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Conventional and Alternative Disinfection Methods of *Legionella* in Water Distribution Systems – Review

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Abstract **– Prevalence of *Legionella* in drinking water distribution systems is a widespread problem. Outbreaks of *Legionella* caused diseases occur despite various disinfectants are used in order to control *Legionella*. Conventional methods like thermal disinfection, silver/copper ionization, ultraviolet irradiation or chlorine-based disinfection have not been effective in the long term for control of biofilm bacteria. Therefore, research to develop more effective disinfection methods is still necessary.**

Keywords **– Disinfection, drinking water, *Legionella*.**

I. Introduction

Microbiological contamination of drinking water is a widespread problem, which causes regular outbreaks of waterborne diseases worldwide. *Legionella* is a typical example of a waterborne pathogen, which can cause different forms of legionellosis [1]–[5]. Clinical manifestations of the *Legionella* caused diseases may vary from mild fever to severe, potentially lethal pneumonia (Legionnaire's disease). *Legionella* and diseases caused by it became topical in 1976 when the first large outbreak of Legionnaire's disease was identified [6]–[9]. Although since then various methods for *Legionella* control have been used, prevalence of *Legionella* in man-made water distribution systems continues to present challenges to public health officials and water suppliers [6], [10]. Studies on occurrence of *Legionella* in drinking water distribution systems report high overall prevalence of *Legionella*. For example, in Italy 22.6 % of the surveyed water distribution systems were contaminated with *Legionella* [11], 26 % in Germany [12], 30 % in Finland [13] and 42 % in Latvia [14]. Results of these studies indicate that *Legionella* in water distribution systems is still an unsolved problem. Conventional water disinfection methods, such as thermal disinfection, use of chlorine, chlorine dioxide, monochloramine, metal ions and other methods [5], [15], [16] have been widely investigated in a large number of studies. Results show that complete elimination of *Legionella* from a water distribution system is difficult to achieve with any disinfection approach. Since each conventional disinfection method has advantages and disadvantages, the optimum method has not been identified. Insufficient disinfection efficacy followed by occasional outbreaks of *Legionella* caused diseases and identification of chlorine as a source of potentially harmful disinfection by-products [8], [17], [18], [19] have led to intensive research to develop alternative disinfection techniques [2], [3], [16]–[18], [20]. Objectives of this paper are to provide an overview of conventional disinfection methods, to assess their advantages and drawbacks, and to provide an insight into research on alternative disinfection approaches.

II. Conventional Disinfection Techniques

A. Thermal Disinfection

The superheat and flush is the oldest method that has been used to control *Legionella* in water distribution systems. For thermal disinfection, it is necessary to flush all outlets, faucets and showerheads of the water distribution system. During several studies, in addition to raising water temperature, faucets and showerheads have been chemically disinfected or replaced, however, such actions have not shown any effect on minimizing the *Legionella* colonization [21].

The temperature of the hot water flushing at distal sites and the duration of the flush are critical for effective thermal disinfection. If the water temperature at the distal outlet does not exceed a critical point or the duration of flushing is too short, the disinfection procedure is likely to fail. Both these parameters have been investigated in various studies [8], [19], [22]–[24] which have shown that one log reduction in *Legionella* counts at 70 °C can be achieved in less than one minute. At 60 °C the required flushing time is less than 5 minutes [8], [19]. Likewise, different authorities and professional organization guidelines suggest that the temperature of the hot water flushing the distal sites should be greater than 60 °C, which has been proved as inhibitory for *Legionella* [8], [19], [22].

Although the thermal disinfection is widely used, there is no consensus yet regarding the required duration of the flush. Different sources recommend flushing time from 5 minutes up to 30 minutes [8], [21], or more [25], though several studies have shown no significant effect of flushing time less than 30 minutes [21].

