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Original paper

Study of biofilm creating bacteria in drinking water of Ahvaz city in Iran

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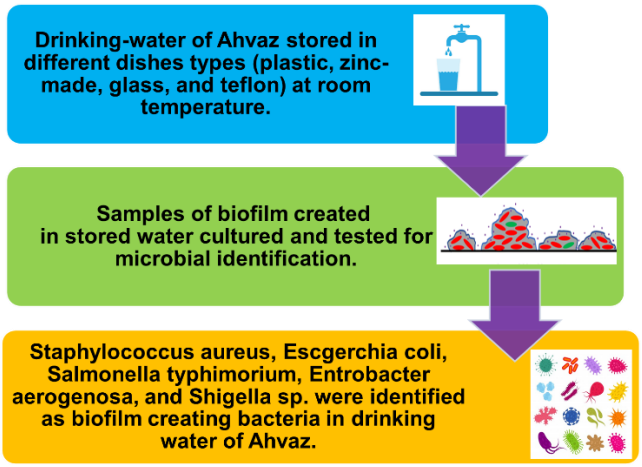
GRAPHICAL ABSTRACT



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ABSTRACT

In order to investigate the biofilm creating bacteria in drinking water of Ahvaz, Iran, 4 different types of frequently used kitchen dishes (made up of plastic, glass, zinc, and teflon) were used for storage of drinking water under the same conditions at room temperature (25 °C) in triplicate order. After the formation of the slime layer, microbiological tests were performed. Results showed that after 3 days, the biofilm layer was created. The biofilm creating bacteria of studied water belonged to both the gram-negative and gram-positive groups and were identified as follows: Staphylococcus aureus, Eschershia coli, Salmonella typhimorium, Entrobacter aerogenes, and Shigella sp. Results showed that the plastic dishes had the highest rate of bacterial growth and E. coli with 65 % of the growth was the most abundant bacteria of the investigated biofilm. It could be concluded that even in purified drinking water there were bacteria with the ability to create biofilm which needs more attention to purification processes and water storage in consideration with the quality of the dishes.

1. Introduction

Today, one of the important issues in protecting public health is the supply of safe drinking water for consumers. Although various methods of water purification can be achieved in terms of appearance, microbial and chemical qualities of water, maintaining this desirable quality during water transfer and distribution operations is still a challenge in water purification technology (Percival et al. 1998). The quality of drinking water in consumer tap water is affected by distribution lines, storage and home appliances installed by consumers, and there are concerns about the deterioration of water quality and the occurrence of secondary microbial and chemical contamination during water transfer and distribution operations (Nikaeen and Mirhendi. 2004). The formation of biofilms on the surfaces of water pipelines, reservoirs, and household devices for treatment and processing of water is one of the most significant and important problems in this regard. Biofilms or biological membranes, one of the oldest forms of life on earth, are a very thin layer of microorganisms that stick and grow to the surface (Szymanska. 2003). Although biofilm development is desirable in some systems, there are many concerns with regard to biofilms in medicine, industry, and aquatic systems (Costerton et al. 1987). Increases in friction resistance of liquids in aqueous systems, the creation of biologic sediments (Flemming. 2002), and creating a taste and smell in water from the problems associated with the growth and development of biofilms (Mirhendi and Nikaeen. 2004). The major concern about biofilm in water systems is resistant to disinfection, which makes biofilms a suitable shelter for the growth of opportunistic and pathogenic bacteria (Percival et al. 1998). The main objective of the present study was to investigate the

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possibility of the creation of biofilm and the identification of biofilm creating bacteria in kitchen dishes filled with drinking water of Ahvaz, Iran.

2. Materials and methods

2.1. Testing water and sampling of biofilm

Drinking water of Ahvaz, Iran was used from a domestic tap and stored in different kitchen dishes (made up of Plastic, Glass, Zinc, and Teflon) of the same size under the same conditions at room temperature (25 °C) in triplicate order. All dishes were previously sterile by kept in the oven at 160 °C for 2 h. All dishes were washed by distilled water prior to the beginning of the experiment (Rivardo et al. 2009).

Dishes were continuously observed for the slime layer of biofilm. After 3 days, the layer was observed and the sampling was performed. The sterile cotton swap was used for sampling and followed by culture on blood agar (BA) culture media (Yari et al. 2009).

2.2. Bacterial culture and identification

In order to identify the bacteria of the biofilm, culture samples on BA culture media were stored in an incubator for 24-48h and the growing colonies were investigated through specific culture media. After conducting the gram staining for identifying the gram-negative and positive bacteria, which showed the cultured samples have both groups, biochemical tests used for identification of cultured samples as follow: culture on McCancy culture media (MAC), broth lactose culture media (BL), eosin methylene blue culture media (EMB), salmonella-shigella culture media (SSA) and mannitol salt agar culture media (MSA). All experimental cultures were performed as triplicate order (Table 1). In order to comparison of the mean values, independent sample t-test ± SD was analyzed using SPSS 16.0.

Table 1. Use experimental Culture media for identification of bacteria.

