Microbial colonization and water Quality evaluation in Drinking water Distribution systems: A study in Sulaimani city

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Abstract

The present study aimed to study the factors affecting biofilm formation in drinking water distribution system. Our Results reveal that most pipe surfaces in water distribution systems are colonized by microorganisms. Heterotrophic bacteria, *coliform*, and *Enterobacteriaceae* have been isolated and enumerated from the biofilm in distribution systems. Also, this study investigates some safety aspects of drinking water in some locations in Sulaimani city. The safety parameters covered some physicochemical parameters that affect biofilm formation, and the results were ranged as follows; pH between 7 – 7.7, Electrical conductivity (EC) between 239– 942 µS cm $^{-1}$, Total Dissolved Solid (TDS) between 673 and 201 mg.L^{-1}, Nitrate (NO $^{-3}$) between 1.10 - 4.90 mg.L-1, Ammonia (NH $_3$) between 0.06-0.44 mg.L^{-1}, total Phosphate (PO_4) between 0.0 – 0.44 mg.L^{-1}, Sulfate (SO_4) between 11-97 mg.L^{-1}, Iron (Fe) between 0.02-0.07, Biological Oxygen Demand (BOD) between 0.09- 0.040 mg.L^{-1}, Chemical Oxygen Demand (COD) between 0.11- 0.76 mg.L^{-1}, Temperature(temp.) between 12-27 ^{o}C, turbidity is < 1 for all samples of drinking water, taste, odor and color are in satisfactory limit. In this study, the physicochemical values except free chlorine are found within the permissible limit of the World Health Organization (WHO), and all the water samples do not pose any water quality problems regarding physicochemical parameters.

Keywords: Drinking Water Distribution Systems, Heterotrophic Bacteria, Membrane Filtration, Biocorrosion, Disinfectant.

Introduction

Safe drinking water is a basic need, and its availability is a top-priority issue worldwide. The drinking water treatment plant's main challenge is delivering a microbiologically and chemically safe product [15]. Although disinfection practices remove most microorganisms in raw water, the treated water is not sterile, and low levels of microorganisms persist in the water when entering the distribution networks. In drinking water distribution systems (DWDS), more than 90% of the total biomass can be found in matrix-enclosed microbial colonies, growing attached to pipe walls, called biofilms, with only up to 5% of the biomass freely suspended in the bulk water [15]. A biofilm is a thin layer of microorganisms that adheres to a surface and produces a slimy matrix of extracellular polymeric substances. Despite residual disinfectant, biofilms develop in all drinking water distribution systems (DWDS) [24]. Biofilm formation is a complex process that involves the attachment of microorganisms to a surface, the production of Extracellular Polymeric Substances (EPS), and the development of a three-dimensional structure. The EPS surrounding the microorganisms in the biofilm protects microorganisms and helps the biofilm adhere to surfaces and resist removal [5]. Biofilm growth in distribution systems could also increase flow resistance, affecting the network's hydraulic efficiency in the long run. Moreover, biofilms in many cases, secrete acid metabolites that corrode concrete and metallic pipes. Biofilms can potentially cause health risks in drinking water if they contain harmful microorganisms such as pathogenic bacteria, viruses, and protozoa. These microorganisms can cause illness if ingested, and the presence of biofilms can make it more difficult to control their proliferation. Biofilms can also harbor other contaminants, such as heavy metals, that can risk human health. To minimize the risk of illness from biofilms, it is essential to maintain the drinking water distribution system regularly and adequately test for harmful microorganisms and other contaminants. This can include maintaining proper disinfection levels, using appropriate filtration systems, and regularly flushing and cleaning the distribution system [24]. Biofilms in drinking water distribution systems (DWDS) have been studied intensively in recent decades. Therefore, a wide assortment of reviews has been published on this topic, covering aspects such as the hygienic quality of DW, as well as its development and control. However, in Sulaimani City, there is no sufficient study on the biofilms in drinking water distribution systems (DWDS); this study aimed to identify the occurrence of biofilm in DWDS pipes and examining the factors affecting their formation provides new and relevant information on the public health problems associated with the presence of biofilms in DWDS and describes current and emergent strategies for their control.

Material and Methods

1. Biofilm Sampling and Detachment from the Inner Surface of the Pipe

The biofilm samples formed on the inner surfaces of the pipe material were scraped with sterile cotton swabs and transferred to tubes containing 10 ml sterile normal saline, then vortexed for 3 min to detach the biofilm.