Thermal disinfection may be only temporary, and recolonization of *Legionella* is predictable after superheat-and-flush procedures [19], [21], [25], [26]. Recolonization of the water system may occur within days, weeks [22], [24] or months [19], [25] after disinfection, especially in cases when circulating temperatures are returned to level of 45 °C to 50 °C [19]. Maintaining the temperature at 60 °C after superheat-and-flush has been reported to be effective in delaying recolonization of bacteria [8], [19]. However, it is not a widespread approach due to economic reasons, even if increase of the fuel and energy costs for maintaining higher hot water temperature (60 °C) has not been proven, since at higher temperature less hot water is used to maintain water at comfortable temperature [8], [19].

One of the main advantages of thermal disinfection is that it requires no special equipment, so it can be initiated expeditiously, which is a notable advantage in an outbreak situation, when *Legionella* must be eradicated from the water distribution system immediately [19], [21]. Another advantage is that this is the least expensive method among various widely used disinfection methods. In case of thermal disinfection, the greatest expenses are personnel costs [19].

The main disadvantages of thermal disinfection method are that it is a time-consuming procedure and numerous personnel are involved in monitoring distal sites, water tank temperatures, and flushing times [19]. Inability to affect biofilm bacteria [22] and short-term effect of disinfection are the major disadvantages of thermal disinfection, as well as the fact that it is not suitable for large buildings, where temperatures >60 °C at distal outlets cannot be reliably maintained [21], [24].

B. UV Irradiation

UV was first used for disinfecting drinking water in the early 1900ies but was not then further developed due to high costs, poor equipment reliability, maintenance problems and the implementation of chlorination, which was cheaper, more reliable and left disinfectant residual [1], [9], [27].

UV irradiation acts by producing thymine dimers in DNA, which hampers DNA replication [1], [8], [9]. Owing to technological improvements and due to the increased information on the production of hazardous oxidation by-products during chlorination and ozonation, this disinfectant is regaining popularity [18], [27], still it has not been widely used in drinking water disinfection because it leaves no residual to provide protection against potential downstream contamination [8], [10].

In contrast to oxidative disinfection processes with chemicals (e.g., chlorine and ozone), the efficacy of UV disinfection is not affected by conditions like temperature, pH and reactive organic matter [27]. Efficacy of UV disinfection may be reduced by the impact of various materials (biofilm, deposits, turbidity etc.) on UV light transmittance [8], [28].

Studies of the effect on *Legionella* have shown that UV alone is insufficient to control *Legionella*, and other actions, such as periodic hyperchlorination, superheat and flush or hydrogen peroxide treatment would have to be used along with UV irradiation [8], [19], [25]. Similarly, efficacy of UV disinfection can be increased by prefiltration, thus delaying recolonization of *Legionella* for about 3 months in contrast to several weeks in which recolonization occurs in case of UV irradiation without prefiltration [19].

UV light units usually are installed near the point-of-use, such as showerheads and faucets [8], [19], [25], therefore this method may be not suitable for disinfection of the entire building because bacteria persist in biofilms within dead ends and stagnant sections of the system [19]. There have been very few studies focused on the impact of UV disinfection on biofilm and its growth in water supply systems, and the results of these studies have shown that UV treatment did not have a consistent impact on the biofilm [27], [29].

The advantages of UV disinfection are easy installation, no danger of overdosing, production of no toxic or mutagenic/carcinogenic by-products (unless UV irradiation is followed by chemical disinfection), no taste and odour problems, no adverse effect on plumbing, no need to handle and store toxic chemicals, and small space requirement [1], [9], [19], [28].

Some disadvantages are lack of disinfectant residual in the treated water, which means that additional water treatment procedures are required, which causes additional expenses; biofilm formation on UV lamp surface, lower disinfection in high turbidity effluents, or potential problems due to photoreactivation of UV-treated microbial pathogens [1], [8], [9], [19].

C. Metal Ions (Copper/Silver Ionization)

Heavy metals such as copper and silver ions are bactericidal agents, which form electrostatic bonds with negatively charged sites on the organism's cell wall. These bonds create stresses leading to distorted cell wall permeability. This action, coupled with protein denaturation, leads to cell lysis and death [19], [30].

