

Impact of Pipe Materials and Chlorination on Planktonic and Biofilm Cells of *Listeria monocytogenes*

Bahaa Hemdan^{1,*}, Mohamed Sedik², Mohamed Kamel¹ and Gamila E. El-Taweel¹

¹Water Pollution Research Department, National Research Centre, Dokki, Giza, Egypt;

²Agricultural Microbiology Department, Faculty of Agriculture, Cairo University, Cairo, Egypt

Abstract: This work aimed to determine the influence of different chlorine doses to inactivate of *Listeria monocytogenes* biofilm cells collected from six types of pipe materials, which are commonly used in household at different biofilm ages. A laboratory-scale simulated distribution system, that consists of a design based on six different pipe materials which are: polyvinyl chloride (PVC), polypropylene (PP), polyethylene (PE), iron (I), copper (Cu) and rubber (R). Six samples of *L. monocytogenes* biofilm were collected from the previous designed system at three different biofilm ages (10, 40 and 90 days-old). The collected biofilm samples were exposed to different chlorine doses (0.2, 0.6, 1.0, 1.4, 1.8, 2.2, 2.6 and 3.0 mg/l) at 10 min, and then total bacterial counts using pour plate method was enumerated. The results showed that the log reduction of 90 days-old of *L. monocytogenes* biofilm formation, when exposed to 3.0 mg/l of chlorine dose, on six pipe materials; PVC, PP, PE, I, Cu and R was 3.96, 4.16, 4.21, 4.17, 4.32 and 4.03 CFU/cm², respectively with the initial count of biofilm cells was 10⁶ CFU/cm². While the log reductions of *L. monocytogenes* planktonic cells were: 6.20 CFU/ml (complete reduction) at the same dose of chlorine dose. The present study concluded that the biofilm cells are more resistant to chlorine dose than planktonic cells. Moreover, the biofilm of 10 days-old is more sensitive than 40 days-old followed by 90 days-old that grows in different pipe materials. In addition, chlorine effectiveness depends on its concentration, the exposure time, nature of pipe materials and the age of biofilm.

Keywords: Biofilm, pipe materials, drinking water, *Listeria monocytogenes*, chlorine.

INTRODUCTION

Listeria monocytogenes is a Gram-positive, facultative anaerobic, non-spore-forming and rod-shaped bacterium with an optimal growth temperature in a range of 30-37°C. *L. monocytogenes* is considered as a food-borne pathogen causing a listeriosis disease to human beings and animals by ingestion [1, 2]. It is widely distributed in different environments and it can be found in water, soil, animal fecal matter and sewage [3]. Also, it has been reported as being capable of attaching and developing biofilm on various surfaces, for example, stainless steel, polymers, rubber gaskets, plastic surfaces, polypropylene, and glass [4, 5].

Biofilm is a microbial consortia embedded in a self production of exopolymer matrix composed mainly of exopolysaccharides (EPS). It is composed of microbial cells, enzymes, proteins, EPS and nucleic acids. The microbes living in these matrix benefit from nutrient and water supplies improved by lateral gene transference [6, 7] and protection against adverse environmental conditions such as a desiccation and chemicals, including detergents, disinfectants and antibiotics [8, 9]. Additionally, biofilm can function as a reservoir for pathogens and also as a source for disease outbreaks [10]. Additionally, it is a functional, three-dimensional

consortia of microbial cells enveloped within extracellular polymers [11]. The microscopic examination of biofilm is made by structure using three dimensional (3-D) data such as Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) [12, 13].

The extracellular polymeric substances (EPS), which are excreted by microbes, are a complex gelatinous matrix of extracellular polymers containing exopolysaccharides, proteins, nucleic acids, lipids and humic substances. Most microorganisms in water distribution systems exist in biofilm on the inner surfaces of the pipelines [14]. The formation of biofilm is influenced by several factors such as concentration and quality of disinfectants, nutrients, water flow velocity, hydraulic conditions, temperature and pipe materials [15, 16]. Pipe materials can influence biofilm formation, especially in its early stages [17, 18]. In addition, Flemming *et al.* [19] estimated that only 5% of bacterial counts are present in the drinking water distribution network which can be detected in bulk water, which is commonly used for its quality control and the high percentage of bacterial densities (95%) adhere to the inner surface of pipe walls.

Many problems in drinking water pipes are due to the biofilm growth [20], and the persistence of pathogens [21]. Moreover, the biofilm growth can cause corrosion of the pipe, deterioration of water quality and organoleptic problems [22, 23]. The pipe material used in water distribution systems is another important factor that influences the proliferation of the distribution system of the biofilm. It has been

*Address correspondence to this author at the Water Pollution Research Department, National Research Centre, Dokki, Giza, Egypt; Tel/Fax: +202/33377164; E-mail: bahaa_nrc@yahoo.com

found that some pipes frequently experience some problems [24].

The most commonly used element in water disinfectant is chlorine, which is considered as a highly effective, highly soluble, more stable, easy to use, low cost and potent oxidizer. Moreover, it has been a residue to avoid regrowth of the microorganisms [25, 26].

L. monocytogenes biofilm was more resistant to antimicrobial agents and disinfectants, such as chlorine, ozone, hydrogen peroxide, and quaternary ammonium compounds [27-29]. Therefore, the main purpose of this research was to evaluate the effectiveness of different doses of chlorine to inactivate *L. monocytogenes* biofilm cells of six types of pipe materials, which are commonly used in the household at different biofilm ages.

MATERIALS AND METHODS

Experimental Model Water Distribution System

The present study was performed by using a laboratory-scale simulated distribution system. The designed system consisted of six different sets, of identical drinking water distribution pipes which are used up till now. The pipe materials were: polyvinyl chloride (PVC), Polypropylene (PP), polyethylene (PE), Iron (I), copper (Cu) and rubber (R). The length of distribution pipe was one meter with an internal diameter of 3 cm. Tap water was pumped from the contact tank (30 liters of tap water) with a flow rate of 1.0 l/min into the simulated drinking water distribution pipe using a peristaltic pump. The culture of *Listeria monocytogenes* strain ATCC 25152 was used as inoculum, whereas the concentration of viable cells was (10^5 CFU/ml) of pumped water in the contact tank.

Preparation of Bacterial Suspensions

Strain of *L. monocytogenes* ATCC 25152 (planktonic cells) was cultured in a Brain-Heart Infusion Broth (OXOID, UK) and incubated at 37°C during 24h. After the incubation, the suspensions were homogenized in the vortex. The initial counts of the inocula (6 log CFU /ml), which were used for biofilm formation, were determined by using the standard plate count method.

Biofilm Sampling

The biofilm samples that have been formed on the inner surface of the pipe material were collected after 10, 40 and 90 days. The biofilm samples were scraped with sterile cotton swabs, which were transferred to tubes containing 10 ml sterile water, and vortex agitator for 2 min. The detached biomass (biofilm suspensions) was diluted with a sterile saline solution for bacterial enumeration. The results were reported as the number of CFU/cm² [30].

Quantification of Biofilm Bacterial Cells

The enumeration of total cells for biofilm samples was performed by the pour plate technique and by using Plate Count Agar medium (OXOID, UK). The replicate plates for biofilm samples, taken from six different pipe materials and three different ages were analyzed for each dilution within

the appropriate count between 30 and 300 CFU, which were selected for enumeration [31].

Electron Microscopy (EM) Examination

Six biofilm samples of *L. monocytogenes* were developed on six different pipe materials after 90 days. They were examined by transmission electron microscopy (TEM) model JEM 2100- HRTEM. As for the investigation of the total samples by the negative contrast method, biofilm cells suspensions were applied to a Formvar coated grid stabilized by carbon. The samples were stained with 1% water solution of the ammonium molybdate (Sigma, United States) for 30 Sec [32].

