

Effect of Corroded and non Corroded Pipe Materials on Biofilm Formation in Water Distribution Systems

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Abstract: The effect of pipe materials on biofilm formation in drinking water distribution system was studied using simulated drinking water distribution system. Biofilm was grown on different pipe materials under laminar flow rate (Reynolds number: 2000). The parameters analyzed were the numbers of cultivable bacteria using chromogenic media. The impact of different pipe materials were assessed after the biofilms reached steady-state. The total numbers of *Salmonella* Typhimurium biofilm was mostly higher than *Listeria monocytogenes* biofilm cells. The steady-state of *L. monocytogenes* and *S. Typhimurium* biofilm grown on PVC, PP and PE pipe materials was achieved in 70 days. While, steady-state biofilm formed in R material was at 60 days of *L. monocytogenes* and 80 days of *S. Typhimurium*. In addition to, steady-state biofilm formed in I pipe material was achieved in 60 days of *L. monocytogenes* with total numbers of biofilm cells (10^8 CFU/cm²) and 70 days of *S. Typhimurium* with total numbers of biofilm cells (10^9 CFU/cm²). In case of biofilm formed in Cu, *L. monocytogenes* and *S. Typhimurium* biofilm have no steady-state. These results concluded that, the plastic-based materials are the most appropriate to be used for carrying the drinking water. While, the iron pipes are not recommended for carrying water in DWDS.

Key words: Biofilm • Pathogenic bacteria • Pipe materials • Flow rate • Steady-state • EPS

INTRODUCTION

Listeria monocytogenes and *Salmonella* spp. are considered as important pathogenic bacteria, which transmitted via aquatic environments and food matter [1]. *L. monocytogenes* is one of the most important causative agents of the serious disease such as listeriosis to animals and humans [2]. *Salmonella* spp. can cause several diseases such as typhoid, para-typhoid, salmonellosis and gastroenteritis for warm blooded animals [3]. In addition to that, these bacteria have ability to adhere and form biofilm on different material such as metal, glass, polyethylene and rubber surfaces [4, 5].

Biofilm is a complicated mixture of microorganisms surrounded by gelatinous layer of extracellular polymeric substances (EPS), which are produced by them. Biofilm occurs and accumulates usually in wet surfaces of drinking water distribution system (DWDS) is unavoidable [6]. Also, it is found on all exposed surfaces

materials to water during different steps in drinking water production and distribution [7].

Most disease outbreaks in the several part of the world can cause by the consumption of contaminated drinking water [8]. Many outbreaks caused by water transmitted pathogens is not limited in many developing countries even developed countries are affected. These outbreaks with public health risks occurred due to failure drinking water treatment plants and distribution system, which failed to maintain an adequate level of disinfectant to prevent the growth of pathogens and/or harbored the pathogens. Waterborne outbreaks have been due to *Escherichia coli* O157:H7, *Campylobacter* spp., *Helicobacter pylori*, *Salmonella* Typhimurium, *L. monocytogenes*, *Cryptosporidium parvum*, *Giardia* and some viruses (norovirus, calcivirus and enterovirus) in Canada, France, Italy, England, Finland, Switzerland, Northern Ireland, Norway, Belarus, New Zealand, Poland and United States [9].

DWDS are complicated engineering systems consisting of pipes, storage vessels, fittings and valves. DWDS are made of a variety of different materials such as cast iron, polyvinyl chloride (PVC) and polyethylene (PE) that interact with the bulk water [10]. Therefore, the present study aimed to compare of biofilm formation and growth rate for two bacterial pathogens (*L. monocytogenes* and *S. typhimurium*) in different pipe materials. Also, to study effect of pipe materials and laminar flow rate on biofilm formation.

MATERIALS AND METHODS

Experimental Design and Biofilm Monitoring: Two sets of *in vitro* laboratory-scale simulated drinking water distribution system (DWDS) model were designed. As shown in Figure (1), the designed system consisted of six different identical pipes which can be used in the drinking water distribution till now in closed system. The most common of pipe materials used in experimental design were corroded metal based materials such as iron (I) and copper (Cu) and non corroded plastic based material such as polyvinyl chloride (PVC), polypropylene (PP), polyethylene (PE) and rubber (R). The length and internal diameters of each pipes was one meter and 3cm. The pipe divided to 9 coupons and the length of every one was 10 cm. In two models, the design of sampling ports allows coupons to be added and removed without emptying or even stopping the system (Hemdan *et al.*, 2015). The biofilm was formed under laminar flow rate (Reynolds number: 2000) to simulate the conditions found in real DWDS.

