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Evaluating efficiency of radio waves for microbial removal in water samples

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Original Article

Abstract
The most common used methods for water disinfection were chemicals like chlorine, ozonation, ultraviolet radiation, membrane processes, etc. Water disinfection using irradiation techniques is new in water treatment industry which has been developed recently. The aim of the present study was to investigate radio frequency (RF) efficiency for the inactivation of total coliform (TC), fecal coliform, and heterotrophic bacterial count of water pellets. Tap water samples were taken from School of Public Health, Tehran University of Medical Sciences and irradiated using hydropad device, steam KLEAR model S-38 (1.2 W and frequency of 120-200 kHz). Microbial concentration was measured in cycles 1, 5, 10, 15, 20, 25, 30, 35, and 40 in 1 and 2 h contact time. Indicator bacteria were counted using plate count method and multiple fermentation tube technique. According to the microbial results, after 40 cycles and without chlorine residual, TC, fecal coliform, and heterotrophic bacteria were reduced by 86, 90, and 85%, while after 15 cycles and 0.8 mg/l chlorine residual, removal rate was 89, 91, and 89%, respectively. Furthermore, it was observed that after 2 h of contact time, TCs, fecal coliforms, and heterotrophic plate count were reduced by 78.2, 80, and 60%, respectively. Although RF efficiency in water disinfection has not been studied, our findings suggested its possible use due to more than 75% efficiency. From the standpoint of practical use, more studies should be done, especially to find a fine synergist agent, determining power, frequency, and suitable contact time and also the method should be modified.
KEYWORDS: Drinking Water, Radio Waves, Disinfection, Coliform

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Introduction

Disinfection is the most important and common step of water treatment process for ensuring the health of the community. Numerous chemicals have been used for water disinfection, the most famous and common of which is chlorine due to its high efficiency in bacterial removal. In addition, simple control of the process and the residual chlorine remaining in distribution systems is other factors that make its use popular.[1] After commencing drinking water disinfection with chlorine compounds in 1904, the occurrence of outbreaks associated with the consumption of contaminated water decreased.[2,3] On the other side, by using chlorine and formation of more toxic and harmful byproducts (carcinogens, mutagens) such as trihalomethanes and haloacetic acids and their adverse effects on aquatic systems and human health, some studies have addressed the

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relationship between long-term exposure to chlorine by products and risk of carcinogenic side effects.[4,5] Furthermore, considering the scientific experiences about microbial immunity against chemical disinfectants and human sensitivity about the health and environment, it is necessary to find some alternative disinfectants using nonchemical materials with complete pathogen removal.[6,7]

There is an old and well-known method for water purification using electromagnetic. Radio frequency (RF) is a kind of 300 kHz-300 GHz electromagnetic wave and unlike ionizing radiation; it does not have any ionization ability and molecular change in the cell structure. The amount of photon energy in RF is not strong enough to make the ionizing operations. This energy may provoke vibration of large molecules or may cause polarization of molecules and atoms. RF field affects the ions and molecules and may change the electrical flux distribution which finally can change their direction in space. RF waves can potentially disinfect and sterilize pest and various substances in food, agricultural, and environmental components due to its high penetrating power and increasing temperature.[8,9]

RF effects are divided into two categories of thermal and non-thermal. In the case of thermal effects, entry the radio waves into the water sample can destroy the bacteria by changing the energy of waves to heat. Nonthermal effects induce electromagnetic currents into the living tissue which can provoke vibration of large molecules and result in the polarization of molecules and atoms. Fields with lower frequency cause little energy absorption and less heat increase and the results are related to the induced current, while the fields with higher frequency are mostly in the heat form.[7,8] Studies have shown that weak RF fields, especially those with low frequencies, change the functional properties of the membrane structure and responses to cellular stimulation. These fields do not make any changes in membrane potential; however, they penetrate into the cell membrane and affect the structure and function of the cytoplasm.[10-12] In an electric field, a potential difference is formed across the membrane by opposite charges attracting each other on either side of the membrane; thus, the membrane becomes thinner. When intensity of the electric filed is sufficiently high, formation of pores in the membrane will eventually rupture the cell.[13,14]

RF efficiency in removal and inactivation of bacterial species, agricultural pests, etc., has been investigated in several studies. Kim et al. examined RF for the inactivation of Salmonella and Escherichia coli on red and black pepper. Results showed 29.4-80.2% decrease in Salmonella and E. coli in black pepper and 38.3% to more than five log decreases of pathogens in red pepper, respectively.[15] Previous studies have investigated the RF and microwave radiation efficiency for the inactivation of Listeria, E. coli and other microbial community in contaminated milk and biofilms. The results showed that, due to the retention time and power of RF, heating was powerful enough to inactivate bacteria.[16-18] The aim of this study was to determine RF efficiencies in the removal and inactivation of total coliform (TC), fecal coliform, and heterotrophic plate count (HPC) as microbial indicators of water samples. Different contact times and microbial load were also examined.

Materials and Methods

This experimental study investigated the efficiency of hydropad device in reducing the microbial load of water samples containing TCs, thermotolerant coliforms (TTC), and HPC. Providing RF, the hydropad device, steam KLEAR model S-38 made in England, was used with the dimensions of 3 × 7 × 11 cm, power of 1.2 W, and frequency of 120-200 kHz. Hydropad is an electronic device that releases an electric field induced in the piping system without the use of electrodes. System performance made the RF of 120-200 kHz by a four wing frequency transformer. This frequency was induced to the water by strontium ferrites around the tubes and the coil winding which made the first orbit and generated the sinuses voltage of 5-35 V with the wavelength of 1200-1600 m in the tube. In these experiments, two 70 L plastic tanks provided with taps on them were used. The tanks were joined together in series with iron tubes of 2 inch in diameter and 6 m in length. Hydropad device was placed between the two tanks and a pump named LONKLY SIRO (model no LSR 40-6s/180, F PN10 class), model F PN10, was used for water circulation. Figure 1 shows the used pilot with details.