Determination of Exopolysaccharides (EPS) at 90 Days Old

The extraction of EPS from biofilm samples was carried out by using cation exchange resin method according to Michalowski *et al.* [33]. The polysaccharide content of crude EPS was determined by using phenol-sulfuric acid method and by the following the protocol described by Dubois *et al.* [34].

Effect of Different Chlorine Concentration on Planktonic Cells and Different Ages of *L. monocytogenes* Biofilm

Six biofilm samples, which were removed from different pipe materials (PVC, PP, PE, I, Cu and R) in different ages (10, 40 and 90 days old), were exposed to eight different chlorine doses (0.2, 0.6, 1.0, 1.4, 1.8, 2.2, 2.6 and 3.0 mg/l) for 10 min. The biofilm cells and planktonic cells were sub-cultured into 100 ml Tryptic Soya broth (OXOID, UK) and incubated at 37°C for 24 h and then centrifuged at 3000 rpm for 20 min. The pellets were washed three times using sterilized distilled water and then the pellets were suspended in 10 ml sterile distilled water.

Determination of Residual Chlorine

Nine flasks (250 ml conical flask with quick fit stopper) containing 100 mL sterilized distilled water were inoculated with 0.5 ml of bacterial biofilm suspension, which was formed on six pipe materials at different ages and then it was inoculated by different chlorine doses and uninoculated chlorine water flask was used as a control. The determination of residual chlorine was carried out by DPD method according to [31].

Statistical Analysis

The relationship between chlorine doses, residual chlorine and log counts of *L. monocytogenes* biofilm cells was carried out by using regression coefficient analysis (R²). All the data was transformed in decimal logarithms and it was processed by SPSS version 14.0, by the computer application.

RESULTS

The results of this study explained the characteristics of biofilm formation by *L. monocytogenes*. In addition, the relationship between the effect of chlorine doses and its residual on log reduction of *L. monocytogenes* on different pipe materials during different ages were also explained.

Characteristics of Biofilm

The kinetics of the biofilm formation of *L. monocytogenes* and the biofilm on different pipe materials were explained by using the transmission electron photomicrograph (Fig. 1). The photomicrograph indicated that the cells were embedded in a polymer matrix and exopolysaccharides. Also, it was produced from the biofilm cells in different shapes. From Fig. (1), it was found that the pipe materials were affected by the quantity and shape of the exopolysaccharides produced from biofilm cells.

When comparing the amount of exopolysaccharides that was produced by the cells of biofilm formation on different types of pipes in both TEM and determination of EPS results, it is clear that, *L. monocytogenes* biofilm grown on copper pipe ($203.7 \mu\text{g}/\text{cm}^2$) produced lower amount of exopolysaccharides than the others types of pipes Fig. (1-E). Also, the results of exopolysaccharides amount of PVC, PP, PE, iron, Cu, Rubber at 90 day-old was 384.2, 390.6, 375.4, 411.5, 203.7 and 289.3 $\mu\text{g}/\text{cm}^2$, respectively.

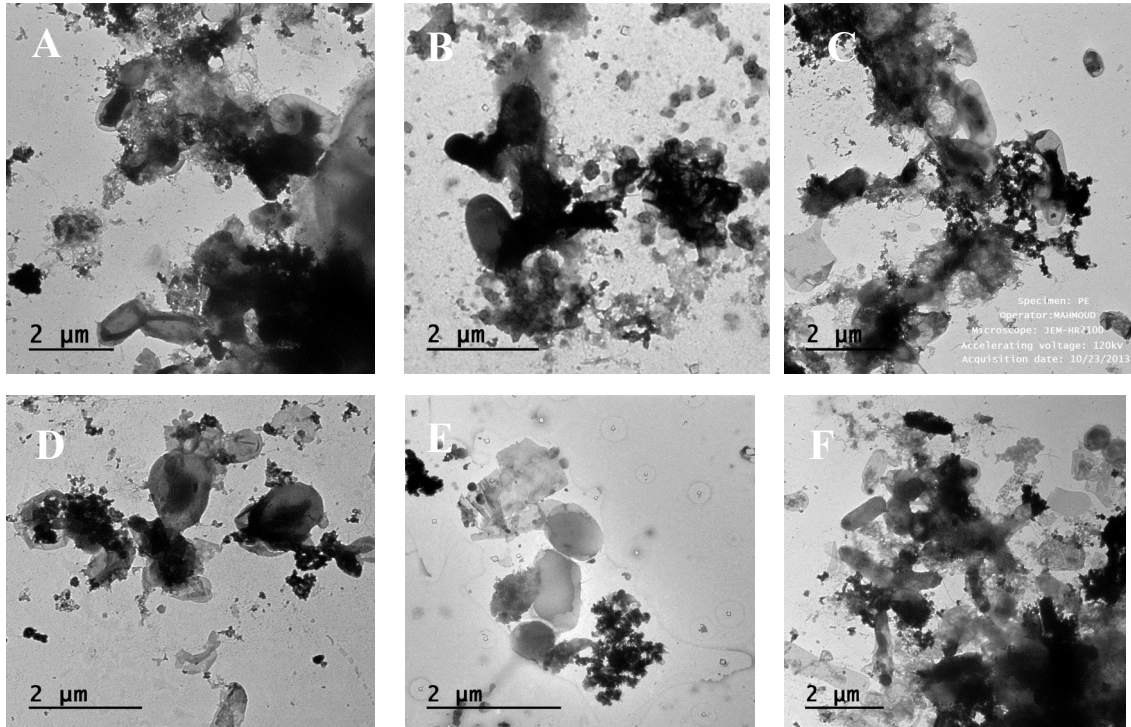


Fig. (1). Transmission electron photomicrograph of biofilm formation of *L. monocytogenes* on different pipe materials (A) PVC, (B) PP, (C) PE, (D) I, (E) Cu and (F) R.