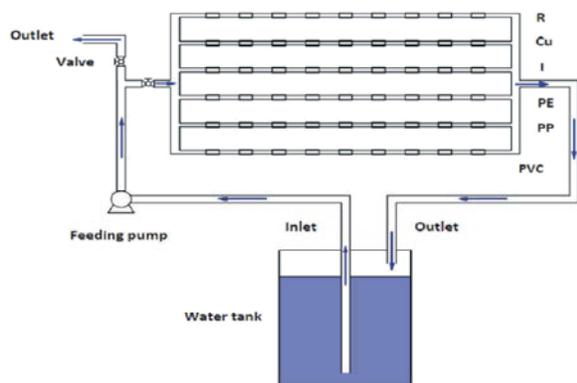


Fig. 1: Layout of *in vitro* simulated DWDS model [11] (Hemdan *et al.*, 2015)

The Reynolds number was calculated as a function of multiple ducts design, using the hydraulic equivalent diameter (D_h), defined as [12]:

$$D_h = 4 \times \text{Flow area/wetted perimeter}$$

For DWDS model with single duct; (1)

$$\text{Flow area} = \pi/4 \times d^2$$

where d , is the semi circular duct diameter

$$\text{Wetted perimeter} = \pi \times d/2 + d$$

The Reynolds no. depends on hydraulic diameter is:

$$Re = (D_h \times u \times \rho) / \pi$$

where;

- u : Flow velocity (m/s)
- ρ : Fluid density (kg/m^3)
- π : Fluid viscosity (kg/m.s)

For multiple ducts with n ducts; (2)

$$D_h = 4 \times \text{Flow area/wetted perimeter}$$

for n multiple ducts,

$$\text{Flow area} = \pi/4n \times d^2$$

$$\text{Wetted perimeter} = \pi \times d/2 + d$$

The biofilm experiment was carried on for 90 days after the biofilm reached a steady-state on each pipe material.

Biofilm Sampling: Six biofilm samples which formed in tested pipe materials were analyzed every 10 days to 90 days. Samples were collected and scraped from the inner surface of coupons (10 cm^2) of tested pipe materials using sterile cotton swabs [13] (Marques *et al.*, 2007).

Counting of *L. monocytogenes* and *S. typhimurium*

Biofilm Cells: According to APHA [14] (2012), the biofilm samples were diluted appropriately from tenfold serial dilutions depending on the cell concentration. Culture-based enumeration method was used to enumerate biofilm cells. By using spread plate method, Hicrome *Listeria* selective agar (HLSA) was used to count *L. monocytogenes* biofilm cells. While Hicrome Improved *Salomonella* agar (HISA) was used to enumerate *S. Typhimurium*. All selective used media were purchased from Oxoid-UK. Biofilm accumulation in all experimental design was expressed in CFU/cm^2 .

Measurements of Exopolysaccharide of Biofilm EPS:

EPS of *L. monocytogenes* and *S. Typhimurium* biofilm samples were analyzed every 10 days to 90 days. EPS was extracted from six biofilm samples using cation exchange resin method according to Denkhaus *et al.* [15] (2007); Michalowski *et al.* (2009). According to the protocol described by Dubois *et al.* [16] (1956), the polysaccharide contents of crude EPS was determined using phenol-sulfuric acid method.

Statistical Analysis: Bivariate person analyses were performed to estimate correlation between biofilm ages, counts of biofilm cells and polysaccharides amounts. Statistical calculations were based on a confidence level equal or higher than 95% (a *P* value ≤ 0.05 was considered statistically significant).

