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Investigation of Bacterial Regrowth and Residual Chlorine in Household Stored Water in Saveh-Iran

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Background & Aims of the Study: Saveh city -located in the semi-arid area in Iran- has brackish water and it was forced to use reverse osmosis desalting systems. Water is not distributed through the distribution network to homes so, people have to buy water from certain places and transfer water through household storage vessel. They have to buy more water and keep it at home for more than 1 week. Therefore, the probability of microbial contamination or the regrowth of microorganisms rise. The aim of this study was to evaluate the changes in the free residual chlorine (FRC) and the number of bacteria in desalinated water samples that collected from water kiosk system that provide desalinated water for the public.

Material & Methods: Samples collected at the beginning and the end of the water network and stay for 9 days at room temperature and similar condition as public stored at home. The samples analyzed for FRC, total coliform (TC), fecal coliform (FC), heterotrophic plate count (HPC) and organic matter for 9 days.

Results: The results indicated that FRC concentration at the beginning of water network was 1 mg/L and after 9 days it was reached to 0.5 mg/L at the end of the water network. The amount of UV254 at the end of water network was 0.0134 cm^{-1} and after 9 days it was reached to 0.0125 cm^{-1}. There aren't detected any TC and FC in samples even at beginning of the examination and also, after 9 days storage of water. The number of HPC colony for samples at the end of the network it reached 22 CFU/ml, after 9 days.

Conclusion: It was concluded that desalinated water that stored at household storage vessel in Saveh has good quality and meets standard limit value until 9 days. It should be noted that this conclusion depends on various conditions, including various environmental factors. However, Water treatment officials need to pay more attention to the amount of chlorine remaining in drinking water in Saveh because after 9 days, there was still 0.5 mg/L of residual free chlorine in the water.

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Background

Today, safe drinking water is right for all people. This is one of the basic needs of people in terms of health and sustainable development. Therefore, governments are obliged to supply and distribute healthy drinking water to the citizens. World population growth, industrial and agricultural development, in addition, lack of proper water management has led to water scarcity in many countries. As a result of these events some countries have turned to water reuse from different resources like wastewater reuse approaches (1-3). Another option for compensation and supply of urban drinking water is to turn towards desalination from brackish or saline waters by different methods (4-7). Desalination is a process to convert high TDS water (saline or brackish water) into low TDS potable drinking water. At present, most desalination plants were operated by semi permeable membrane techniques like reverse osmosis (RO) in many countries and cities. This method has the advantage including low-energy consumption, user friendly and high efficiency. RO is the separation process to separate pure water and salt solution through a semi permeable membrane by air pressure (8).

In addition to many method of water supply for cities or rural section like raw water resource change, raw or treated water transition from nearby towns, Water supply with mobile tanker there is another concern for this matter entitled decentralized or centralized water systems. Decentralized systems for water supply refer to single household, domestic (point-of-use systems) or small-scale systems in community (like small RO system that used for desalination in houses). Centralized systems for water supply refer to a big plant or system that Provides water for the city (likes water treatment plant).

Each of these has its own advantages and disadvantages. Some believe that decentralized drinking-water systems are an important element in the process of reaching the Millennium Development Goals and centralized systems are often deficient or nonexistent in developing and transition countries (9). Also, some believe that centralized water, wastewater and storm water services for urban areas has been common practice for over 100 years and they have been in use since the mid-1800s for water and waste water services but in recent years alternative technical solutions can improve the efficiency and infrastructure functions in urban water management, especially in water scarce areas (10).

Centralized water services have ensured adequate water supply, sanitation and drainage services to its inhabitants in cities (11). Also, the typical centralized system can limit the potential to adapt water supply systems to local opportunities and needs (10). So, in some condition these water infrastructures are considered to be unsuitable to address future challenges (12). On the other hand the conventional approach to the water service provision does not comply with the more recent aspirations of ecologically sustainable development (13). Decentralized water infrastructure is an alternative to the conventional centralized systems for implementing sustainable water infrastructure in urban setting (14).

the benefits of decentralized systems can be as 1- reducing the cost of infrastructure for long distance transport and treatment of water and wastewater, along with unnecessary treatment to potable standards of water used for purposes other than drinking and bathing; 2- more efficient use of resource; 3- improving service security; 4- reducing water systems' risk; 5- reducing operation and maintenance cost; 6- good response at emergency conditions (15).