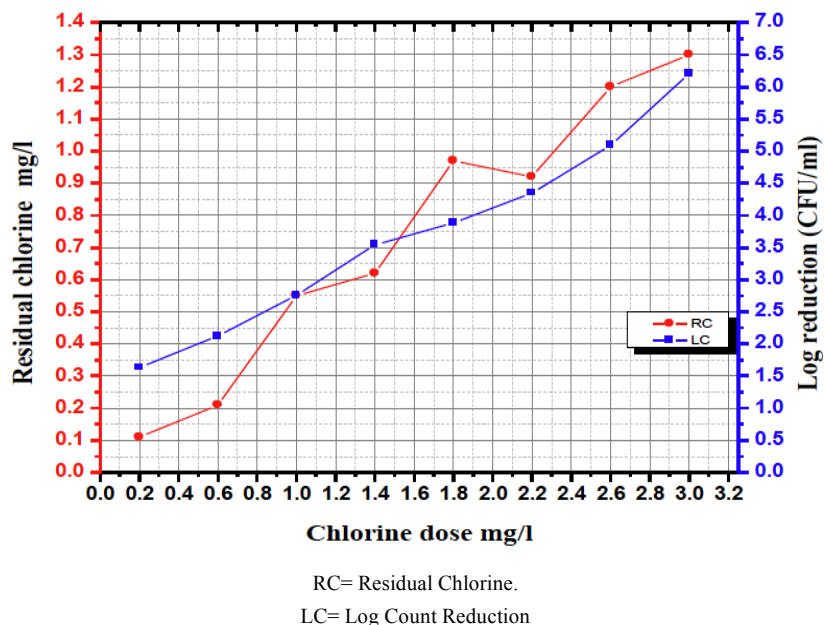
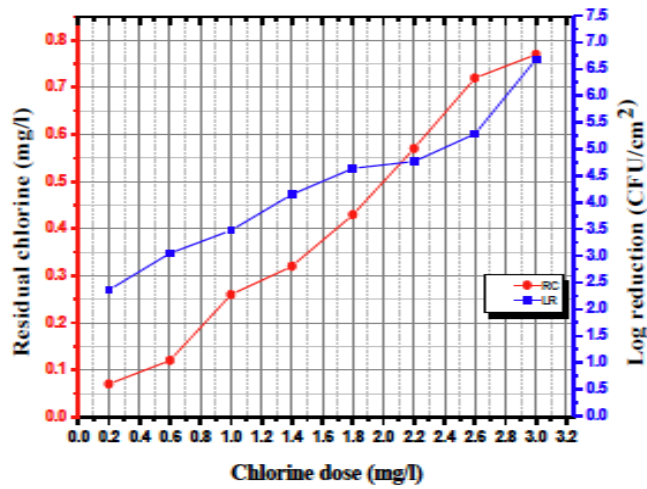
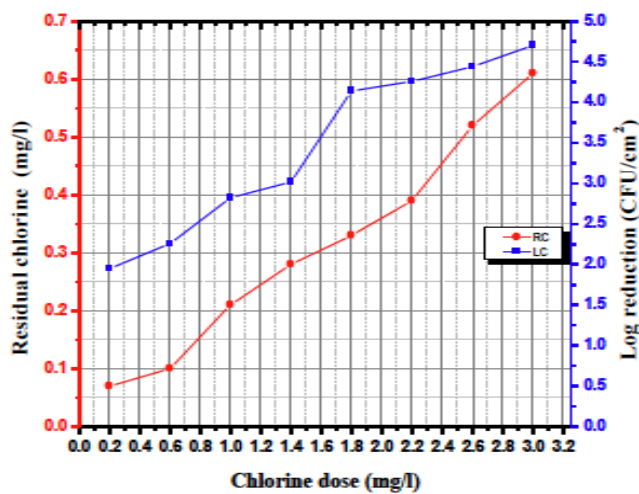


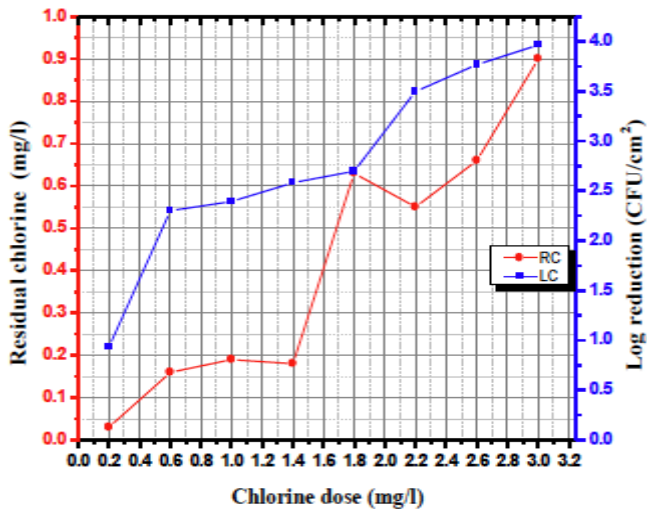
Fig. (2). The relationship between the chlorine doses, residual chlorine and log reduction of *L. monocytogenes* planktonic cells.



(A)



(B)



(C)

RC= Residual Chlorine
LC= Log Count Reduction

Fig. (3). The relationship between the chlorine doses, residual chlorine and log reduction of *L. monocytogenes* biofilm cells on PVC pipes (A) 10 days-old, (B) 40 days-old and (C) 90 days-old.

Effect of Different Chlorine Doses and its Residual on *L. monocytogenes* Planktonic Cells

In this study *L. monocytogenes* ATCC 25152 was used as a reference strain (planktonic cells). The initial count of planktonic cells of *L. monocytogenes* (1.6×10^6 CFU/ml) was exposed to eight chlorine doses (0.2, 0.6, 1.0, 1.4, 1.8, 2.2, 2.6 and 3.0 mg/l). The obtained results showed that the most effective chlorine dose was 3.0 mg/l, which led to complete inactivation (100% removal) (Fig. 2).

Biofilm Cells Formed on Different Pipe Materials in Different Ages

The six samples of *L. monocytogenes* biofilm cells were scraped from six different pipeline materials (PVC, PP, PE, I, Cu and R) in three different ages (10, 40, and 90 days-old) and were exposed to the same eight chlorine doses (Figs. 3-8).

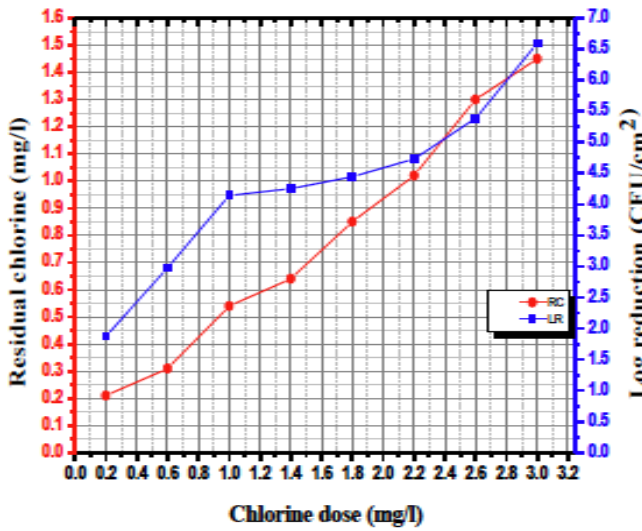
As for the *L. monocytogenes* biofilm cells were collected from PVC pipeline in different ages (10, 40, and 90 days-old), it is clear that, the initial log counts of 10 days-old biofilm cells were 6.6 CFU/cm². In this age, 3.0 mg/l of chlorine dose was able to complete the removal of cells (Fig. 3A), while the initial logs count of 40 and 90 days-old of biofilm cells were 6.6 and 6.3 CFU/cm², respectively. The log reduction was reached to 4.7 and 3.9 CFU/cm², respectively at the obtained ages (Figs. 3B, C).

In the case of PP pipeline, the initial count of biofilm formation of *L. monocytogenes* at 10 days-old was 6.5 CFU/cm² (Fig. 4A). Results demonstrated that, 3 mg/l of chlorine dose was able to do a complete removal of the initial log counts. While 40 and 90 days-old biofilm cells (the initial count 6.6 and 6.5 CFU/cm²) the log reduction was 4.71 and 4.16 CFU/cm², respectively (Fig. 4B, C).

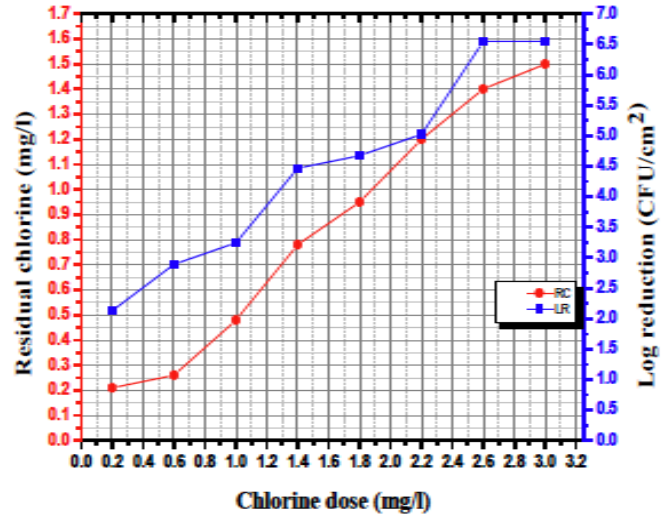
By regarding the biofilm cells of *L. monocytogenes* growing on the PE pipe, the initial log counts of different ages were, respectively, of : 6.5, 6.3 and 6.7 CFU/cm² in 10, 40 and 90 days-old,. The results indicated that, 3 mg/l of chlorine dose was able to reduce the biofilm cells (4.21 and 5.50 log) for 90 and 40 days-old (Fig. 5B, C) Whereas, the biofilm cells of 10 days-old *L. monocytogenes* were completely removed (6.5 log CFU/cm²) (Fig. 5A).