RESULTS AND DISCUSSION

DWDS are composed of a variety of materials and may harbor potential pathogens within surface-attached microbial biofilm [17]. Biofilm formation is a significant problem in many types of watery treatment systems and it is transported with the flow and this eventually deteriorates the water quality [18]. In DWDS, biofilm accumulated by the microorganisms causes several troubles in final product of water quality such as obnoxious taste and odor, increased turbidity, reduced water stress and flow, encourage biocorrosion and release pathogenic bacteria, which is a major public health concern [19]. Due to the complexity water transported systems, the biofilm construction study in DWDS is unachievable. So, the use of designed system is considered as one of the approach to recognize the dynamics of biofilm formation and study effect of pipe material and its ages on biofilm growth rate. Whereas, the pipe material used in DWDS is one of the most crucial issue which can effect on biofilm pattern [20]. In this study, two sets of designed DWDS model were used to monitor and observe biofilm formation of *L. monocytogenes* and *S. Typhimurium* on tested pipe materials, under one distinct hydrodynamic condition (laminar flow rate). The designed DWDS model operates in horizontal position in semi continuous recycling and closed mode.

Regarding biofilm formation on plastic-based materials, results graphically illustrated in Figure (2, 3, 4) showed that, steady-state of *L. monocytogenes* and *S. Typhimurium* biofilm on PVC, PP and PE pipe material was achieved at 70 days-old. While, the biofilm cells counts at this age was 10^7 CFU/cm² for *L. monocytogenes* and 10^8 CFU/cm² for *S. Typhimurium*. Additionally, the highest amounts of exopolysaccharide of *L. monocytogenes* biofilm grown on PVC, PP and PE were 384.5, 390.6 and 375.4 $\mu\text{g}/\text{cm}^2$, respectively. While, *S. Typhimurium* biofilm were 206.5, 213.6 and 219.0 $\mu\text{g}/\text{cm}^2$, respectively.

Data represented in Figure (5) showed that, the accumulation of biofilm on R material, results revealed that steady-state of *L. monocytogenes* was reached to 10^7 CFU/cm² at 60 days-old. While, in *S. Typhimurium* biofilm

was reached to 10^8 CFU/cm² at 80 days. The maximum amounts of exopolysaccharide of *L. monocytogenes* and *S. Typhimurium* biofilm were 289.3 and 198.5 $\mu\text{g}/\text{cm}^2$. From the obtained results, it can be noticed that growth rate of *S. Typhimurium* biofilm was more than *L. monocytogenes* biofilm. Although, exopolysaccharide amounts of *L. monocytogenes* biofilm was greater than *S. Typhimurium* biofilm. These results verified the results of Stepanovic' *et al.* [21] they recorded that, the quantities of biofilm produced by *Salmonella* spp. were greater than those produced by tested *L. monocytogenes*. Also, many researchers reported that, the relationship between EPS production and biofilm growth rate depend on the type of bacteria involved in biofilm accumulation (Turakhia and Characklis, 1988; Hemdan, 2015) [21-5].

These findings are in agreement with those Hemdan [5] (2015) who found that in spite of the counts of *S. Typhimurium* biofilm cells was greater than *L. monocytogenes* biofilm; the production of EPS in *L. monocytogenes* biofilm was higher than *S. Typhimurium* biofilm. Also, Evans *et al.* [22] reported that the microorganisms produced small quantity of EPS when they are quickly growing and consuming nutrients. This is as a result of EPS production was increasing when the biofilm cell density started to decrease. Consequently, the high amount of EPS production led to decrease biofilm cell densities. This is due to the death of those cells that have spent more energy on EPS synthesis than they can increase at the low oxygen tensions in the depth of the biofilm [23- 24].