Infrastructure investment and replacement is a gradual process and changing the whole infrastructure is not economically or environmentally sustainable (16). With a legacy of centralized infrastructure solutions in combination with growing investment in decentralized infrastructure solutions, the result is a hybridization process where mix of centralized and decentralized systems co-exists. However, there is limited practical or scholarly understanding of how to enable such transition of traditional urban water systems towards hybrid systems (17). Today many country and city especially in arid area forced to use desalination by various methods like membrane process. Although this process has good efficiency but their rejected water or wastewater has very bad effect on environment. In many countries this process operated as centralized so, uniform monitoring is done on water quality and more precise management is done on the sewage.

Saveh is one of the city of Arak provinces with about 280000 populations in 2017. This city has semi arid weather. The water resources of this city (surface and ground water) are brackish water so, desalination is one of the most important processes for supplying drinking water. In this city, drinking water is not distributed through the distribution network to homes. People have to buy water from certain places and transfer water through containers (mostly polyethylene containers as 20 liter volume).Drinking water is provided by three main methods, including a small central desalination plant, private sector, and household reverses osmosis. Central desalination plant produced water about 1000 m^3/d (Total dissolved solid was 185 mg/L) and delivered drinking water through districted water network that is provided to supermarkets and water kiosk system (an intelligence system that deliver specific amount of water by payment with electronic card). People are forced to move their water through 20-liter containers to their homes and stored it for several days (at least 2 to 3 days and in some situation until 5 or 7 days). Therefore, the probability of microbial contamination or the growth of microorganisms rises.

Aims of the study:

In this study, the effect of time (storage time of water in container) on microbial growth, organic matter changes and residual chlorine has been investigated to determine the effect of duration on water quality.

Materials & Methods

Study area and sampling points

The central desalination plants in Saveh produced drinking water and delivered it through supermarkets and water kiosk system (an intelligence system that deliver specific amount of water by payment with electronic card) to people. In this study water kiosk system (WKS) was selected as a drinking water source for sampling (Fig. 1). Two points at beginning and end of water network that delivered water were selected for sampling (Fig. 2). At usual method for measuring the microbial quality, some chemicals such as thiosulfate is used for neutralization of the residual chlorine, or disinfection of faucet, tap and components is necessary before sampling. In this study to simulate the actual state of people's water harvesting these actions were not taken. Sampling was done from the beginning and the end of the network. At each point, two samples of 20 liters were collected and immediately transferred to the lab.

Figure 1) water kiosk system that delivered drinking water to people

Experimental methods and analytical methods

Two samples of 20 liters were collected from the beginning and the end of the network and they stored and used as usual people use. They were consumed during 9 days. During this time, two liters of water were taken from the container each day and examined for total coliform (TC), fecal coliform (FC), Heterotrophic Plate count

(HPC), free residual chlorine (FRC) and organic matter as UV 254 nm. Also, for each point (beginning and the end of the network) two situations as closed and opened cap of vessel were investigated to identify the effect of open situation on FRC concentration and microbial pollution. Tacked sample at the beginning of water network placed at two 20th liter vessels, one with open cap and one with closed cap that nominated as A1 and A2, respectively. Also, tacked sample at the end of water network placed at two 20th liter vessels and nominated as B1 (open cap at the end of water network) and B2 (closed cap at the end of water network).

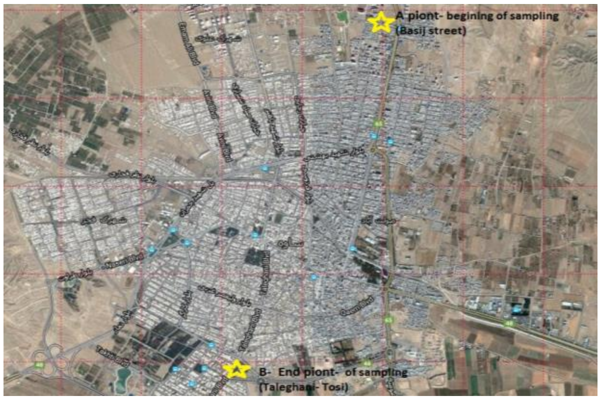

Figure 2) Two point of sampling in Saveh desalinated water network distribution system as beginning and end of system

All the experiments were conducted according to the Standard Methods for the Examination of Water and Wastewater (18). UV254 nm was analyzed by DR 6000-HACH LANGE after filtering the samples by a 0.45µm membrane filter. Free residual chlorine was analyzed by FTC-420 Free & Total Chlorine Meter as DPD (diethyl paraphenylene diamine) method (19).