Culture media	Incubation duration and conditions	Control organisms	Expected results
MSA	35 °C, aerobic, 24 h, 48 h	Staphylococcus oreus 25923	Growth of colonies with yellow margin
		Staphylococcus epidermis 12228	Growth of colonies with red margin after 48h
		Proteus mirabilis 12453	No growth
SSA	35 °C, aerobic, 24 h	Salmonella typhimorium 14028	Growth of colonies with/without black centers
		Shigella flextery 12022	Growth of colourless colonies
		Entrococcus fecalis 29212	Complete inhibition of growth
		Eschershia coli 25922	Inhibition of growth (complete or partial); in case of growth: pink to red colonies with sedimentation
MAC	35 °C, aerobic, 18-24 h	Eschershia coli 25922	Growth of pink colonies
		Proteus mirabilis 12453	Growth of colourless colonies, inhibition of swarming
		Salmonella typhimorium 14028	Growth of colourless colonies
		Entrococcus fecalis 29212	Partial growth inhibition
BL	35 °C, aerobic, 18-24 h	Shigella sonei 929	Growth after re-culture, growth could be inhibited by selenite media
		Eschershia coli 25922	Inhibition of growth (complete or partial) after re-culture
EMB	35 °C, aerobic, 18-24 h	Salmonella typhimorium 14028	Growth of colourless to amber colour colonies
		Eschershia coli 25922	Growth of blue to black colonies with metal appearance
		Entrococcus fecalis 29212	No growth (or partial growth)
BA	35 °C, aerobic or with CO_2 flow, 18-24h	Streptococcus pyogenes 19615	Beta haemolysis growth
		Streptococcus pneumonia 6305	Alfa haemolysis growth
		Staphylococcus oreus 25923	Growth
		Eschershia coli 25922	Growth

3. Results and discussion

The formation of a biofilm layer is a major concern in drinking water supplies, medical and food facilities. It has been shown that a wide range of microorganisms, mainly bacteria have the ability of the formation of biofilm. Results of the present study showed that even in purified water of Ahvaz City, Iran, there was a considerable amount of bacteria that could form a significant layer of the biofilm in different commonly used kitchen dishes. Results showed that all experimental dishes had the ability for the formation of biofilm, but some differences were observed. Results of the storage of studied water in different kitchen dishes showed that after 3 days the biofilm layer appeared. Results of the cultured samples showed that the number of total bacteria in the biofilm layer was increased after 5 days of the experiment (Fig. 1). The culture of samples from day 3 showed that the highest presence of bacteria belonged to the plastic dish, and the Teflon dish showed no growth on day 3 (Fig. 1). After 5 days of the experiment, the Teflon dish develops the slime layer of biofilm (Fig. 1).

In another word, the results of the present study showed that biofilm in the plastic dishes was created and grow more rapidly than the other experimental dishes. The results of the statistical analyses showed that over time, there was a significant difference between growths in different dishes. The significant differences of 99 % about the number of the bacteria were observed in plastic, and zinc made dishes ($p<0.01$), which was showed that the biofilm layer of these two experimental dishes grew during the experiment time. It has been revealed that bacteria have the affinity of attachment to hydrophobic surfaces such as plastics more than the attachment to the hydrophilic surfaces such as glass or metals (Zottola. 1994). It is also mentioned that one of the reasons for the formation of biofilm on a surface is the roughness of the surface which is suitable for attachment of the bacteria. It has been studied that the main density of attached cells to

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a smooth surface of a glass is significantly lower than a scratchy one (Characklis et al. 1990).

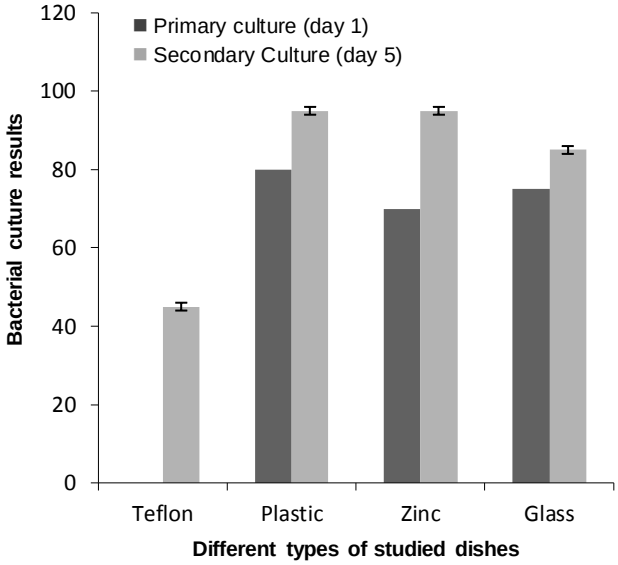

Fig. 1. Results of the primary culture (day 1) from four experimental dishes and results of the secondary culture (day 5) from four experimental dishes.