Metal-based material such as iron and steel pipes have been used in DWDS for several centuries throughout the world and are subjected to corrosion, causing deterioration of potable water quality due to unwanted chemical and biochemical reactions [25]. By concerning of corroded metal-based materials, the steady-state of *L. monocytogenes* and *S. Typhimurium* biofilm which formed in I pipe material was achieved at 60 and 70 days-old. While, the biofilm cells counts at this age was 10^8 CFU/cm² for *L. monocytogenes* and 10^9 CFU/cm² for *S. Typhimurium*. Moreover, the greatest exopolysaccharide quantities of *L. monocytogenes* and *S. Typhimurium* biofilm were 411.5 and 274.9 $\mu\text{g}/\text{cm}^2$ (Figure 6). While, in case of biofilm developed on Cu pipe material, the results of this study demonstrated that, *L. monocytogenes* and *S. Typhimurium* biofilm grown on Cu material had no steady-state in biofilm formation. Whereas, the highest growth rate of *L. monocytogenes* was reached to 10^6 CFU/cm² at 70 days then the growth rate decreased to 10^4 CFU/cm² at 90 days. Also, the maximum counts of *S. Typhimurium* biofilm was reached

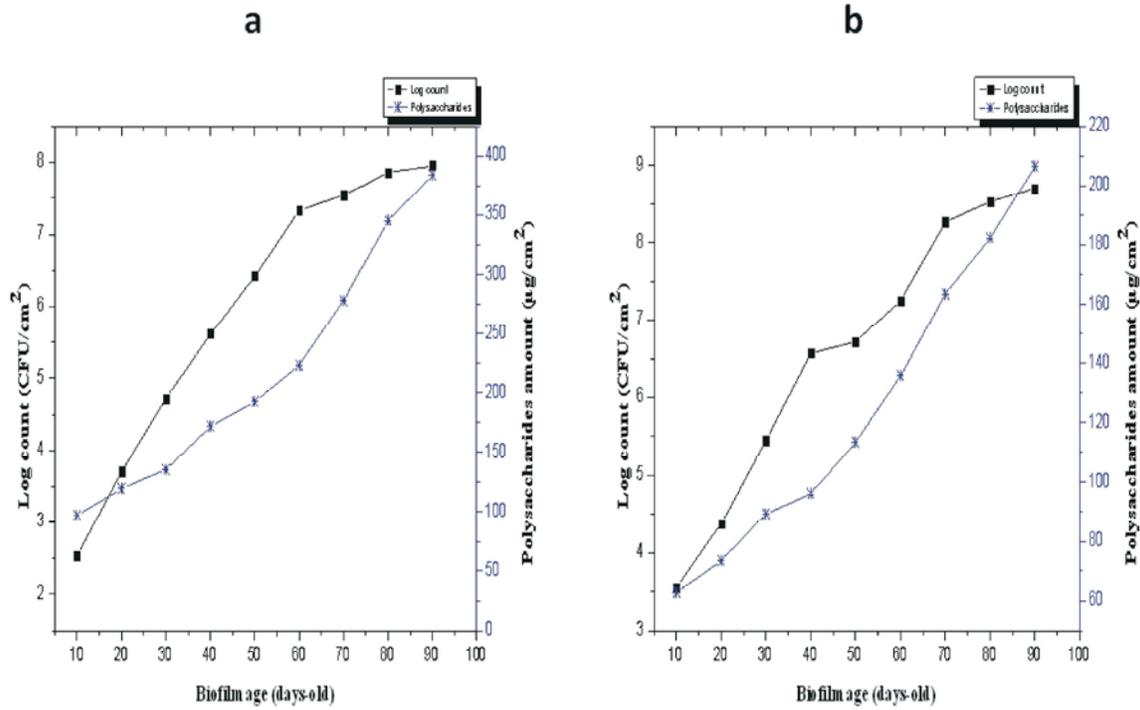


Fig. 2: Effect of PVC material and biofilm age on growth rate and exopolysaccharide amounts of *L. monocytogenes* (a) and *S. Typhimurium* biofilm (b)