Total and fecal coliform (E. coli) were estimated by most probable number (MPN) method with 9 tubes was used to determine coliform. Multiple tube fermentation included possible, confirmatory, and complementary stages. For bacterial growth, lactose broth medium was used in this method. The tubes were incubated in 35 °C ±0.5 for 24-48 h and then, from each positive tube inoculate (by loop) was added to brilliant green and EC broth media. Then, the tubes with brilliant green medium were incubated at 35 °C ±0.5 for 24-48 h and the tubes containing an EC medium incubated in serology water bath at 44 °C ±0.5 for 24 h. Both media with gas were marked as positive. Value is reported as MPN per 100 ml of sample (20).

HPC of water samples were estimated by spread plate method and dilution plate method technique using on R_2A agar plates. HPC medium was melted by heating in boiling water and then allowed to cool in a water bath to 44 - 46°C. Then 15 ml of the agar medium was poured into the petridish. When the media has solidified, the plates were inverted and placed in refrigerator. Serial dilutions were prepared by peptone water and 1 ml of the sample or dilution was spread on plate. They incubated at 35 °C for 24 to 48 hours. After the appropriate length of incubation, suitable plates from different dilutions were selected and the visible colonies were counted by a colony counter. Then the average colonies were counted and expressed as colony forming unit per ml of water.

Results

Changes in free residual chlorine, organic matter and qualitative microbial properties of the samples are shown in Figures 3 -6 and Tables 1 and 2 after 9 days of storage.

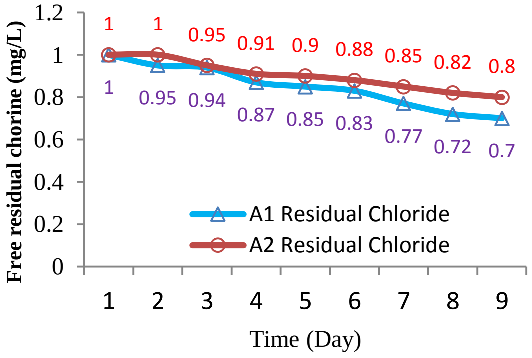

Figure 3) Changes in free residual chlorine during 9 days for open lid (A1) and closed lid (A2) at the beginning of water network.

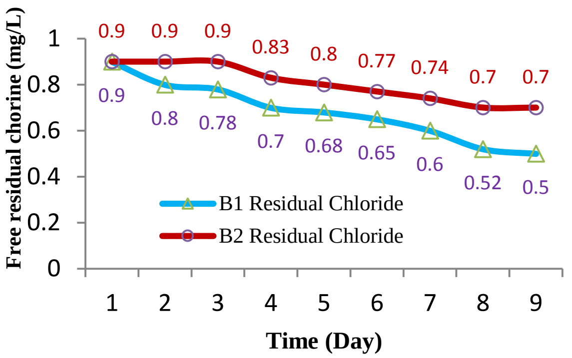

Figure 4) Changes in free residual chlorine during 9 days for open lid (B1) and closed lid (B2) at the end of water network.

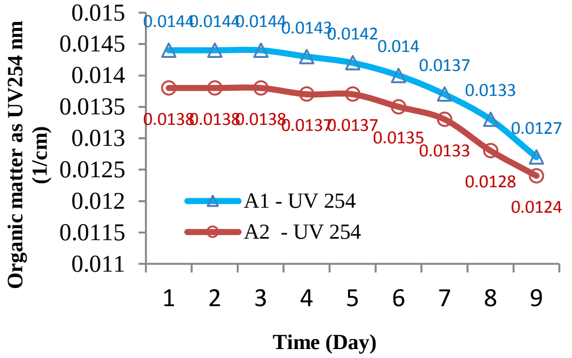

Figure 5) Changes in UV254 during 9 days for open lid (A1) and closed lid (A2) at the beginning of water network.