Results of the gram staining showed that grown bacteria of all dishes were gram-negative and gram-positive both (Fig. 2). Results of specific biochemical tests and specific cultures showed that the biofilm bacterial community was composed of Eschershia coli, Entrobacter aerogenes, Staphylococcus aureus, Salmonella sp., and Shigella sp. (Fig. 3). The results also showed that among the observed bacteria, E. coli had the highest number and growth, and after that, the number and growth of the observed bacteria were as follows: Salmonella sp.>Shigella sp.>Entrobacter aerogenes>Staphyolcoccus aureus (Fig. 3). In a previous study, September et al. (2007) showed that biofilm creating bacteria in drinking water supplies of southern Africa were Aeromonas, Pseudomonas, Klebsiella, and Enterobacter, no Salmonella or Shigella were detected. Another study on drinking water supplies showed that the water has microorganisms with the ability to creation of a biofilm layer on the surfaces of the pipelines (Boe-Hansen et al. 2003). On the other hand, Bomo et al., (2004) demonstrate that biofilm of the drinking water pipelines could contain the Aeromonas. It has also been revealed that the bacteria of biofilm have the catalytic ability in chemical and microbial activities which could lead to the corrosion of the pipelines and water reservoirs (Anand et al. 2014).

In another study on biofilms in drinking water, Juhna et al. (2007) showed that drinking water pipelines have the capability for the formation of biofilm containing E. coli. Besides the bacterial diversity of the water which could affect the biofilm community members, it has been revealed that the composition and chemistry of the surface could have a significant role in the formation of biofilm.

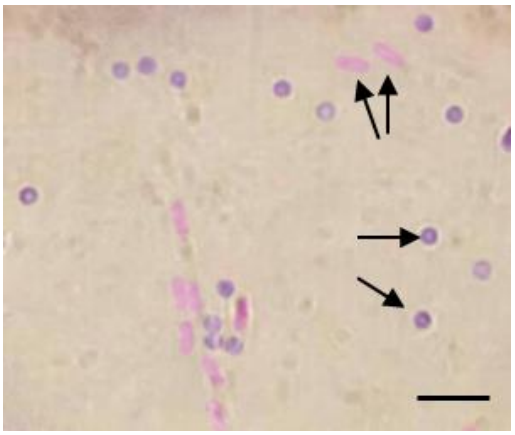

Fig. 2. Gram staining photomicrograph of the sample from the biofilm, rod-shaped gram negative and cocci gram positive bacteria were observed.

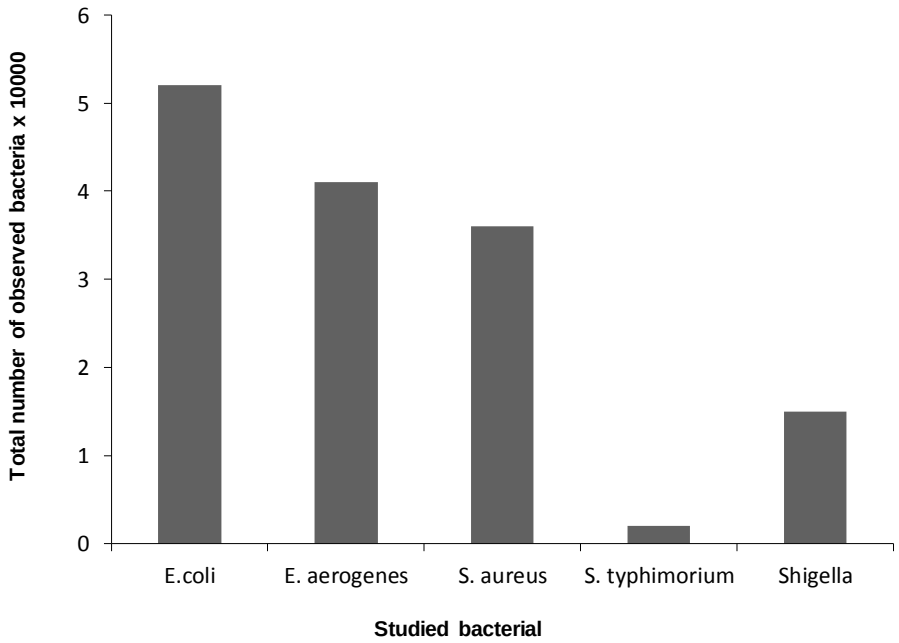

Fig. 3. The total number of observed bacteria in all experimental dishes.

4. Conclusions

In conclusion, the results of the present study showed that the drinking water of Ahvaz has the potential to create a biofilm layer which is a sign of weakness in the water purification process. Results also showed that the type of water container (dish) is important on the formation time of the biofilm layer, as in the present study the first and last dishes that started to grow a biofilm layer were plastic and Teflon made respectively. Such results could be considered for the pipelines and water reservoirs in order to decrease the possibility of biofilm creation alongside the proper purification processes. The number and species of the observed bacteria showed that the biofilm layer could be formed even at short periods of time which would be considered as an alarm for the low hygienic condition of the drinking water supply.

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By the supports of the previously published data, such short term biofilm could also grow to bypass the time and the bacterial community it could also become more complicated and resulted in corrosion of the water supply system, contamination of the drinking water, and finally the health problems in domestic consumers.

Acknowledgment

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