2. Enumeration of Bacteria in Biofilm Samples at Different Locations

The detached bacteria were diluted with sterile saline, and the enumeration was done by the pour plate technique using a nutrient agar medium for Heterotrophic bacteria. Then, the plates were incubated at 22°C for 72 hours and 37°C for 24 hours to detect HPC [10]. The same method was used to detect coliform using MacConkey agar, eosin methylene blue agar for *E. coli*, and violet red bile agar for *Enterobacteriaceae* bacteria, and plates were incubated at 37℃ for 24 hours. After the incubation period, Colonies Forming Units (CFU) were counted, and the number of bacteria was recorded.

To calculate the number of bacteria per surface area (CFU/cm^2), the average number of the bacteria CFU of the same dilution is multiplied by the reciprocal of the dilution used and divided by the volume of the aliquot pipetted to get the CFU /ml as follows: -

$$\text{CFU/ml} = \text{CFU/plate} \times \text{dilution factor/aliquot (ml)}$$

Then CFU/ml is multiple by the volume of the solution and divided by the area of the pipe to get cfu\cm^2 as follows: -

$$\text{CFU/cm}^2 = \text{CFU/ (ml)} \times \text{solution volume (ml)/ pipe area (cm}^2\text{)}$$

3. Physio-Chemical Analysis of Potable Water:

Physico-chemical parameters were determined manually in triplicate for the drinking water collected from different location such as temperature ℃, Total Dissolved Solids (TDS), Iron, Nitrate (NO_3), pH, Sulfate (SO_4), Turbidity, Ammonia (NH_3), Electrical conductivity (EC), Chloride, Phosphate (PO_4), BOD and COD using standard procedure by APHA [1].

4. Statistical Analysis

Obtained data from the study were statistically analyzed using a Completely Randomized Design (CRD) within three replications, and the means were compared according to the Least Significant Difference (LSD0.01). Also, for each studied parameter, the grand means and confidence interval with a probability of 99% were calculated, and a (t-test) at a significant level α=0.01 was performed to test the difference of Coliform detection by most probable number and membrane filtration.

Results and Discussion

Prevalence of Heterotrophic Bacteria in Pipe Biofilm Samples at Different Locations

The results showed in table 1 cleared that 96% of water samples from different locations were contaminated with Heterotrophic bacteria that incubated at 37^{0}C. Meanwhile, 100% of the water samples from different locations were contaminated with Heterotrophic that incubated at 22ºC. Based on the obtained results, it was found that the number of HPC at 22ºC and 37℃ in all pipeline biofilms was high; this may be due to the presence of an old biofilm in the DWDS pipeline. Biofilm growth is a common process in DWDSs due to various chemical and biological processes during distribution, including corrosion and disinfectant depletion [9]. Our results are in agreement with Manuel *et al*. [11], who found that the number of HPC in two years old biofilms was higher than that of one-year biofilms. These results are incompatible with absent-free chlorine in this study. Sence Control of biofilm formation in DWDS has been mainly achieved by means of chemical disinfection like chlorine.

The chemistry of the pipe wall may also play a role in microbial attachment. For example, both inorganic and organic nutrient concentrations may be much higher at the wall than in bulk solution due to adsorption; this would increase microbial growth. The pipe wall also provides a protective environment for bacteria to grow, especially in older pipes. Bacteria can attach more easily because the surface has become rough due to pitting, corrosion, scaling, or chemical deposition [17]. Once attachment begins, bacteria produce extracellular polymers that can self-perpetuate the attachment process. The polymer matrix can also cause rapid chemical reduction of disinfectants near the biofilm surface, providing a shield for bacteria against most disinfectants [16].

Prevalence of Enterobacteriaceae in Pipe Biofilm Samples at Different Locations

Table 1 cleared that 92% of the samples were contaminated with *Enterobacteriaceae* bacteria and a higher number that reached (1 x 10^8) CFU/cm^2 was observed at 8 locations, namely L1, L2, L3, L4, L5, L8, L9, and L 22. *Enterobacteriaceae* in pipe biofilm are persisted disinfection treatment and transmitted to consumers through the DWDS. These results explain to us the presence of *Enterobacteriaceae* in drinking water samples.

Prevalence of coliform Bacteria in Pipe Biofilm Samples at Different Locations

In Table 1, the results revealed that 92% of samples were highly contaminated with coliform, and just 8% of samples were free of the coliform. Also, it's clear from the same table that all samples from different locations were free of fecal coliform. Coliforms are not necessarily pathogenic, but their presence indicates the potential for fecal contamination and the need for further testing [3].