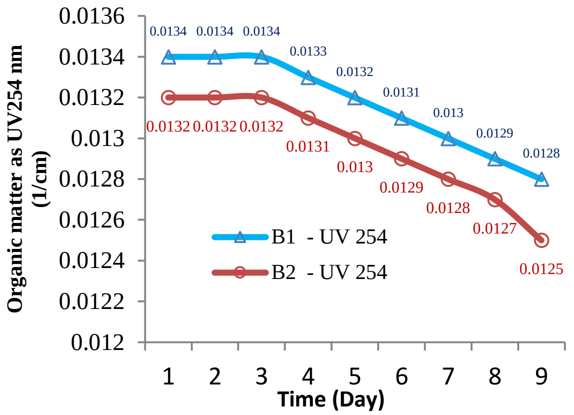

Figure 6) Changes in UV254 during 9 days for open lid (B1) and closed lid (B2) at the end of water network.

Table 1) Microbial quality of stored water in household vessel during 9 days at the beginning of the water network

Day	Vessel position	Total coliform	Fecal coliform	Heterotrophic Plate
1	open	0	0	0 (±0.00)
	closed	0	0	0 (±0.00)
2	open	0	0	0 (±0.00)
	closed	0	0	0 (±0.00)
3	open	0	0	0 (±0.00)
	closed	0	0	0 (±0.00)
4	open	0	0	3 (±1.4)
	closed	0	0	1(±0.00)
5	open	0	0	2 (±0.7)
	closed	0	0	2 (±0.00)
6	open	0	0	5 (±1.4)
	closed	0	0	2 (±0.7)
7	open	0	0	7 (±1.4)
	closed	0	0	2 (±0.7)
8	open	0	0	8 (±1.4)
	closed	0	0	2 (±0.7)
9	open	0	0	8 (±1.4)
	closed	0	0	3 (±0.7)

Table 2) Microbial quality of stored water in household vessel during 9 days at the end of the water network

Day	Vessel position (closed/open)	Total coliform (MPN/100ml)	Fecal coliform (MPN/100ml)	Heterotrophic Plate count (CFU/ml)
1	open	0	0	0 (±0.00)
	closed	0	0	0 (±0.00)
2	open	0	0	0 (±0.00)
	closed	0	0	0 (±0.00)
3	open	0	0	13 (± 2.12)
	closed	0	0	2 (±0.7)
4	open	0	0	15 (±2.12)
	closed	0	0	2 (±0.7)
5	open	0	0	15 (±2.12)
	closed	0	0	3 (±0.7)
6	open	0	0	17 (±1.4)
	closed	0	0	4 (±1.4)
7	open	0	0	18 (±2.12)
	closed	0	0	5 (±1.4)
8	open	0	0	21 (±1.14)
	closed	0	0	5 (±1.4)
9	open	0	0	22 (±2.12)
	closed	0	0	6 (±1.4)

Discussion

Residual chlorine

Across the world, chlorine is used to disinfect drinking-water and swimming pool water (21). The taste and odor thresholds for this chemical matter in distilled water are about 5 and 2 mg/L, respectively (22). In aqueous solution, chlorine reacts to form hypochlorous acid and hypochlorites that known as free residual chlorine (FRC). The concentrations of these ions are approximately equal at pH 7.5 and 25 °C. in most disinfected drinking water it is presented at concentrations of 0.2–1 mg/L (23). The World Health Organization has set a health-based guideline maximum value of 5 mg/l for chlorine as a residual disinfectant in drinking water. However, in most disinfected drinking water, it appeared at concentrations of 0.2–1 mg/L (24).

The results of the free residual chlorine (FRC) analysis of desalinated water in Saveh (Fig. 3 and 4) showed that at the beginning of water network FRC was 1 mg/L and after 9 days it was reached to 0.7 and 0.8 mg/L at opened and closed cap vessel, respectively. Also at the end of water network FRC was 0.9 mg/L and after 9 days it was reached to 0.5 and 0.7 mg/L at opened and closed cap vessel, respectively. As results showed the amount of FRC at the Saveh water network was high to some extent and after 9 days it reached to 0.5 mg/L.

Factors like dissolved organic carbon, Fe^{2+}, Mn^{2+}, NO_2 -, H_2S, H_2SO_3, ammonia, high temperatures and also corroded pipes, dead ends, long storage time and poor maintenance standards are known to cause residual chlorine decay in drinking water distribution network (25,26). Previous studies showed that the free chlorine concentration gradually decreases over time (27) by storing the chlorinated water. Also, the storage of water in the refrigerator leads to reduce THMs significantly (28). The results of this study demonstrated that the amount of residual chlorine is decreasing over time and along the length of the path that it was accordance with Ecura's study path (19) but at the end of the network and also after 9 days it t remained high (0.5 mg/L). However, the concentration of 1 mg/L of residual chlorine in treated water with reverse osmosis appears to be high, and in a study conducted in Japan, various measures have been taken to reduce residual chlorine to 0.5 mg /L (29).

