  
**TNO EN 13 623**

<table>
<thead>
<tr>
<th>Formulation Concentration [% w/v]</th>
<th>Contact Time</th>
<th>Log Nc [log cfu/ml]</th>
<th>Log Nd [log cfu ml]</th>
<th>ME 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>24 hours</td>
<td>7.1</td>
<td>&lt;2.0</td>
<td>&gt;5.1</td>
</tr>
<tr>
<td>0.02</td>
<td>24 hours</td>
<td>7.1</td>
<td>&lt;2.0</td>
<td>&gt;5.1</td>
</tr>
<tr>
<td>0.2</td>
<td>24 hours</td>
<td>6.5</td>
<td>&lt;2</td>
<td>&gt;4.5</td>
</tr>
<tr>
<td>1.0</td>
<td>24 hours</td>
<td>6.5</td>
<td>&lt;2</td>
<td>&lt;4.5</td>
</tr>
<tr>
<td>0.2</td>
<td>1 hour</td>
<td>7.78</td>
<td>3.00</td>
<td>4.8</td>
</tr>
<tr>
<td>1.0</td>
<td>1 hour</td>
<td>7.78</td>
<td>&lt;2</td>
<td>&gt;5.78</td>
</tr>
<tr>
<td>2.0</td>
<td>5 minutes</td>
<td>8.6</td>
<td>&lt;2.0</td>
<td>&gt;6.6</td>
</tr>
</tbody>
</table>

Cfu=colony forming units.

Microbiocidal effect at 30º C, ME 30= $\log N_c - \log N_d$.

Nc=the number of cfu ml of the test mixture without the product under test.

Nd=the number of cfu ml of the test mixture after the action of the product under test.

At the INSTITUT PASTEUR, tests were performed at 20 and 30ºC, during 5 minutes, according to the European norm EN 1276, on Legionella pneumophila serotypes 1 and 6. The formulation presented for both microorganisms a delta log N> 5 at a concentration of 0.1%. This institute also showed that the formulation can be diluted either in distilled or in hard water.
Delta LOG N >5: 20ºC: 5 min.: 0.1% of the formulation (diluted with distilled water)
Delta LOG N >5: 30ºC: 5 min.: 0.1% of the formulation (diluted with hard water)

The French laboratory CAPSIS also tried the formulation* on the amoebae Naegleria fowleri (the killing amoebae which grows in cooling towers). At a concentration of 10%, at 37º C, at pH 7 and for one hour of contact, the population of Naegleria fowleri fell from 15,000 to less than 25 cysts/l.

TOXICOLOGICAL ASPECTS

In France, the laboratory EVIC-CEBA performed a toxicological evaluation on a dilution of the formulation* recommended for the water distribution system surface disinfection. This dilution presented 3% of hydrogen peroxide (ready to use).

The laboratory demonstrated that the formulation is not dangerous by inhalation and tested in accordance with Directive 67/548/EEC is not a skin irritant or skin sensitiser, is not dangerous by ingestion and is not an eye irritant.

PRACTICAL APPLICATIONS OF THE FORMULATION IN BUILDING WATER SYSTEMS

In the United Kingdom this formulation has Ministry of the Environment Approval for use as an emergency disinfectant in public water supplies. This means that it can be applied up to a level consistent with the silver level in drinking water allowed by the EC regulations – equivalent to around 200ppm of the product for a maximum of 90 days.

On the continent where there are no storage tanks in buildings, and the complete mains water to buildings must be treated, the formulation is applied for 24 hours at a concentration of 200 ppm and then removed from the system.

In the United Kingdom we can either isolate the treatment from the potable water system, and treat only the non potable water on a continuous basis, or we can treat the complete water supply on a discontinuous basis. The European experience is generally that a 24-hour treatment conducted twice per annum is sufficient to prevent biofilm development. (This may increase to 3 treatments if the distribution pipework is plastic or the quality of the incoming water is poor).

In the United Kingdom constant dosing of non potable water in multioccupancy buildings has eradicated persistent Legionella problems. Discontinuous dosing of water systems where the water may be drunk has also been successful.

The formulation* can therefore be used to replace chlorine dioxide for legionella control. Chlorine Dioxide while it has approval for constant dosing into potable water systems in the United Kingdom is unlikely to be effective on biofilm at the dosage levels which can be used...
(up to 0.5mg/l total oxidant).