The reason for the presence of coliform in a high number may be related to the protection effect of biofilm that enabled the growth and development of coliform in the pipeline of the DWDS [3].

Table 1. Numbers CFU/ cm^2 of Enterobacteriaceae, Coliform, *Escherichia Coli*, and heterotrophic bacteria at 37 ℃ and at 22 ℃ in Biofilm Samples at Different Locations

Locations	*Enterobacteriaceae*	Coliform	*Escherichia Coli*	HPC at 37 ^{O}C	HPC at 22 ^{O}C
L1	1 x 10^{8}	1 x 10^{8}	ND	9 x 10^{6}	5x 10^{5}
L2	1 x 10^{8}	1 x 10^{8}	ND	1 x 10^{8}	1 x 10^{8}
L3	1 x 10^{8}	1 x 10^{8}	ND	1 x 10^{8}	1 x 10^{8}
L4	1 x 10^{8}	1 x 10^{8}	ND	1 x 10^{8}	1 x 10^{8}
L5	1 x 10^{8}	1 x 10^{8}	ND	8x 10^{6}	1 x 10^{8}
L6	7 x 10^{6}	1 x 10^{8}	ND	1 x 10^{8}	6 x 10^{5}
L7	5 x 10^{5}	9 x 10^{5}	ND	4 x 10^{5}	1 x 10^{8}
L8	1 x 10^{8}	6 x 10^{5}	ND	1 x 10^{8}	7 x 10^{5}
L9	1 x 10^{8}	1 x 10^{8}	ND	1 x 10^{8}	1 x 10^{8}
L10	2 x 10^{5}	0	ND	0	5 x 10^{5}
L11	2 x 10^{5}	2 x 10^{5}	ND	2 x 10^{6}	8 x 10^{6}
L12	5 x 10^{6}	3 x 10^{5}	ND	6 x 10^{6}	1 x 10^{8}
L13	5 x 10^{5}	4 x 10^{5}	ND	9 x 10^{5}	7 x 10^{5}
L14	3 x 10^{6}	2 x 10^{6}	ND	2 x 10^{5}	1 x 10^{8}
L15	6 x 10^{6}	3 x 10^{5}	ND	6 x 10^{6}	7 x 10^{6}
L16	4 x 10^{6}	6 x 10^{5}	ND	2 x 10^{6}	4 x 10^{6}
L17	4 x 10^{5}	2 x 10^{6}	ND	4 x 10^{5}	6 x 10^{5}
L18	5 x 10^{6}	2 x 10^{6}	ND	3 x 10^{6}	4 x 10^{6}
L19	3 x 10^{6}	4 x 10^{6}	ND	3 x 10^{5}	4 x 10^{5}
L20	2 x 10^{5}	6 x 10^{6}	ND	3x 10^{5}	4 x 10^{5}
L21	2 x 10^{5}	4 x 10^{6}	ND	3 x 10^{5}	4 x 10^{5}
L22	1 x 10^{8}	3 x 10^{5}	ND	3 x 10^{5}	4 x 10^{5}
L23	3 x 10^{6}	6 x 10^{5}	ND	5 x 10^{6}	7 x 10^{5}
L24	0	2 x 10^{5}	ND	5 x 10^{6}	7 x 10^{6}
L25	0	0	ND	4x 10^{5}	1 x 10^{5}
LSD $_{0.01}$	4 x 10^{7}	NS	ND	4 x 10^{6}	4 x 10^{6}
Grand Mean	5 x 10^{6}	7x 10^{7}	ND	6 x 10^{7}	7 x 10^{7}
Lower limit 99%	3 x 10^{5}	3 x 10^{6}	ND	4 x 10^{6}	5 x 10^{5}
Upper limit 99%	7 x 10^{7}	1 x 10^{8}	ND	7 x 10^{7}	8 x 10^{7}

Analysis of Chemical Parameters of DWDS Samples at Different Locations

Drinking water with good quality is very important to improve the lives of people and to prevent diseases [6]. Pollution of surface water comes from many sources, such as the discharge of waste disposal from agriculture, industries, and municipalities. Sometimes, run-off also brings mud, leaves, and human and animal wastes into surface water bodies. These pollutants may enter directly into the surface water and contaminate it. The surface water of Dukan Lake is the main source of drinking water in Sulaimani province and may be polluted with wastewater from different sources, so the present study has been undertaken to determine the physicochemical parameters in some locations of Sulaimani province.