As shown in Fig. (6), it can be explained that the biofilm formation of *L. monocytogenes* on the iron pipes revealed more resistant to different chlorine doses in all ages. On the other hand, the high level from chlorine dose (3 mg/l) was not able to cause a complete inhibition of biofilm cells, not only for 90 days-old biofilm (older biofilm), but also for 10 days-old biofilm (younger biofilm). In addition, the initial log count of the obtained ages was, respectively, 6.5, 6.7 and 6.8 CFU/cm² at 10, 40 and 90 days-old.

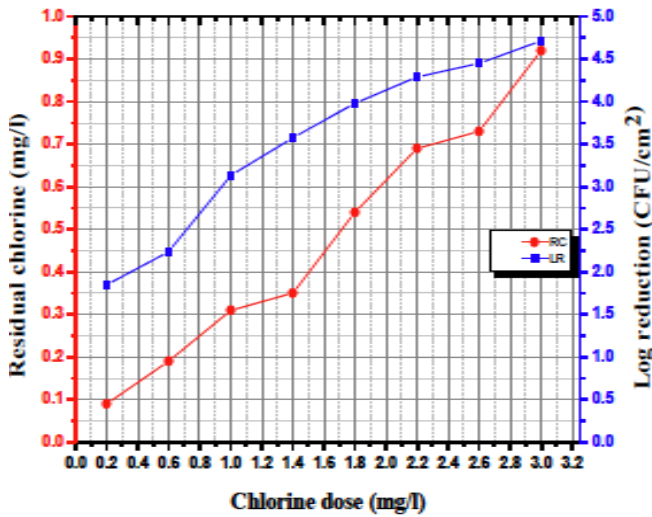
In case of biofilm formation of *L. monocytogenes* on Cu pipe materials, the results revealed that 10 days-old biofilm cells were completely removed (6.3 log reduction) at 2.2 mg/l of chlorine doses, While 40 and 90 days-old of biofilm cells were completely inactivated at 3 mg/l. Whereas, the initial log count was 6.3, 6.5 and 6.1 CFU/cm² in different ages, respectively (Fig. 7).



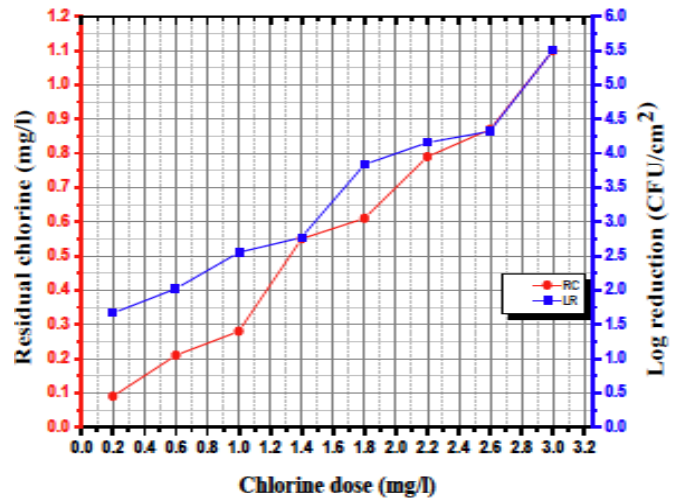
(A)



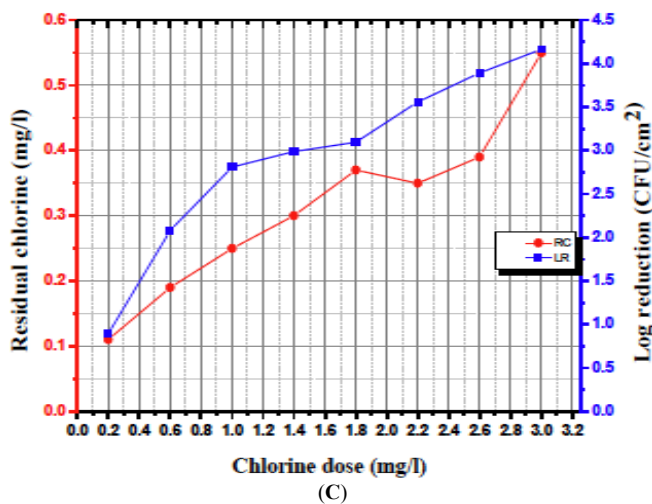
(A)



(B)

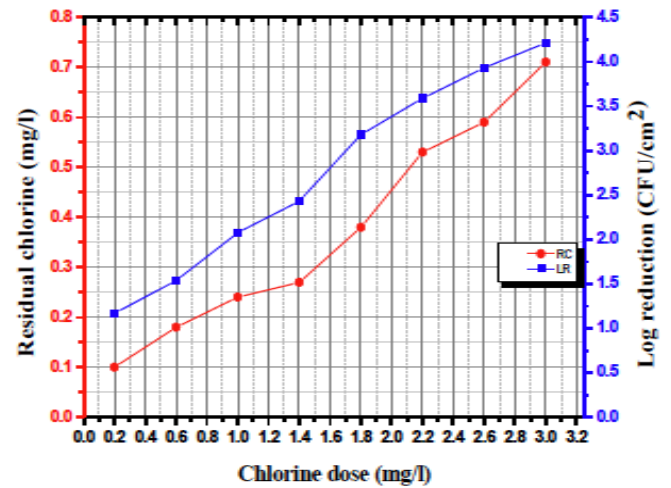


(B)



(C)

RC= Residual Chlorine
LC= Log Count Reduction

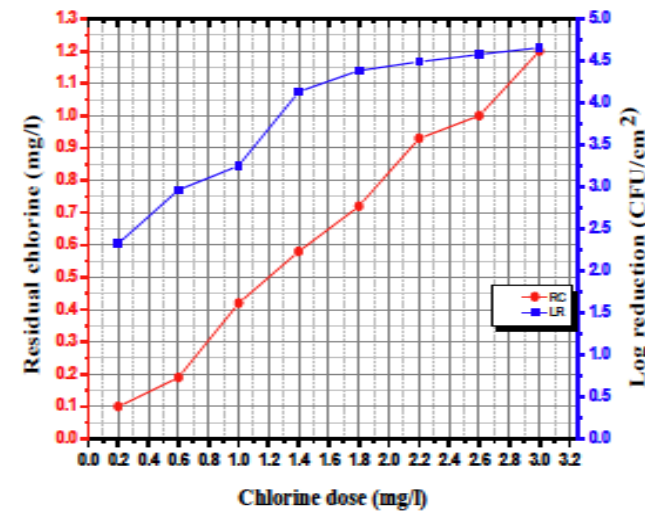


(C)

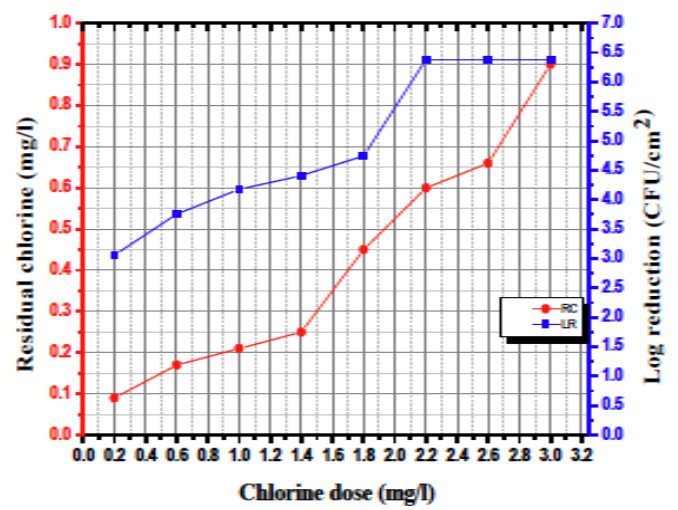
RC= Residual Chlorine
LC= Log Count Reduction

Fig. (4). The relationship between the chlorine doses, residual chlorine and log reduction of *L. monocytogenes* biofilm cells on PP pipes (A) 10 days-old, (B) 40 days-old and (C) 90 days-old.