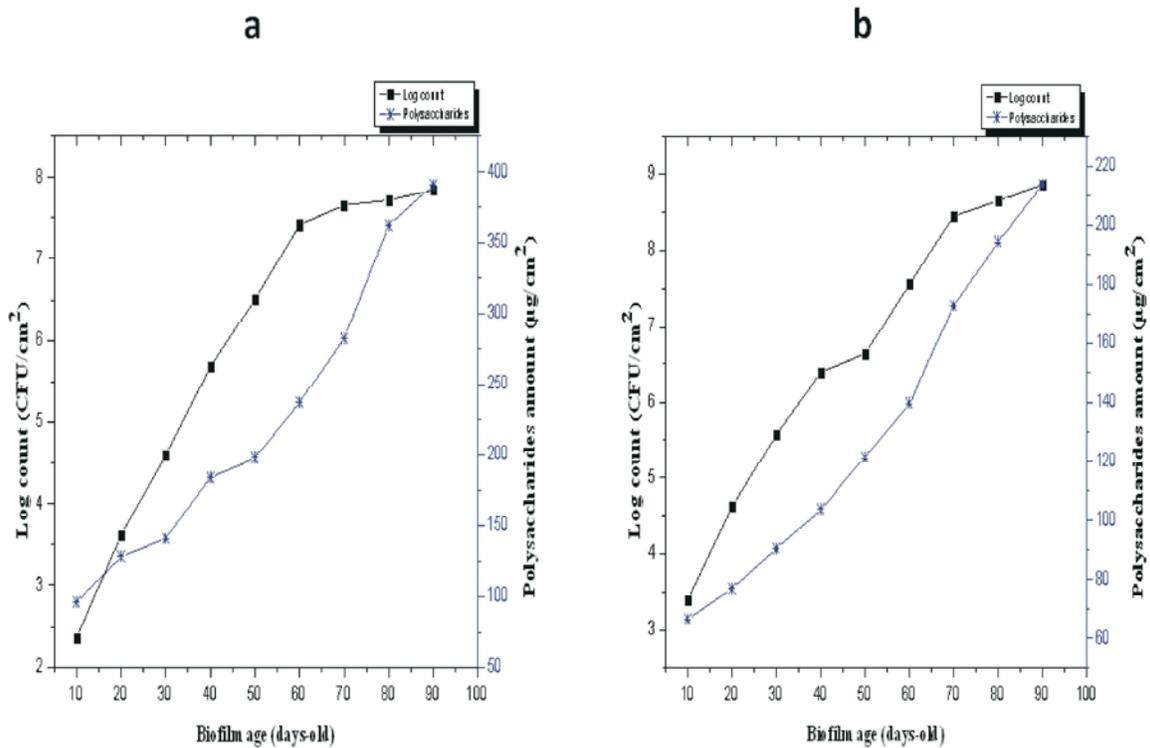


Fig. 3: Effect of PP material and biofilm age on growth rate and exopolysaccharide amounts of *L. monocytogenes* (a) and *S. Typhimurium* biofilm (b)