Metal ions can be added to water electrolytically or as metal salts. For ease of operation ions are frequently introduced electrolytically, therefore the electrodes of ionization unit must be periodically cleaned of the accumulated scale [8], [19].

Most studies have been based on the combined use of two metal ions [4], [8], [22], [30]. However, several studies have been performed on both separate and combined use of copper and silver ions against *Legionella* [31]. 6-log reduction of *Legionella* in 2.5 h is reported with copper ion alone at 0.1 mg/L. Silver ion is also reported to be effective, but it takes more time (8 h with concentration at 0.1 mg/L) to inactivate microorganisms [31]. The combination of both ions has been found to be synergistic [8], [31], similarly intermittent use of copper and silver ions is also reported to be successful [8].

Maintaining high temperatures in the water system can improve effectiveness of the copper/silver ionization, whereas effectiveness at high levels of pH is still questionable [30], [32].

Although in several studies copper/silver ionization failed to reduce colonization with *Legionella* [22], in most cases good efficacy for *Legionella* control during one year after activation of ionization unit is reported [8], [19], [21]. Cases of failure probably may be explained with the low concentrations of ions. Another reason could be high pH of the water, which may be an important factor in efficacy of copper and silver in controlling *Legionella* [32].

Periodic monitoring of metal ion concentrations is necessary for drinking water to ensure that the concentrations are below the maximum allowed contaminant level. It should be noted that in some countries local regulations do not allow the use of copper in its range of effectiveness, thus excluding adequate use of copper/silver ionization [8], [30], [31].

Advantages of copper/silver ionization include relatively low cost, easy installation and maintenance [8], [19], [32].

Disadvantages are that the electrodes accumulate scale and should be cleaned regularly to ensure maximum performance. In addition, the level of copper and silver ions may fluctuate. Excessive ion levels may cause discoloration of water and surfaces [8], [19], [30]. Monitoring of ion levels should be performed routinely. Long-term treatment with copper and silver ions could theoretically result in the development of resistance to these ions [8], [19], [22]. Like other conventional disinfection methods, copper/silver ionization is not able to completely eradicate *Legionella* from the plumbing system, since it persists in biofilm [30].

D. Chlorine

Chlorine, like some other oxidizing agents (chlorine dioxide, chloramines, ozone and hydrogen peroxide), is commonly used for drinking water disinfection. Chlorination has been the most favoured method in water industry, providing both primary and residual disinfection, though due to concerns about harmful disinfection by-products, other disinfection methods are being explored [5], [8], [17], [33].

Chlorine can be added to water as a gas, as a liquid (mainly as either sodium hypochlorite or purified hypochlorous acid); or as a solid (most commonly as calcium hypochlorite) [8], [28].

Chlorine affects respiratory and transport activities and nucleic acids in bacteria leading to their inactivation [1], [8].

Chlorine reduces and controls *Legionella* populations as long as the residual concentrations are maintained. However, to continuously control *Legionella*, much higher chlorine concentrations than typically found in domestic drinking water, are needed [8], [19]. Shock hyperchlorination can be applied for disinfection of water system, followed by replacing the water in the system after 1–2 h with fresh water and maintaining around 1 mg/L of chlorine concentration in the water. If shock hyperchlorination is used, recolonization of *Legionella* will occur after chlorine levels decrease [24].

Different studies show that chlorine is more effective against *Legionella* at higher temperatures [28], [34], with no turbidity effect, although chlorine decays faster at the higher temperature [19]. Chlorine efficacy also decreases rapidly at high pH, or in the presence of organic and nitrogen contaminants. The peak efficacy of chlorine against pathogens is observed around pH 6 [28], [35].

In order to improve disinfection efficacy, multiple disinfection approaches may be used. Synergy between chlorine and ultraviolet light or copper and silver ions has been observed, so chlorination may be combined with other disinfection modalities at a much lower concentrations of chlorine [19].

The main advantage of chlorination is that it provides a residual disinfectant concentration throughout the water distribution system so that colonization of *Legionella* at distal sites could be minimized [19].