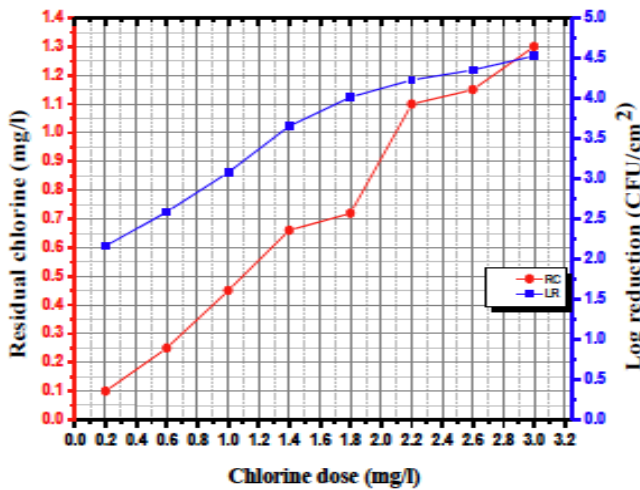
Fig. (5). The relationship between the chlorine doses, residual chlorine and the log reduction of *L. monocytogenes* biofilm cells on PE pipes (A) 10 days-old, (B) 40 days-old and (C) 90 days-old.



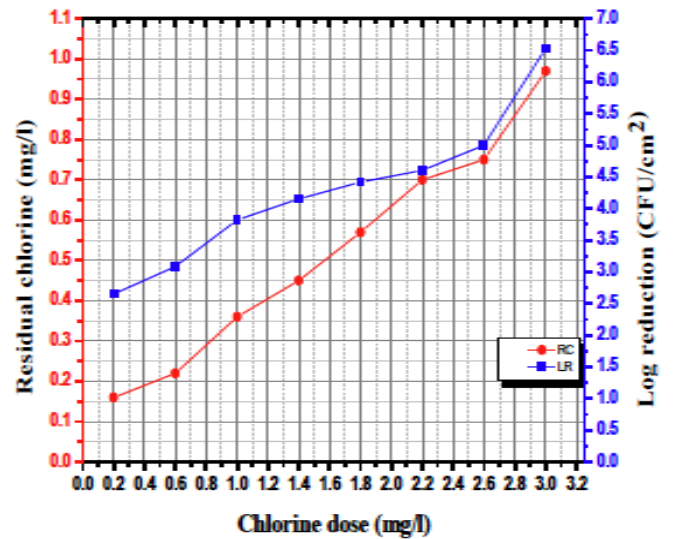
(A)



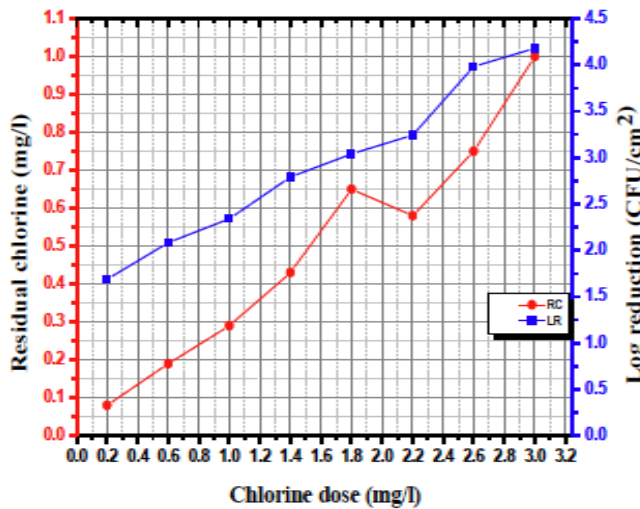
(A)



(B)

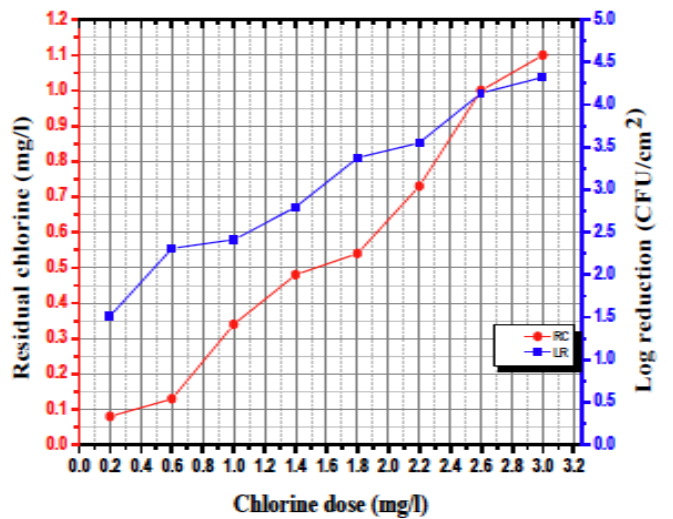


(B)



(C)

RC= Residual Chlorine
LC= Log Count Reduction

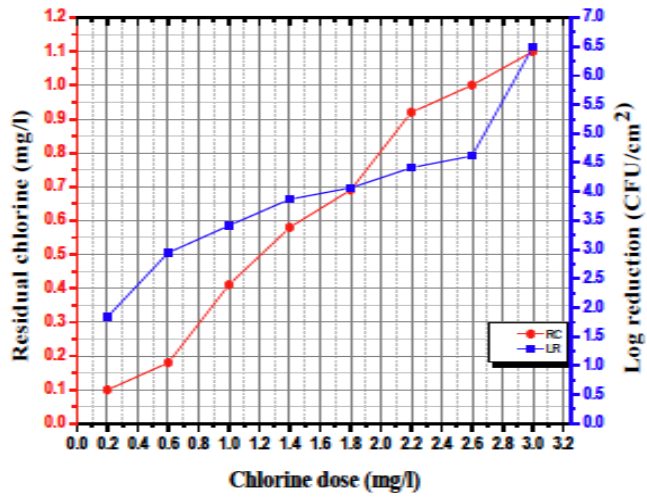


(C)

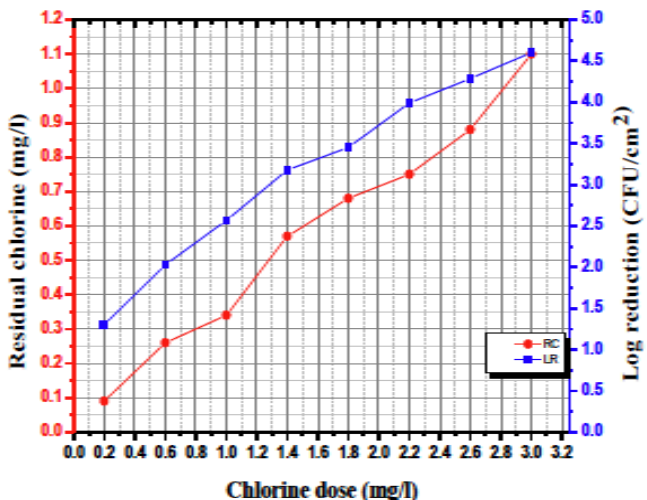
RC= Residual Chlorine
LC= Log Count Reduction

Fig. (6). The relationship between the chlorine doses, residual chlorine and the log reduction of *L. monocytogenes* biofilm cells on I pipes (A) 10 days-old, (B) 40 days-old and (C) 90 days-old.