The formulation* has other advantages in building water. It is safe to use, it is easy to use - it can be dosed using conventional dosing and control equipment, it does not impart taste, taint or smell to water and it can be easily measured (standard peroxide test strips or colorometric tests.)

It should be stated that the World Health Organisation commenting on silver levels, which are acceptable in drinking water, have stated that “The low levels of silver in drinking-water, generally below 5 µg/litre, are not relevant to human health with respect to argyria (illness resulting from excessive silver in the human body).

On the other hand, special situations exist where silver salts may be used to maintain the bacteriological quality of drinking-water. Higher levels of silver, up to 0.1 mg/litre (this concentration gives a total dose over 70 years of half the human NOAEL (No Obvious Adverse Effect Level) of 10 g), could be tolerated in such cases without risk to health.”. The EPA in the United States have relaxed the allowable level of silver in drinking water to 100 ppb.

A number of facilities management companies are starting to treat systems with this formulation and it is hoped that a paper giving information on practical applications of the formulation will be available early next year.

The formulation* has been successfully used as a cooling water biocide in both large and small cooling systems. Because of its stability the chemical is not lost in the airflow through the cooling tower (c.f. chlorine and chlorine dioxide). It can be dosed in proportion to the make up water flow and is ideal for getting rid of biofilm and the Legionella which it hosts. It is not aggressive to most common materials of construction.

CORROSION ASPECTS

The French laboratory CETIM evaluated by immersion the corrosion / passivation aspects of our* hydrogen peroxide containing a silver salt on stainless steel 304 L, copper and galvanized steel. These materials are commonly used in the manufacturing of the pipes for the distribution systems. The table 4 summarizes the data.

**Table 4**

Corrosion data of a 1% hydrogen peroxide formulation containing a silver salt on several materials

<table>
<thead>
<tr>
<th>Immerged material during 6 periods of 170 hours at 40 degrees</th>
<th>Average corrosion rate in mm/year</th>
<th>U Schweitzer criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stainless Steel 304L</td>
<td>4.12 $10^{-4}$</td>
<td>Very good resistance towards corrosion</td>
</tr>
<tr>
<td>Copper</td>
<td>0.021</td>
<td>Very good resistance towards corrosion</td>
</tr>
<tr>
<td>Galvanized steel protected by</td>
<td>0.62</td>
<td>Good behaviour towards</td>
</tr>
</tbody>
</table>
The case of galvanized steel was investigated further. The corrosion rate was high during the two first periods corresponding to zinc oxidation. After these two first periods, the rate of corrosion rapidly decreases and reaches a stable level of 0.3 mm / year.

In order to see if the hydrogen peroxide formulation containing a silver salt has a passivation effect, the CETIM followed during the immersion time the free potential of galvanized steel without zinc protection. With demineralised water, the electrochemical potential decreased constantly from — 670 mV to — 710 mV and the metallic surface is affected by corrosion. The addition of a hydrogen peroxide formulation containing a silver salt modifies the potential which immediately performs a delta of 650 mV towards positive values. Final stabilisation of the potential is between + 100 and + 200 mV.

The substrate surface is thus passivated when the hydrogen peroxide formulation is added. This is confirmed by other experiments like the measurement of the polarisation resistance and the follow up of the intensity / potential curve.

**CONCLUSIONS**

Legionella pneumophila living in the biofilm when inhaled can cause damage in the lungs as these microorganisms can grow and proliferate in human cells just as they do in protozoa.

A 50% hydrogen peroxide formulation* containing a silver salt has been developed. The enhancement of the activity of hydrogen peroxide by silver salts is described in the literature.

This formulation shows a fast (5 minutes) efficacy at 0.1% at 20º-30º C on Legionella pneumophila according to European norms. Amoebae Naegleria fowleri can also be treated. On the other hand, a ready to use dilution of the formulation* containing 3% hydrogen peroxide is of very low toxicity towards mammals. The formulation* which is completely chlorine free can be injected into domestic or industrial water distribution systems through a practical dosing device. The use of this formulation* is permitted by Dutch organisations like KIWA and CTB, by the Secretary of State for the Environment in the UK and by the Direction Generale de la Santé in France.

Research goes on to assess the efficacy on other microorganisms and new formulations are under investigations.
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