Free chlorine

Free chlorine was absent in all examined samples, and the reason may be due to the long residence time of water in the storage tank (around 48-72 hours). However, if the residence time is too long, chlorine losses, which endanger its disinfection, may be produced [13]. For this reason, it is necessary to maintain a residual chlorine level not only during the disinfection process but also during storage. In this case, the water feeding the tank is consumption water that is duly chlorinated, but during its storage, the chlorine existing may disappear, and supplementary intakes maintaining its level in accordance with the current regulation for human consumption waters are required [13]. Also, Free chlorine may be degrading due to reactions with organic and inorganic compounds (ammonia, Br−, I−, SO3 2−, NO2 −, and Fe (II)) in bulk water, corrosion products, pipe materials, and even through interactions with microorganisms and their EPS.

Total Ammonia Nitrogen (TAN)

Data in Table 2 reported that the average values were generally below 1 mg.L^{-1}, with annual maximum concentration ranging from (0.06-0.44 mg.L^{-1}). All water samples are considered safe for drinking purposes. The National Academy of Science recommends, and many European nations have adopted, a drinking water standard of 0.5 mg.L^{-1} (ppm).

Total Phosphate (PO_4^{-3})

Phosphate enters our waterways from a variety of sources, primarily from sewage and industrial discharges and agricultural land from organic and inorganic fertilizers [26]. The value of phosphate in the water samples lies between 0.0 to 0.44 mg.L^{-1}. In this present study, the phosphate values are found within the permissible limit (0.1 mg.L^{-1}) of WHO. The phosphate values of all the water samples do not pose water quality problems.

Sulfate (SO_4^{-2})

Sulphates can be found naturally in several minerals, such as gypsum ($CaSO_4 2H_2O$), epsomite ($MgSO_4.7H_2O$), and barite ($BaSO_4$). High concentrations of sulphate may cause gastrointestinal irritation, particularly when magnesium and sodium ions are also present in drinking water resources [13]. The results in the Table 3 showed that the sulphate values for the drinking water samples are between 11 and 97 mg.L^{-1}. The maximum value 97 mg.L^{-1} is noted at location 1, and the minimum value of sulphate 11 mg.L^{-1} is indicated at location 2. The permissible limit of sulphate in drinking water, according to WHO, is 200 mg.L^{-1} [26]. Accordingly, the sulfate content of water in all the locations during this investigation was within the safe margin for drinking purposes.

Iron (Fe)

The presence of iron in water is one of the most frequent reasons for customers' complaints due to aesthetic issues (yellow, brown, and black or stains on laundry and plumbing fixtures), one important source of iron in drinking water is from old corroded cast-iron water mains, historically the material used most commonly in supply networks. Table 2 showed that the Iron concentration among locations was not significant, and the concentrations of iron in all drinking-water samples were less than 0.3 mg.L^{-1} (WHO in 1996b) [25][26]. For that, all the investigated water samples were acceptable for Iron content, and they are safe for drinking purposes.

Nitrate (NO_3^-)

The majority of the organic and inorganic sources of nitrate in the environment are waste discharges, animal slurries, and synthetic fertilizers. The results of nitrate concentration at all locations ranged between 1.1 mg l^{-1} at L23 and 4.9 mg l^{-1} at L1, as shown in Table 3. Overall, the locations are in a normal range. WHO [26] and EU [8] suggested the maximum contamination level of nitrate in drinking water to be lower than 50 mg l^{-1}. Therefore, all the water samples are considered safe for drinking purposes.

Hydrogen Ion Potential (pH)

Estimation of Water pH is an indicating factor through which the suitability of water for various purposes can be determined [19]. As shown in Table 3, the highest pH value was 7.7 at L2, L5, and L22, while the lowest pH value was 7 at L14, and statically the deference among locations in pH value was not significant. Comparing these pH values to the guideline values recommended by WHO is between (6.5-8.5) for water quality standards [25][26]. The pH values of water samples during the studied period were within acceptable levels.

Total Dissolved Solids (TDS)

The total dissolved solids in water are due to the presence of sodium, potassium, calcium, magnesium, manganese, carbonates, bicarbonates, chlorides, phosphate, organic matter, and other particles. The importance of TDS in drinking water lies in developing a particular taste in the water, and at higher concentrations, reduces its portability [4]. The results in Table 2 showed that the highest value of TDS was 673 mg.L^{-1} at L23, and the lowest value of it was 201 mg.L^{-1} at L4. The maximum acceptable concentration of TDS in drinking water, according to WHO guidelines, is below 1000 mg.L^{-1}. Accordingly, all the water samples in all locations were within the acceptable level for drinking purposes.