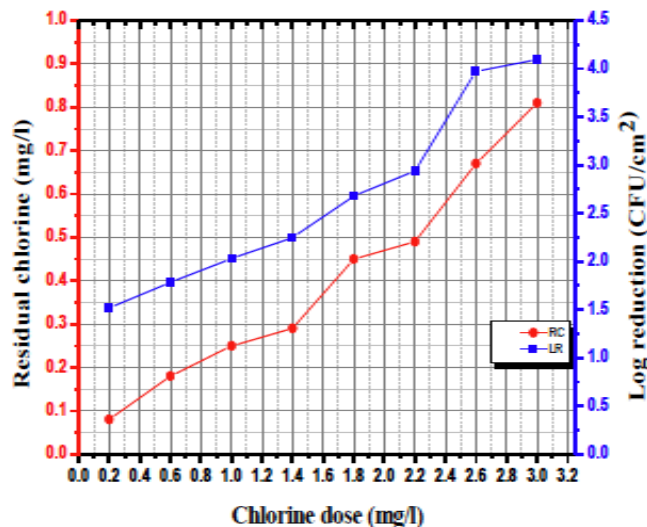
Fig. (7). The relationship between the chlorine doses, residual chlorine and log reduction of *L. monocytogenes* biofilm cells on Cu pipes (A) 10 days-old, (B) 40 days-old and (C) 90 days-old.



(A)



(B)



(C)

RC= Residual Chlorine
 LC= Log Count Reduction

Fig. (8). The relationship between the chlorine doses, residual chlorine and the log reduction of *L. monocytogenes* biofilm cells on R surface (A) 10 days-old, (B) 40 days-old and (C) 90 days-old.

Additionally, when the biofilm cells of *L. monocytogenes* were grown on the inner surface of R materials, the initial log count of three different ages was 6.4, 6.6 and 6.6 CFU/cm², respectively. The results of biofilm cells, which scraped after 10 days, were completely reduced, when exposed to 3 mg/l of chlorine dose. On the other hand, the log reduction after 90 and 40 days-old of biofilm cells were, 4.0, and 4.6 CFU/cm² (Fig. 8).

Finally, from the obtained results (Figs. 2-8), it is clear that the most effective chlorine dose was 3.0 mg/l. Also, the residual free chlorine dose ranged between 0.55 - 1.5 mg/l.

Statistical Analysis

The statistical analysis using regression coefficient (R²) expresses the relationship between residual chlorine and log reduction count of biofilm cells in different ages (Table 1).

DISCUSSION

L. monocytogenes is widely spread throughout the land and water environments. It is often isolated from samples of different sources such as soil, feces, water, decaying plant material, vegetables, and silage [35]. A high proportion of bacteria *L. monocytogenes* was observed in the treated household and industrial sewage. The contaminated sewage plays an essential role in the transmission of *Listeria* in the water environment. It leads to their presence in rivers, lakes, sea and groundwater. The transmissions of *L. monocytogenes* through water can cause a dangerous disease, called listeriosis, for human beings and animals [36]. The literature data determined the frequency of *L. monocytogenes* in water samples reaches up to 62% [37]. It is also notable that these bacteria, owing to a high resistance to unfavorable external conditions, are able to survive in the environment for a long time [38]. *L. monocytogenes* was able to produce biofilm on the surfaces and it was rapidly adaptable to changing environmental conditions [39]. Due to this fact, the presence of *L. monocytogenes* bacilli in water ecosystems may be a cause of the sporadic or epidemic listeriosis incidence, which poses a serious hazard for the health of people and animal [40].

In this study by using TEM, it is clear that the biofilm cells, which grow in different pipe materials, are embedded in a polymer matrix and in exopolysaccharides. Moreover, exopolysaccharides were produced from the biofilm cells in different shapes. In addition, *L. monocytogenes* biofilm grows on copper pipe produced lower amounts of exopolysaccharides than the others. Therefore, EPS plays a vital role in the build-up of biofilm [41, 42]. In comparison to many other biofilm studies, they showed cells surrounded in a heavy, slimy polymeric matrix layers [43, 44].

There are many factors that affect the formation of biofilm such as the type, the doses of disinfectant, the nature and concentration of organic and inorganic compounds in the water, type of pipe composition and water temperature. Momba *et al.* [45] demonstrated that, a variety of disinfection processes such as ozonation, chlorination, monochloramination, hydrogen peroxide, and UV irradiation are used to prevent the formation of biofilm on the surface of pipeline composition in water distribution networks. The efficiency

Table 1. Regression coefficient analysis between residual chlorine and log reduction count of biofilm cells that formed on different pipe materials at different ages.

Tested Pipe materials	R ² Biofilm ages (day-old)		
	10	40	90
PVC	84.7	84.6	73.3
PP	88.1	84.2	91.7
PE	92.7	92.6	94.9
I	74.5	79.0	85.9
Cu	75.8	74.4	84.1
R	84.1	93.2	92.3

of these biocides in the prevention of biofilm production varies because of their different abilities to penetrate the biofilm. Also, they reported that the pipe material plays a very crucial role in the maintenance of a high water quality because it can supply the bacteria in the water with nutrients through the leaching of organic compounds.

The usage of chlorine as a disinfectant was to prevent and control the biofilm formation. It was indicated that, the residual chlorine, after the disinfection of *L. monocytogenes* biofilm by 3.0 mg/l chlorine dose, ranged between: 0.55-1.5 mg/l with a contact time of 10 min although the EPA demonstrated that the maximum residual chlorine level was 4.0 mg/l [46]. On the other hand, the effectiveness of disinfectant residual depends on the concentration, the contact time and the presence of microorganisms [47].

There are various investigations showing that, the adequate disinfectant residuals can control the biofilm accumulation [48]. Momba [49] found a large increase of biofilm microorganisms on test coupons in the absence of a disinfectant residual. The WHO mentioned that the risks from disinfection by-products to health are very small, in comparison with insufficient disinfection. Therefore, the development of safe and effective alternative disinfection methods is highly desirable. The chemical disinfection, especially chlorine, is considered as the most common approach to prevent the development of biofilm in the water distribution system. The microbes, which have passed through the treatment stage, can be destroyed or irreversibly inactivate by chlorination, so as to ensure microbiologically potable water [25].

The results of this study showed that the most effective chlorine dose was 3.0 mg/l, which led to a complete inactivation of planktonic cells and biofilm cells, which were collected from different pipe materials in 10 days-old, except in the case of used iron pipeline. Also, the biofilm formations of *L. monocytogenes* on iron pipes in all ages were more resistant to different chlorine doses. This may be due to the iron pipes corrosion that influences the efficiency of chlorine for the eradication of bacterial biofilm [50, 51]. Additionally, LeChevallier *et al.* [52] found that the corrosion of metal based pipes decreases the efficiency of residual chlorine in the plumbing system. In spite of an adequate corrosion control and biological treatment of water to reduce assimilable organic carbon levels and consistently maintained chlorine

residuals; the development of biofilm on the inner surface of iron pipe rapidly occurred and more diverse microorganisms occurred than on plastic polyvinyl chloride (PVC) pipes [50, 53].