The main disadvantages associated with chlorination are short-term effectiveness, corrosiveness, chlorine by-products and chlorine toxicity. Short-term effectiveness can be explained by the presence of *Legionella* in amoeba, which may be resistant to chlorine or that chlorine does not penetrate biofilm well [19], [20], [28], [34]. The corrosiveness of chlorine should be considered with respect to the pipes and materials used in the water system. This problem could be minimized by chemical coating of all hot water pipes with sodium silicate precipitate, but it will increase the initial and maintenance costs of water distribution system [19], [24]. Disinfection by-products, formed following the reaction of chlorine with precursors, such as natural organic matter (mainly humic and fulvic acids), and extracellular products from microorganisms, such as algal cells, include trihalomethanes, haloacetic acids and haloacetonitriles. Some disinfection by-products are suspected mutagens/carcinogens or teratogens. There is also the possibility of an association of water chlorination with increased risk of cardiovascular diseases [1], [17], [19].

E. Chlorine Dioxide

Chlorine dioxide is a green–yellow gas, which has to be manufactured from sodium chlorite and a strong acid in situ because it decomposes readily and presents toxicity hazards when stored [8]. It inactivates bacterial pathogens by disrupting the outer membrane of bacteria or by interfering with protein synthesis [1].

Chlorine dioxide use in water treatment is becoming more common because it forms much less trihalomethanes and haloacetic acids than free chlorine and does not react with ammonia to form chloramines [1], [28], [36].

Chlorine dioxide is more effective at higher temperature and higher pH. However, the pH effect on inactivation is less pronounced than observed with chlorine [8], [28], [35].

There are opposing views on the efficacy of chlorine dioxide treatment, which may reflect the interaction of other important factors. Several studies have shown significant decrease of *Legionella* colonization after chlorine dioxide disinfection [24], [35], [37] while other have reported no significant effect and recommend for additional treatment [1], [24]. Similarly, influence of chlorine dioxide on biofilm is unclear. Although in most cases better inactivation of biofilm bacteria is reported than in case of chlorination, complete eradication of *Legionella* from water system has not been observed [1], [6], [24], [32], [34].

Chlorine dioxide is reduced in water to form two inorganic disinfection by-products – chlorite and chlorate. Chlorite is of greater health concern than chlorate. Both chlorite and chlorate may combine with haemoglobin to cause methemoglobinemia. However, direct ingestion or inhalation of chlorine dioxide or its by-products can result in irritation of the digestive tract or chronic respiratory deficiencies, and skin and nasal irritations [1], [28].

The main advantages of chlorine dioxide are relatively low costs and less harmful disinfection by-products [1], [32], [37]. Uncertainties regarding effectiveness of chlorine dioxide against *Legionella* are a significant disadvantage [32].

F. Chloramines

Popularity of chloramines, especially monochloramine, have increased because of concerns of harmful chlorination by-products [8]. Chloramines produce the same disinfection by-products as chlorine but in lower amounts [36]. Chloramines are not as reactive as chlorine with iron and corrosion products, they are more stable and their residual concentration is kept for longer periods.

Although some studies have shown that the use of monochloramine could be the most effective chlorine-based disinfection for *Legionella* control, in case if a residual is correctly maintained [32]; most of reports indicate that chloramines are less effective than free chlorine [8], [32]. For that reason, some reports suggest combined use of chlorine and monochloramine in order to obtain higher disinfection and reduced disinfection by-products [1], [8], [36]. It should be noted that combined residual chlorine requires much longer contact time than free residual chlorine to achieve the same degree of elimination of pathogens. Also high temperatures accelerate loss of disinfectants and pipe-biofilm nitrification accelerates monochloramine decay [6], [17], [32], [36].

The main advantages of chloramines are less disinfection by-products and better inactivation of bacteria in the biofilms [1], [8], [23], [34], [38], which could be a considerable advantage if long distribution system needs to be disinfected. However, long-term assessment is still needed to establish efficacy of disinfection with chloramines.