Biological Oxygen Demand (BOD)

The BOD values of water samples shown in table 3 ranged from (0.40 -0.09) mg.L^{-1}. The maximum acceptable concentration of BOD in drinking water, according to WHO, is 3 mg.L^{-1}, so all the investigated water samples in all locations were acceptable for BOD content, and they are safe for drinking purposes.

Chemical Oxygen Demand (COD)

COD is commonly used to indirectly measure the concentration of organic substances in water [2]. The results in Table 3 showed that the highest value of the COD in drinking water was 0.76 mg.L^{-} at L21, and the lowest value was 0.11 mg.L^{-} at L13. The standard value of COD in drinking water is not more than 120 mg.L^{-1}, according to the Notification of the Ministry of Science [25][26]. Therefore, all the investigated water samples were acceptable for COD content, and they are safe for drinking purposes.

Table 2. Chemical Characteristics of Water Samples at Different Locations.

Locations	NH_3 mg.L^{-1}	PO_4^{-2} mg.L^{-1}	SO_4^{-2} mg.L^{-1}	Fe mg.L^{-1}	NO_3^- mg.L^{-1}	pH	TDS mg.L^{-1}	BOD mg.L^{-1}	COD mg.L^{-1}
L1	0.06	0.41	97.00	0.02	4.90	7.40	603.00	0.39	0.55
L2	0.09	0.40	11.00	0.02	3.50	7.70	244.00	0.30	0.41
L3	0.22	0.39	21.00	0.02	2.20	7.60	244.00	0.38	0.53
L4	0.19	0.24	22.00	0.07	2.10	7.50	238.00	0.09	0.29
L5	0.10	0.10	28.00	0.02	3.10	7.70	201.00	0.31	0.45
L6	0.08	0.18	20.00	0.02	2.50	7.60	233.00	0.13	0.40
L7	0.17	0.38	96.00	0.02	4.00	7.30	512.00	0.38	0.52
L8	0.17	0.00	27.00	0.02	3.80	7.40	233.00	0.36	0.53
L9	0.23	0.01	24.00	0.02	2.70	7.40	240.00	0.36	0.50
L10	0.44	0.00	24.00	0.02	2.50	7.50	244.00	0.08	0.13
L11	0.15	0.14	21.00	0.02	3.30	7.40	245.00	0.12	0.18
L12	0.16	0.18	25.00	0.02	4.10	7.40	232.00	0.20	0.25
L13	0.18	0.17	24.00	0.02	2.80	7.40	233.00	0.18	0.11
L14	0.21	0.15	24.00	0.02	2.90	7.00	242.00	0.15	0.16
L15	0.15	0.15	21.00	0.02	1.90	7.20	232.00	0.17	0.19
L16	0.20	0.13	20.00	0.02	1.80	7.30	432.00	0.12	0.34
L17	0.13	0.38	33.00	0.02	1.70	7.40	345.00	0.16	0.31
L18	0.38	0.27	47.00	0.02	2.87	7.10	523.33	0.15	0.33
L19	0.41	0.28	40.00	0.02	1.20	7.50	438.00	0.31	0.44
L20	0.43	0.24	22.00	0.02	2.30	7.20	432.00	0.29	0.32
L21	0.21	0.44	84.00	0.02	2.40	7.10	569.00	0.34	0.76
L22	0.30	0.36	55.00	0.02	1.50	7.70	543.00	0.22	0.54
L23	0.23	0.19	79.00	0.02	1.10	7.30	673.00	0.40	0.32
L24	0.38	0.11	69.00	0.02	2.10	7.10	321.00	0.24	0.18
L25	0.32	0.15	32.00	0.02	1.80	7.40	438.00	0.33	0.40
$LSD_{0.01}$	0.24	0.34	6.69	NS	0.76	NS	14.14	0.14	0.21
Grand Mean	0.22	0.22	38.64	0.02	2.60	7.38	355.61	0.25	0.37
Lower limit 99%	0.16	0.14	24.22	0.02	2.07	7.27	273.99	0.19	0.28
Upper limit 99%	0.29	0.29	53.06	0.03	3.13	7.49	437.24	0.31	0.46