In this study, in the case of biofilm formation of *L. monocytogenes* on copper pipes, all biofilm ages (10, 40 and 90 days-old) were completely inactivated by using 3.0 mg/l of chlorine dose. This may be due to the release of copper residuals that have antibacterial properties expressed by the damage of cell membranes and nucleic acid structure [54]. So, the copper plumbing material is widely used. This may be due to the easy fittings with different pipe varieties [55, 56]. Metal-based materials can form the corrosion products on pipe surfaces and release metals into water as a result of chemical or biological reactions [57, 58]. Moreover, Lehtola *et al.* [59] found that, the biofilm formation in copper pipes was slower than in polyethylene (PE) pipes, and that copper ions led to the decrease of the count of microorganisms in water while plastic pipes such as PE, which have been used recently, are more economic pipes than traditional metal plumbing materials. But, the most disadvantage of PE is the release of biodegradable organic compounds and phosphorus, which can enhance microbial regrowth and biofilm accumulation.

When comparing the ages of different pipe materials, the results showed that, after 40 days-old, it can be more resistant to the dose of chlorine. It could be revealed that, the presence of EPS, which coated biofilm cells, protected the cells from any disinfectant or antimicrobial agents. The EPS matrix is prevented by the penetration of antimicrobial agents from reaching the microorganisms within the biofilm by the diffusion limitation and/or the chemical interaction with the extracellular proteins and polysaccharides [6, 60, 61] whereas the biofilm EPS plays a vital role to protect the biofilm cells towards antimicrobial agents [62]. Biofilm cells are more resistant than planktonic populations as they include chlorine and antibiotics [63, 64]. Moreover, chlorine is proved to be less effective on older *L. monocytogenes* biofilm [65].

CONCLUSION

It could be concluded that, *L. monocytogenes* biofilm was more resistant to chlorine when compared to planktonic

cells. Older biofilm is more difficult to remove than younger biofilm and planktonic cells. Chlorine is less effective on older *L. monocytogenes* biofilm. As for the residual chlorine, 1.0 mg/l was found in older *L. monocytogenes* biofilm occurrence on all tested plumping materials, except the copper pipe, that was found 1.1 mg/l.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

The authors would like to thank and appreciate *Dr. Gamila H. Ali*, professor of Hydrobiology for her technical support in this study.

REFERENCES

- [1] Chen BY, Pyla R, Kim TJ, *et al.* Prevalence and contamination patterns of *Listeria monocytogenes* in catfish processing environment and fresh fillets. *Food Microbiol* 2010; 27: 645-52.
- [2] Pagadala S, Parveen S, Rippen T, *et al.* Prevalence, characterization and sources of *Listeria monocytogenes* in blue crab (*Callinectes sapidus*) meat and blue crab processing plants. *J Food Microbiol* 2012; 31: 263-70.
- [3] Vaid R, Linton RH, Morgan MT. Comparison of inactivation of *Listeria monocytogenes* within a biofilm matrix using chlorine dioxide gas, aqueous chlorine dioxide and sodium hypochlorite treatments. *J Food Microbiol* 2010; 27: 979-84.
- [4] Jeong DK, Frank J F. Growth of *Listeria monocytogenes* at 21°C in biofilms with microorganisms isolated from meat and dairy environments. *Lebensmittel-Wissenschaft und-Technologie* 1994; 27: 415-24.
- [5] Rieu A, Briandet R, Habimana O, Garmyn D, Guzzo J, Piveteau P. *Listeria monocytogenes* EGD-e biofilms: no mushrooms, but a network of knitted chains. *Appl Environ Microbiol* 2008; 74: 4491-7.
- [6] Maha TF, O'Toole GA. Mechanisms of biofilm resistance to antimicrobial agents. *Trends Microbiol* 2001; 9: 34-9.
- [7] Goller CC, Romeo T. Environmental influences on biofilm development. *Curr Top Microbiol Immunol* 2008; 322: 37-66.
- [8] Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms. *Lancet* 2001; 358: 135-8.
- [9] Costerton JW. Bacterial attachment to surfaces. In: Eckey DC, editor. *The biofilm primer*. Berlin: Springer 2007; pp. 36-43.
- [10] Bakaletz LO. Bacterial biofilms in otitis media: evidence and relevance. *Pediatr Infect Dis J* 2007; 26: 17-9.
- [11] McMath SM, Sumpter C, Holt DM, Delanoue A, Chamberlain AH. The fate of environmental coliforms in a model water distribution system. *Lett Appl Microbiol* 1999; 28: 93-7.
- [12] Kämper M, Vetterkind R, Hoppert M. Methods for *in situ* detection and characterization of extracellular polymers in biofilms by electron microscopy. *J Microbiol Methods* 2004; 57: 55-64.
- [13] Barbara V, Chen M, Russell JC, Ivanova EP. Bacterial extracellular polysaccharides involved in biofilm formation. *Mole* 2009; 14: 2535-54.
- [14] Bachmann RT, Edyvean RGJ. Biofouling: an historic and contemporary review of its causes, consequences and control in drinking water distribution systems. *Biofilms* 2006; 2: 1-31.
- [15] Zacheus OM, Lehtola MJ, Korhonen LK, Martikainen PJ. Soft deposits, the key site for microbial growth in drinking water distribution networks. *Water Res* 2001; 35:1757-65.
- [16] Lehtola MJ, Laxandera M, Ilkka T, *et al.* The effects of changing water flow velocity on the formation of biofilms and water quality in pilot distribution system consisting of copper or polyethylene pipes. *Water Res* 2006; 40: 2151-60.
- [17] Waines LP, Moateb R, Moodya AJ, Allenc M, Bradleya G. The effect of material choice on biofilm formation in a model warm water distribution system. *Biofouling* 2011; 27:1161-74.
- [18] Chowdhury S. Heterotrophic bacteria in drinking water distribution system: a review. *Environ Monit Assess* 2012; 184: 6087-137.
- [19] Flemming HC, Percival SL, Walker JT. Contamination potential of biofilms in water distribution systems. *Water Sci Technol* 2002; 2: 271-80.
- [20] Camper AK. Involvement of humic substances in regrowth. *Int J Food Microbiol* 2004; 92: 355-64.
- [21] Emtiazi F, Schwartz T, Marten SM, Krolla-Sidenstein P, Obst U. Investigation of natural biofilms formed during the production of drinking water from surface water embankment filtration. *Water Res* 2004; 38:1197-206.
- [22] Ludmány Z, Borsányi M, Vargha M. Evaluation of biofilms occurring in drinking water distribution systems of Balatonfüred, p. 501–507. In: Simeonov L, Chirila E, (ed.). *Chemicals as international and accidental global environmental threats*. Lavoisier, Cachan Cedex, France 2006.
- [23] Storey MV, Ashbolt NJ. Persistence of two model enteric viruses (B40-8 and MS-2 bacteriophages) in water distribution pipe biofilms. *Water Sci Technol* 2001; 43:133-8.
- [24] Lehtola MJ, Miettinen IT, Lampola T, *et al.* Pipeline materials modify the effectiveness of disinfectants in drinking water distribution systems. *Water Res* 2005; 39:1962-71.
- [25] WHO. *Guideline for drinking-water quality*. (4th ed.). Geneva, Switzerland 2011.
- [26] Simões LC, Simões M, Vieira MJ. influence of the diversity of bacterial isolates from drinking water on resistance of biofilms to disinfection. *Appl Environ Microbiol* 2010; 76: 6673-9.
- [27] Stopforth JD, Samelis J, Sofos JN, Kendall PA, Smith GC. Biofilm formation by acid-adapted nonadoted *Listeria monocytogenes* in fresh its beef decontamination washings and its subsequent inactivation with sanitizers. *J Food Prot* 2002; 65: 1717-27.
- [28] Somers EB, Wong AC. Efficacy of two cleaning and sanitizing combinations on *Listeria monocytogenes* biofilms formed at low temperature on a variety of materials in the presence of ready to eat meat residue. *J Food Prot* 2004; 67: 2218-29.
- [29] Robbins JB, Fisher CW, Moltz AG, Martin SE. Elimination of *Listeria monocytogenes* biofilms by ozone, chlorine, and hydrogen peroxide. *J Food Prot* 2005; 68: 494-8.
- [30] Zhou L, Zhang Y, Li G. Effect of pipe material and low level disinfectants on biofilm development in a simulated drinking water distribution system. *J Zhejiang Univ Sci A* 2009; 10: 725-31.
- [31] APHA (American Public Health Association). *Standard methods for the examination of water and wastewater*, 22nd ed. Washington, D.C. 2011; p. 187.
- [32] Didenko LV, Konstantinova ND, Romanova Yu-M, *et al.* Ultrastructural organization of *Salmonella typhimurium* cells at prolonged tarvation and transition to a noncutable state. *Mol Genet Mikrobiol Virusol* 2000; 3: 21-6.
- [33] Michalowski W, Flemming H, Wingender J. Isolation and analysis of extracellular polymeric substances from drinking water biofilms. Paper presented at the 5th ASM Conference on Biofilms, Cancun, Mexico 2009.
- [34] DuBois M, Gilles K, Hamilton J, Rebers P, Smith F. Colorimetric method for determination of sugars and related substances. *Anal Chem* 1956; 28: 350-6.
- [35] Hansen CH, Vogel BF, Gram L. Prevalence and survival of *Listeria monocytogenes* in Danish aquatic and fish-processing environments. *J Food Prot* 2006; 69: 2113-22
- [36] Czeszejko K, Bogusl A, Wska-Was E, *et al.* Prevalence of *Listeria monocytogenes* in municipal and industrial sewage. *EJPAU* 2003; 6: 12-16.
- [37] Rodas-Suárez OR, Flores-Pedroche JF, Betancourt-Rule JM Quiñones-Ramírez I, Vázquez-Salinas C. Occurrence and antibiotic sensitivity of *Listeria monocytogenes* strains isolated from oysters, fish, and estuarine water. *Appl Environ Microbiol* 2006; 72:7410-12.
- [38] Doumith M1, Cazalet C, Simoes N, *et al.* New aspects regarding evolution and virulence of *Listeria monocytogenes* revealed by comparative genomics and DNA arrays. *Infect Immun* 2004; 72: 1072-83.
- [39] Gandhi M, Chikindas ML. *Listeria*: A foodborne pathogen that knows how to survive. *Int J Food Microbiol* 2007;113:1-15.
- [40] Jeffers GT, Bruce JL, McDonough PL, *et al.* Comparative genetic characterization of *Listeria monocytogenes* isolates from human and animal listeriosis cases. *Microbiology* 2001; 147: 1095-104.