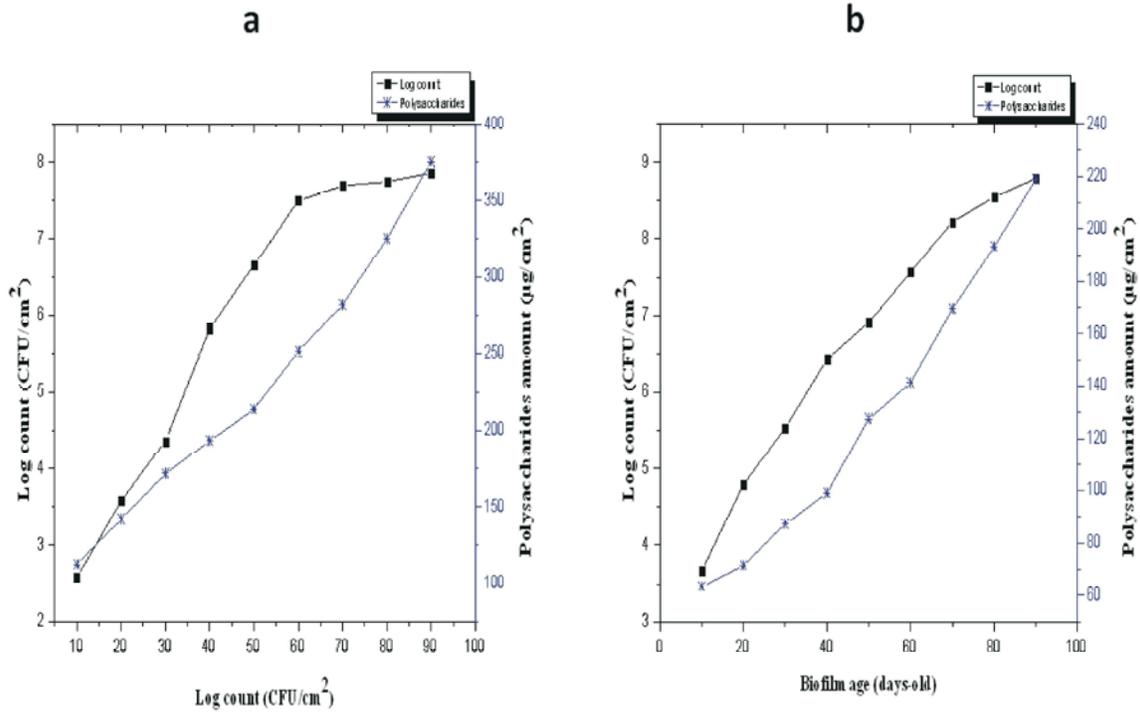


Fig. 4: Effect of PE material and biofilm age on growth rate and exopolysaccharide amounts of *L. monocytogenes* (a) and *S. Typhimurium* biofilm (b)

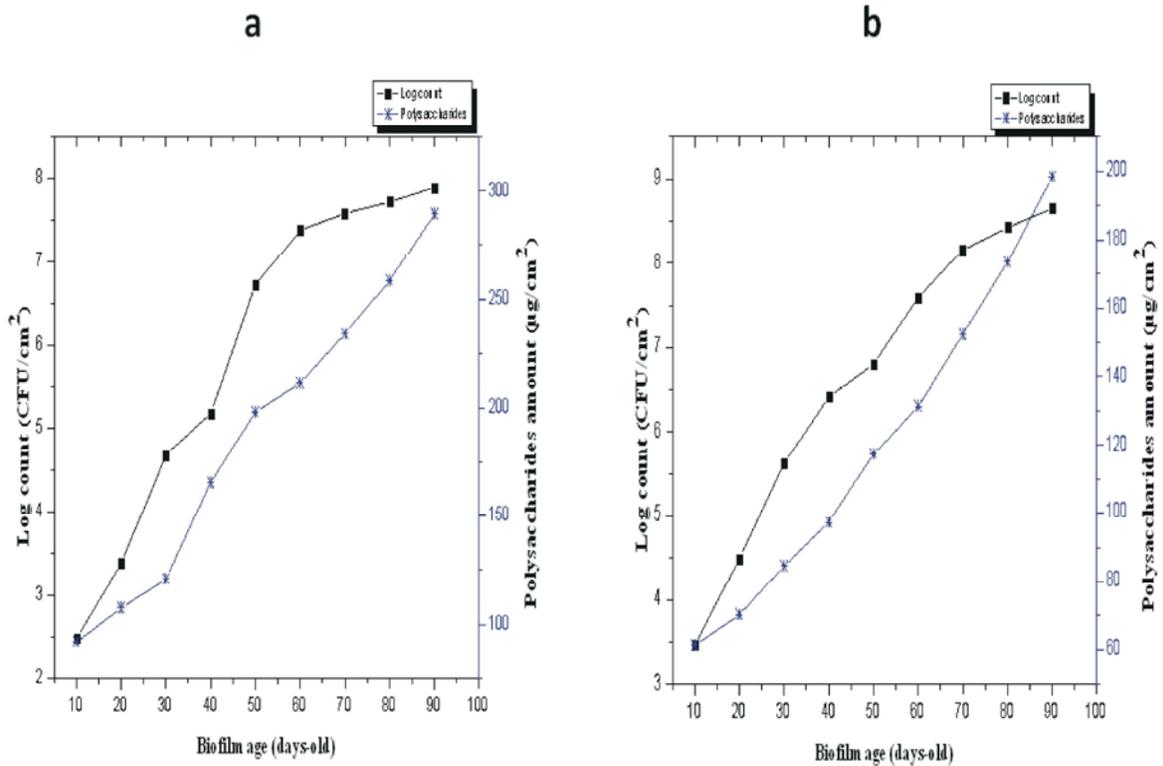
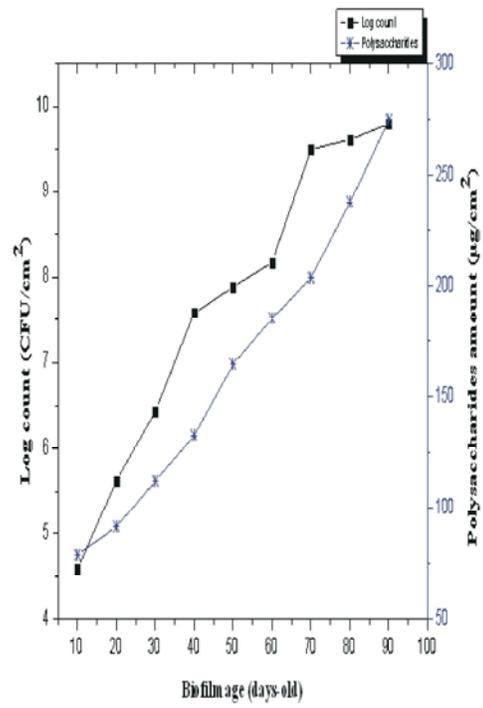
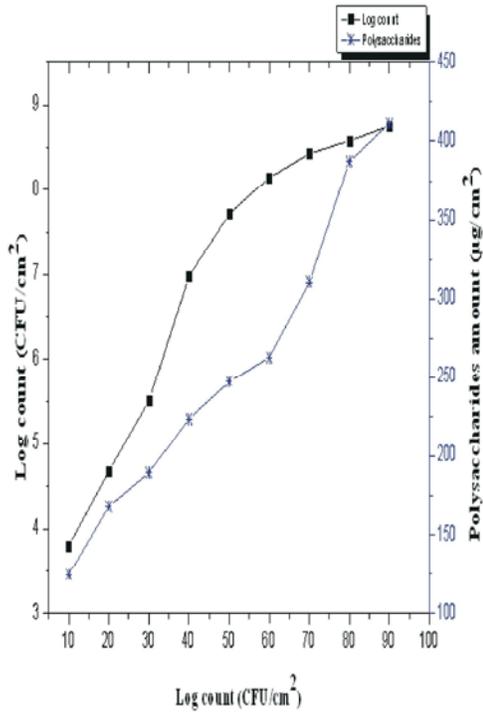