Disadvantages include the risk of anemia for kidney hemodialysis patients, increased populations of other microorganisms (*Mycobacterium* species), presence of nitrogen by-products and increased lead leaching in drinking water [1], [32].

G. Ozone

Ozone inactivates microorganisms via production of hydroxyl and superoxide-free radicals. It affects the permeability, enzymatic activity, and DNA of bacterial cells [1], [8], [35]. Ozone reacts quickly, and therefore inactivation can occur by both gaseous ozone through direct physical contact and dissolved ozone [8].

Effectiveness of ozone is not significantly affected by pH or temperature and it does not interact with ammonia, while the presence of suspended solids can reduce efficacy of ozone [1], [8].

Although ozone is much more powerful oxidant than chlorine, it is not efficient enough for controlling *Legionella* in water systems when used alone [1], [22]. Because ozone does not stay in water sufficiently long to provide a residual effect against potential contamination in the distribution system, it can be used as the primary disinfectant followed by chlorination in order to provide chlorine residual [1], [8], [22].

The main advantage of ozone is that it generates relatively few disinfection by-products, and leaves no taste and odour [36]. Reaction of ozone with bromide ions produces bromate, which is a mutagen and potential carcinogen. Other by-products may include aldehydes, bromoform, and brominated acetic acids, which are not classified as genotoxic carcinogens [1], [28], [39]. Apart from being more expensive than chlorination, the major drawback is the lack of residual disinfection action in water distribution system [1], [8], [22], [36].

III. Research and Development of Alternative Disinfection Methods

A. Electrochemical Disinfection

Electrochemical disinfection is a relatively new concept in disinfection technologies and it is considered one of the most promising alternatives to chlorine providing both primary and residual disinfection [5], [15], [17]. This method does not involve direct addition of chemicals into the water. A low-voltage current is directed across the electrodes causing the formation of oxidizing agents like free chlorine, chlorine dioxide, hydrogen peroxide, ozone and other short-lived oxidants. Through transmission of the reactive energy of the oxidants, the disinfection capacity can be upheld for a while in the water, resulting in some residual activity [5], [10].

The inactivation efficacy of electrochemical disinfection systems is largely dependent on cell configuration, electrode material, electrolyte composition, microorganisms present, mass transfer conditions, and other parameters such as flow rate and current density. The presence of chloride in the electrolyte will increase the cells inactivation efficacy generating highly germicidal active chlorine species [15], [17].

Electrochemical systems, which generate excess amounts of chlorine species, will have the same major disadvantages for drinking water disinfection as chlorine. Question whether electrochemical systems could replace chlorine is still open. Research concerning the generation of disinfection by-products in electrochemically treated water has reported more than 50 % reduction in total trihalomethanes, which could be the major advantage of electrochemical disinfection [10], [17]. Other advantages include onsite generation and avoidance of transportation and storage of hazardous chemicals used in chlorine based disinfection methods, which makes electrochemical disinfection environmentally friendly [10], [15], [17]. The high capital costs of electrochemical systems and high cell voltages associated with relatively low conductivity of water undergoing treatment can also be barriers for implementation [15].

Electrochemical processes are reported to be effective for disinfection, however, biofilm bacteria are found to be extremely resistant to disinfection [15], [19]. Therefore, complete eradication of *Legionella* may not be achieved, what is in line with the results of other disinfection methods [5]. It should be noted that experiments of electrochemical disinfection are performed mostly at lab-scale and further research on its influence on *Legionella* in water, amoebae and biofilms is still necessary.

B. Photocatalysis

Semiconductor photocatalytic processes recently have shown a great potential as a low cost, environmentally friendly and sustainable water treatment technology for bacteria inactivation. Due its low toxicity, chemical and thermal stability, low energy band-gap and low cost, TiO_2 is accepted to be one of the most suitable semiconductors for photocatalysis [16], [18], [33]. Photocatalysis requires activation by ultraviolet light, fluorescent light or visible light [1]. In most lab-scale experiments, ultraviolet light has been used to induce photocatalytic processes [2], [16], [33].