Zeighami study on Serum lipid levels in neighboring communities with chlorinated and non-chlorinated drinking water showed that where chlorine levels in water ranged from 0.2 to 1 mg/L, serum cholesterol and low-density lipoprotein levels were higher in communities using chlorinated water. Levels of high-density lipoprotein (HDL) and the cholesterol/HDL ratio were significantly elevated in relation to the level of calcium in the drinking water, but only in communities using chlorinated water. He believed that chlorine and calcium in drinking-water may interact in some way that affects lipid levels (30). Also, recent studies showed the chlorine in treated water is dangerous to human health and can cause allergic symptoms ranging from skin rash to intestinal symptoms to arthritis (31).

Ecura study results showed that water age has an impact on chlorine concentration as reservoir that is the nearest to the water treatment plant in the distribution network, has intermediate concentrations (0.11 mg/L Cl_2) but reservoir that is the farthest displayed the lowest concentrations (0.0 mg/L Cl_2 at times) (19). Some study introduced two methods for the decay of chlorine that including storage of the samples at ambiance and storage of the samples in the refrigerator. Also, it was demonstrated that FRC dissociates more rapidly when the water is stored in bottles or containers which have no lid because the chlorine evaporates from the water that it is exposed to the air (32). However, in this study, the storage of water with or without lid didn’t have an important effect on FRC dissociation or reduction. Another important point related to TDS concentration and organic matter content that both of them were low in Saveh desalinated water. Therefore, taking into account the high concentrations of chlorine and its high residual after 9 days, as well as reducing the risks of high chlorine intake, measures are needed to reduce the amount of chlorine.

Organic matter

One of the most important parameters in water quality is organic matter. The concentration of natural organic matter (NOM) in water has an important impact on the quality and treatability of water. Chlorine can react with NOM and lead to the formation of potentially carcinogenic disinfection byproducts (DBPs), like trihalomethanes (THMs) (33). Organic matter characterization can be conducted by resin adsorption, size exclusion chromatography (SEC), nuclear magnetic resonance (NMR) spectroscopy, and fluorescence spectroscopy (34). Also, the amount of NOM in water has been predicted with parameters including UV–Vis at 254nm, total organic carbon (TOC), and specific UV absorbance (SUVA).

It has been proven that UV254 can be used as a surrogate parameter for and predictor of dissolved organic carbon (DOC), total organic carbon (TOC), and trihalomethanes (THMs) precursors. This parameter is important in monitoring water treatments plants for the removals of TOC and THM precursors. Measuring of it is simple, rapid and inexpensive (35). The UV light at 254 nm is absorbed by various organic and inorganic molecules, but especially organic compounds with an aromatic structure or compounds that have conjugated C=C double bonds. Aquatic humic matter has these structural features so they absorb more light per unit concentration of DOC (called the specific UV absorbance, or SUVA) than other types of NOM in water supplies, such as hydrophilic acids, hydrophobic bases (e.g., proteins and aromatic amines), and hydrophobic neutrals (e.g., aldehydes). The C=C double bonds are sites for donating electrons, oxidants and disinfectants attack and chemically react with these compounds so, these properties making UV254 nm a particularly good surrogate and predictor of DBP formation and oxidant demand like ozone, chlorine and other oxidants demand (36,37).

Unfortunately, they're not sophisticated analysis device like TOC meter in Saveh city so, for analyzing this parameter samples transported to it province (Arak city). However, we also had to use the UV254 parameter to check the variation of organic matter in drinking water. As indicated in Fig. 5 and 6 the amount of UV254 at the beginning of the water network was 0.0144 cm^{-1} and after 9 days it was reached to 0.0127 cm^{-1} and 0.0124 cm^{-1} at opened and closed lid vessel, respectively. Also at the end of the water network it was 0.0134 cm^{-1} and after 9 days it was reached to 0.0128 cm^{-1} and 0.0125 cm^{-1} at opened and closed lid vessel, respectively. As results showed the amount of UV254 at the Saveh water network was low. Over time, the amount of this parameter is lower and may be due to its consumption by chlorine over time. In water quality control the target value or goal value of UV254 was introduced as 0.03 to 0.035 cm-1 that correlates with TOC ≤ 2 mg/L. Also, it is recommended that treatment plants set treated water UV254 goals of 0.02 to 0.035 cm-1 (38).