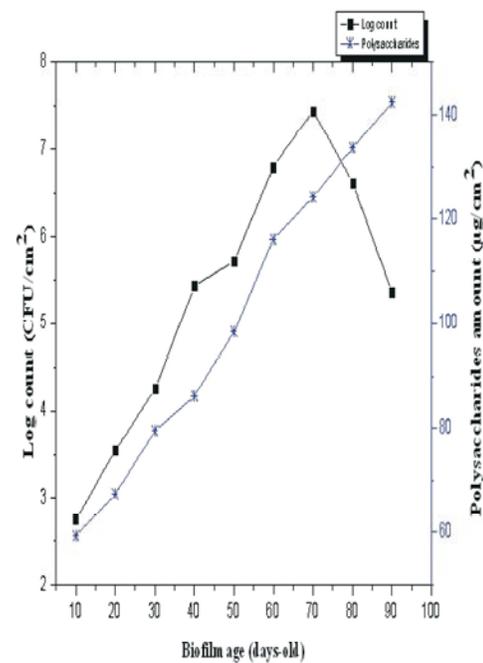
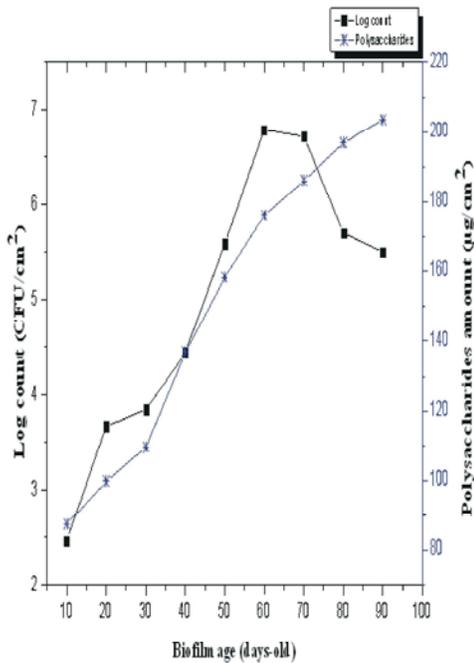
Fig. 5: Effect of R material and biofilm age on growth rate and exopolysaccharide amounts of *L. monocytogenes* (a) and *S. Typhimurium* biofilm (b)