- [41] O'Toole G, Kaplan HB, Kolter R. Review. Biofilm formation as microbial development. *Annu Rev Microbiol* 2000; 54: 49-79.
- [42] Watnick P, Kolter R. Mini-review. Biofilm, city of microbes. *J Bacteriol* 2000; 182: 2675-9.
- [43] Auerbach ID, Sorensen C, Hansma HG, Holden PA. Physical morphology and surface properties of unsaturated *Pseudomonas putida* biofilms. *J Bacteriol* 2000; 182: 3809-15.
- [44] Flemming HC. Mini-review. Biofouling in water systems—cases, causes and countermeasures. *Appl Microbiol Biotechnol* 2002; 59: 629-40.
- [45] Momba MNB, Cloete TE, Venter SN, Kfir R. Influence of disinfection processes on the microbial quality of potable groundwater in a laboratory-scale system model. *J Water SRT-Aqua* 2000; 49: 23-33.
- [46] US EPA (U.S. Environmental Protection Agency). Drinking water contaminant candidate list. 2010. Available from: [http://www.epa.gov/safewater/ccl/ccl3.html/]
- [47] Besner MC, Gauthier V, Servais P, Camper A. Explaining the occurrence of coliforms in the distribution system. *J AWWA* 2002; 94: 95-109.
- [48] Zhang W, DiGiano FA. Comparison of bacterial regrowth in distribution systems using free chlorine and chloramine: a statistical study of causative factors. *Water Res* 2002; 36: 1469-82.
- [49] Momba MNB. The impact of disinfection processes on biofilm formation in potable water distribution systems. Ph.D. Thesis Univ Pretoria South Africa 1997.
- [50] LeChevallier MW, Lowry CD, Lee RG, Gibbon DL. Examining the relationship between iron corrosion and the disinfection of biofilm bacteria. *J AWWA* 1993; 85: 111-23.
- [51] Ainsworth R, WHO (World Health Organization). Safe piped water: managing microbial water quality in piped distribution systems. IWA Pub London 2004; p. 147.
- [52] LeChevallier MW. Coliform regrowth in drinking water: a review. *J AWWA* 1990; 32: 74-86.
- [53] Camper AK. Factors influencing microbial growth in the distribution system: laboratory and pilot experiments. *J AWWA*. Research Foundation, Denver, CO 1996.
- [54] Bruins MR, Kapil S, Oehme FW. Microbial resistance to metals in the environment. *Ecotoxicol Environ Safety* 2000; 45: 198-207.
- [55] Zhang Y, Griffin A, Edwards M. Nitrification in premise plumbing: role of phosphate, pH and pipe corrosion. *Environ Sci Technol* 2008; 42: 4280-4.
- [56] Moritz MM, Flemming H-C, Wingender J. Integration of *Pseudomonas aeruginosa* and *Legionella pneumophila* in drinking water biofilms grown on domestic plumbing materials. *Int J Hyg Environ Heal* 2010; 213:190-7.
- [57] Edwards M, Jacobs S, Taylor R. The blue water phenomenon. *J AWWA* 2000; 92: 72-82.
- [58] Nguyen CK, Powers KA, Raetz MA, Parks JL, Edwards MA. Rapid free chlorine decay in the presence of Cu(OH) (2): Chemistry and practical implications. *Water Res* 2011; 45: 5302-12.
- [59] Lehtola MJ, Miettinen IT, Keinänen MM, et al. Microbiology, chemistry and biofilm development in a pilot drinking water distribution system with copper and plastic pipes. *Water Res* 2004; 38: 3769-79.
- [60] Pearson JP, Delden CV, Iglewski BH. Active efflux and diffusion are involved in transport of *Pseudomonas aeruginosa* cell-to-cell signals. *J Bacteriol* 1999; 181; 1203-10.
- [61] Sutherland IW. The biofilm matrix an immobilized but dynamic microbial environment. *Trends Microbiol* 2001; 9: 222-7.
- [62] Suzuki I. Microbial leaching of metals from sulfide minerals. *Bio-technol Adv* 2001; 19: 119-132.
- [63] Luppens SBI, Reij MW, van der Heijden RWL, et al. Development of a standard test to assess the resistance of *Staphylococcus aureus* biofilm cells to disinfectants. *Appl Environ Microbiol* 2002; 68: 4194-200.
- [64] Schwartz T, Hoffmann S, Obst U. Formation of natural biofilms during chlorine dioxide and U.V. disinfection in a public drinking water distribution system. *J Appl Microbiol* 2003; 95: 591-601.
- [65] Lee SH, Frank J. Inactivation of surface adherent *Listeria monocytogenes* hypochlorite and heat. *J Food Prot* 1991; 54: 46-62.

Received: October 16, 2014

Revised: January 06, 2015

Accepted: January 06, 2015

© Hemdan et al.; Licensee Bentham Open.

This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted, non-commercial use, distribution and reproduction in any medium, provided the work is properly cited.