Analysis of Physical Parameters of DWDS Samples at Different Locations

Temperature ℃

The results in Table 3 cleared that the highest value of temperature in the same table was 27 at L21, and the lowest value was 12 at L2. According to the Dutch Drinking Water Directive [20][21], the temperature of drinking water at the customers' tap is not allowed to exceed 25°C. To limit the regrowth of microorganisms, the World Health Organization also recommends this maximum value; this recommendation is all the more important because drinking water is distributed without additional residual disinfectant [25]. Therefore, all the water samples in all locations in this study were acceptable for temperature, and they are safe for drinking purposes, exception L20 and L21, and this may be due to the relatively warm year with global warming, and it is possible that the pipes were under the direct influence of sunlight, the samples that exceed the temperature limit may be expected [20][21][22][23].

Electrical Conductivity (EC)

The electrical conductance is a good indication of total dissolved solids, which is a measure of salinity that affects the taste of potable water [27]. The conductivity is also affected by a number of variables, including temperature, ionic mobility, and ionic valences. In this investigation, the EC, as shown in Table 3, was ranged between (942- 239) µS/cm. This wide range over the studied period is probably related to the differences in climate, lithology, and geological formation. Similar observations were made by Nabi [14] and Trojan *et al*. [18]. On the other hand, the maximum acceptable level of conductivity, as indicated by the US-EPA [7], is 1000 µS l^{-1}, accordingly all studied water samples were within the permissible range and suitable for drinking purposes.

Turbidity (NTU)

Turbidity is caused by particles suspended or dissolved in water that scatter light, making the water appear cloudy or murky. Particulate matter can include sediment - especially clay and silt, fine organic and inorganic matter, soluble colored organic compounds, algae, and other microscopic organisms [12]. One of the water treatment operator's primary jobs is controlling turbidity [27]. The results shown in table 3 cleared that the water treatment process to control the turbidity of drinking water in all locations in this study was very good.

Regarding taste, odor, and color all samples were acceptable to consumers.

Table 3. Physical Parameters of DWDS Samples at Different Locations

Locations	Temperature ℃	Conductivity µS/cm	Turbidity NTU	Taste	Odor	Color
L1	14	942	<1	Normal	Normal	Normal
L2	12	381	<1	Normal	Normal	Normal

L3	16	382	<1	Normal	Normal	Normal
L4	17	372	<1	Normal	Normal	Normal
L5	13	314	<1	Normal	Normal	Normal
L6	15	364	<1	Normal	Normal	Normal
L7	18	800	<1	Normal	Normal	Normal
L8	19	364	<1	Normal	Normal	Normal
L9	20	375	<1	Normal	Normal	Normal
L10	22	381	<1	Normal	Normal	Normal
L11	21	383	<1	Normal	Normal	Normal
L12	24	362	<1	Normal	Normal	Normal
L13	22	364	<1	Normal	Normal	Normal
L14	23	378	<1	Normal	Normal	Normal
L15	25	362	<1	Normal	Normal	Normal
L16	22	350	<1	Normal	Normal	Normal
L17	20	334	<1	Normal	Normal	Normal
L18	22	548	<1	Normal	Normal	Normal
L19	24	487	<1	Normal	Normal	Normal
L20	26	239	<1	Normal	Normal	Normal
L21	27	641	<1	Normal	Normal	Normal
L22	22	544	<1	Normal	Normal	Normal
L23	24	643	<1	Normal	Normal	Normal
L24	25	346	<1	Normal	Normal	Normal
L25	24	764	<1	Normal	Normal	Normal
$LSD_{0.01}$	5	15	<1	Normal	Normal	Normal
Grand Mean	20.68	456.80	<1	Normal	Normal	Normal
Lower limit 99%	18.34	359.51	<1	Normal	Normal	Normal
Upper limit 99%	23.02	554.09	<1	Normal	Normal	Normal

Conclusion

The physical and chemical properties of water samples at different locations were within normal range according to WHO guidelines. The biofilm counts for heterotrophic bacteria at 22°C were generally higher than those at 37°C. Effective control of biofilm growth in DWDS requires suitable antimicrobial agents and the design of treatment processes to limit the initial presence of colonizing microbial community at the treatment plant. Free chlorine was absent in all examined samples due to the long residence time of water in the storage tank. For this reason, it is necessary to maintain a residual chlorine level not only during the disinfection process but also during storage.

Conflict of interest

The authors declare no conflict of interest.

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