Inactivation of microorganisms by TiO_2 is mainly due to reactive oxygen species such as hydroxyl radicals, superoxide anions and hydrogen peroxide, produced by TiO_2 irradiation. Hydroxyl radicals can induce strand breaks in DNA and can cause damage, which results in cell death [1], [16], [33], [35].

In lab-scale experiments, photocatalysis is approved to be effective for bacteria inactivation; however, regrowth of bacteria may take place if no final disinfection process (e.g., chlorine or chlorine dioxide) is used and optimum environmental conditions for bacteria regrowth occur in the distribution network [16]. Application of photocatalysis for disinfection of drinking water still needs further investigations, especially visible light induced photocatalysis, which would be less costly than the one using UV light.

The major barrier for wide application of photocatalytic disinfection is slow kinetics due to limited light fluence and photocatalytic activity. Excessive levels of turbidity, presence of organic and inorganic constituents (e.g., humic acids, sulphate and nitrate) can reduce photocatalytic disinfection efficiency [1], [18], [33].

C. Fenton Processes

Fenton's reaction is an advanced oxidation process, based in physicochemical processes that generate and use transient chemical species with a very high oxidant potential, such as hydroxyl radical. Fenton's reagent is formed with iron and hydrogen peroxide, reaction does not involve any light irradiation. In the absence of a light source, hydrogen peroxide will decompose by Fe^{2+} ions that present in the aqueous phase, resulting in the formation of hydroxyl radicals [33], [40], [41]. Process of generation of hydroxyl radicals by Fenton's reaction is very sensitive to pH, the ratio of Fe^{2+} to H_2O_2 and the concentration of Fe^{2+} [42], [43]. Fenton's reaction is effective for acidic conditions (optimum pH of 3 – 4), and its efficiency may rapidly decrease at pH above 5 [20], [43], [44]. Phosphates can also affect performance by lowering reaction rate and decrease of the H_2O_2 consumption efficiency [43].

Unlike other disinfection methods, the Fenton's process is effective for biofilm bacteria inactivation, especially in cases when biofilms are grown on corroded surfaces, which means a high quantity of iron is available. In such conditions, rapid inactivation is possible even at pH 5 [20]. Another advantage over chlorine-based disinfectants is the prevention of bacteria regrowth potential without formation of deleterious disinfection by-products. Chemicals involved in the Fenton's process are easily handled, are not toxic or harmful to the environment, and are economically attractive [20], [41], [42], [43]. Another advantage of the Fenton's process is that no energy input is necessary to activate hydrogen peroxide, making the reaction possible at atmospheric pressure and at room temperature [43]. Disadvantages include the need for acidic conditions and formation of large amounts of iron sludge.

D. Photo – Fenton Processes

The photo-Fenton reaction is expedited when light source is present, causing rapid H_2O_2 decomposition by ferrous or ferric ions and resulting in the formation of radicals. All these soluble iron-hydroxyl or iron complexes can absorb not only UV radiation but also visible light. When a light source is present, the rate of photo-Fenton is positively enhanced compared to the dark condition [33], [43]. This is mainly due to the regeneration of Fe^{2+} from the photochemical effect of light and the concurrent generation of the hydroxyl radicals in the system [33].

IV. Summary and Conclusions

Reviewed conventional disinfection methods have been used in water industry for years, however, serious drawbacks like production of dangerous disinfection by-products and inadequate disinfection efficacy necessitate research and development of alternative methods. The main challenge is to promote ability to inactivate biofilm bacteria, which are more resistant to disinfectants than free living bacteria. Advanced oxidation processes (e.g., photocatalysis, Fenton and photo-Fenton like processes) have shown promising results, although it has to be noted that at the moment most experiments are performed at lab-scale and long-term experiments are still needed to prove the observations. Several experiments are carried out testing the effect of disinfectant on coliform bacteria, which are more sensitive to treatment, therefore additional experiments are needed to test disinfection efficacy for *Legionella*. In addition, combined use of different disinfection techniques may be investigated, as well as other novel technologies (e.g., nanotechnologies or plasma technologies).

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