Microbial quality

Total coliform (TC) include organisms that can survive and grow in water. They (excluding Escherichia coli that it is considered as the most suitable indicator of fecal contamination) occur in both sewage and natural waters. Hence, they are not useful as an indicator of fecal pathogens, but they can be used to assess the cleanliness and integrity of distribution systems and the potential presence of biofilms. However, there are better indicators for these purposes. It has been proposed that total coliform could be used as a disinfection indicator. Fecal coliform are found naturally in the human gut and pose no threat to health but their presence can indicate fecal contamination of a water source. heterotrophic plate count (HPC) bacteria are not a direct indicator of fecal contamination; however, they do indicate variations in water quality and the potential for pathogen survival and regrowth (38). HPC widely used when investigating complaints of off-tastes or odors, as changes in HPC populations may indicate proliferation of biofilms, which can be associated with the microbial mediated generation of some organoleptic compounds (39).

The results demonstrated that there aren't detected any total coliform (TC) and fecal coliform (FC) in samples even at beginning of the examination and also, after 9 days storage of water in the room as opened and closed lid vessel (Table 1 and 2). For heterotrophic plate count bacteria, there aren't detected any colony at the beginning and end of water network during 3th and 2nd days of sampling, respectively. The number of heterotrophic plate count colony for samples at the beginning of network in opened and closed lid reached to 3 and 8 CFU/ml respectively after 9 days. Also for samples at the end of the network it reached to 22 and 6 CFU /ml in opened and closed lid, respectively after 9 days. Jaleilzadeh studied the heterotrophic bacteria and coliform in the water of old and new distribution networks. Results of study showed that the highest concentration (15.82 CFU/100 ml) was observed at the end of the old water distribution network, and the lowest concentration (3.45 CFU/100 ml) was observed at the beginning of the new distribution network that it is may be due to the decrease in chlorine concentration in parts of the system that were in end points or blinded areas of the network (40). Amanidaz results on the Interaction between heterotrophic bacteria and coliform, fecal coliform and fecal streptococci bacteria in the water supply networks showed that the average number of total coliform, fecal coliform and heterotrophic bacteria in the end point of water network systems is more than the beginning part (20).

Results of Momba study showed that the length of time mostly resulted in the regrowth of TC and both types of household containers supported the growth and survival of indicator microorganisms due to the bad quality of the intake water before storage. The storage of drinking water for 48 h resulted in the regrowth of TC (41). According to WHO guideline (24), E. coli or thermo tolerant coliform bacteria must not be detectable in any samples in treated water in the distribution system and all water directly intended for drinking. The presence of TC indicates inadequate treatment. The presence of this organism in distribution systems and stored water supplies can reveal regrowth and possible biofilms formation or contamination through ingress of foreign material, including soil or plants. By the way, there aren't any contaminations in Saveh water distribution system and stored water for 9 days. The results demonstrated that by increasing storage time, the number and growth rate of heterotrophic bacteria will increase and similar results have been reported by Burkowska (42). Although, the growth rate has been increasing, the number of HPC has not exceeded the standard value in drinking water (500 CFU /ml). The limit value of 100 colony-forming units (CFU /ml) is not directly correlated to potential health risk. But, it reflects the efficiency of the filtration process; in this sense, it is only indirectly correlated to the lowering of the risk of infection, particularly for gastrointestinal infections that are acquired by ingestion (43).

Conclusion

The concentration of free residual chlorine at the beginning of the water distribution line is higher than that in the end of the line. So, those who live at the beginning of the water distribution network receive more FRC in their water. The FRC concentration reached to at least 0.5 mg/L after 9 days. Maybe it is necessary to reduce FRC concentration at the water storage reservoir before transfer to the water distribution system or water kiosk systems. Microbial quality of desalinated water that stored at household storage vessel in Saveh has good quality and meets standard limit value until 9 days. It should be noted that this conclusion depends on various conditions, including various environmental factors. Water treatment officials need to pay more attention to the amount of chlorine remaining in drinking water in Saveh because after 9 days, there was still 0.5 mg/L of residual free chlorine in water. The response of the company that produced drinking water about the high content of chlorine in treated water during 9 days was low organic matter and total dissolved solids in water. So, it was necessary to do more study in future to prove this claim.

Footnotes

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Conflict of Interest:

The authors declared no conflict of interest.

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