a

b

Fig. 6: Effect of I material and biofilm age on growth rate and exopolysaccharide amounts of *L. monocytogenes* (a) and *S. Typhimurium* biofilm (b)



a

b

Fig. 7: Effect of Cu material and biofilm age on growth rate and exopolysaccharide amounts of *L. monocytogenes* (a) and *S. Typhimurium* biofilm (b)

to 10^7 CFU/cm² at 70 days and decreased to 10^5 CFU/cm² at 90 days. In addition to that, the maximum exopolysaccharide amounts of *L. monocytogenes* and *S. Typhimurium* biofilm were 203.7 and 142.4 µg/cm² (Figure 7). From statistical analysis results, there was found that a positive correlation with significance between biofilm age, biofilm growth rate and produced exopolysaccharide quantities.

Results of present study noticed that, the greatest biofilm growth rate and EPS production was in I pipe material. In contrary, the lowest was found in Cu pipe materials. The main reasons for this, the metal-based materials have a roughness surfaces that allow forming biofilm rapidly. While, plastic-based materials have smooth surfaces which can be decreased the biofilm accumulation. These results were compatible with Niquette *et al.* [26] who found that, plastic-based materials support less biofilm biomass than metal materials. Also, Liu *et al.* [27] reported that PVC as a type of plastic-based material appears more suitable as a plumbing material in drinking water distribution systems than iron material which able to support more complex bacterial diversity than PVC material.

Results summarized that, the biofilm formation in non corroded plastic-based materials was slower than corroded metal-based materials. These results in agreement with Christensen [28]; Chowdhury [29] who found that, DW biofilm grew less on polymeric materials (PE, PVC) than on iron matrices (grey iron, cast iron and galvanized steel). This fact was attributed to iron corrosion products that favor biofilm protection from the effect of flow rate and disinfectants. Also, the accumulation of corrosion products and dissolved substances in the older pipes can increase the roughness of the pipe that favoring the development of biofilm.

In contrast, the obtained results of biofilm formation in Cu pipe materials were less than other tested materials. In spite of the Cu pipe is considered as a corroded metal-based material, the results recorded that, the growth rate of biofilm was lower than tested plastic based materials. This may be revealed that biofilm formation can be caused the corrosion and released the copper ions that can be inhibited the bacterial communities and considered as antimicrobial agents. Consequently, copper pipes are known as one of the most resistant to pollution materials with a property of the toxicity of copper ions to microorganisms, especially for bacteria in biofilm [30].

Moreover, The results also in agreement with the results of Lehtola *et al.* [31-32] Hemdan [5] they found that biofilm grew faster in PE than in copper pipes, but such differences could not be detected in older piping systems; these authors also studied the release of

nutrients from the surface materials to the bulk water and the deleterious effects that this may cause on the water quality and on the efficacy of chlorine disinfection. Also, copper corrodes producing structural failure of water distribution systems and increasing the copper concentration of bulk water to health threatening levels [33]. Thus, pipes with the presence of biofilm the complication of copper with the biomass and the hydrodynamics are the main mechanisms for copper release [34].

CONCLUSIONS

The results of present study concluded that *L. monocytogenes* and *S. Typhimurium* real own a high ability to form biofilm on plastic and metal-based materials. Also, it concluded that, the numbers of *S. Typhimurium* biofilm cells were greater than *L. monocytogenes* biofilm cells. Also, results concluded that biofilm formation in I pipe materials was greater than others. While, the plastic-based materials less encouraged the formation of biofilm. Thus the present study recommended that, the plastic-based materials are the most appropriate to be used for carrying the drinking water. While, the iron pipes are not recommended for carrying water in DWDS. Also, these results could be applied toward the control of water quality in drinking water distribution systems.

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