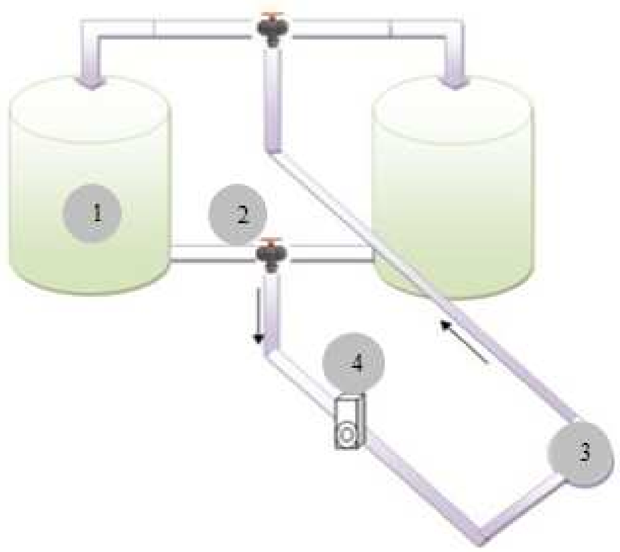

Figure 1. Schematic representation of disinfection pilot: (1) Tank, (2) three-way tap, (3) pump, (4) hydropad device

Water samples were collected from the tap water from School of Public Health. A particular amount of secondary treated, but not disinfected, wastewater from Zargandeh treatment plant, located in north of Tehran, Iran, was added to introduce microbial contamination to water samples and sodium thiosulfate was added to the tap water to neutralize residual chlorine before mixing the sample with wastewater. A proper sample was prepared after completely mixing water and wastewater using a pump. At the beginning of each set of the experiment, sampling was done to determine the initial bacterial count and the efficiency of bacterial removal was measured at the end of each experiment.

Bacterial enumeration was done according to the standard methods for water and wastewater examination (APHA 21ed). Briefly, multiple fermentation tube procedure was used for the TC count (section E9921), bacteria were incubated on lactose broth for 24 h. Fecal coliforms were analyzed by the direct (without enrichment) multiple fermentation tube technique (standard methods, 9221E-2). As mentioned by Nabizadeh et al., the samples were inoculated and incubated on A1-medium and their ability to produce gas was determined. For the enumeration of thermotolerant coliform bacteria, the samples were incubated at 37 °C for nearly 3 h and the tubes were then transferred to a 44.5 °C water bath for 19-21 h.[19] Production of turbidity and/or gas accumulation in the tubes constitutes a positive result. HPC with power plate method (section 9215) was used for the enumeration of heterotrophic bacteria on the R2A agar medium. All the materials were purchased from Merck Company, Germany. Total and fecal coliforms were reported in terms of most probable number at 100 ml and heterotrophic bacteria were reported in terms of CFU/ml. HPC was used for evaluating the microbiological quality of drinking water and controlling water treatment processes, in which different types of colonies of more than 500 were important. All the glassware used in the experiments were sterilized at 180 °C for 2 h.[20]

In these experiments, the water sample which was contaminated with municipal sewage effluent filled the tank number 1. In order to complete mixing of sewage effluent with water, the pump was started and the sample was circulated between the two tanks. Hydropad system was started and the sample circulated from tanks 1 to 2 in contact with the produced waves. The sample cycle from tanks 1 and 2 was named the first cycle and its return from tanks 2 to 1 was called the second cycle. In order to determine the efficiency of RF to inactivate different indicator organisms with different initial concentrations of TCs, TTCs, and HPC, 40 cycles and different sampling times were examined. There was no chlorine residual in all the samples, except for the last cycle, in which RF effect was evaluated beside chlorine residual between 0.5 mg/l and 0.8 mg/l as chlorine. In order to evaluate effect of contact time, only one of the tanks was operated and the effect of 1 and 2 h of contact time was studied.

To determine the initial bacterial load before starting the hydropad device, water sampling was done and TCs, TTCs, and HPC were measured as the base point or cycle zero. Then, hydropad was turned on to determine the efficiency of radio wave on disinfection of target microorganisms. The sample was circulated between two tanks by the pump in cycles 1, 5, 10, 15,…, 40 and the microbial tests were done on the samples once more.

In the second phase of the experiments, the amounts of TCs, TTCs, and HPC were reduced in comparison to the earlier tests and water was circulated in one of the tanks for 2 h, instead of circulating between two tanks. Finally, bacterial removal was investigated in the range of 0-2 h. The experiments were conducted in six phases with different initial microbial loads.

Results and Discussion

Results related to the effect of radio waves on water disinfection using the hydropad device at different microbial concentrations and cycles are presented in table 1. As can be observed, when the initial bacterial concentration was high (HPC = 41000-95000, TTCs = 15000-33000 and TCs = 22000-50000), removal efficiency was very low between 5.2 and 9%, while in lower microbial load (HPC = 6200, TTCs = 2300 and TCs = 3500), the removal efficiency was 19.3, 41, and 28.6% in the 20th cycle and 86, 87, 90% in the 40th cycle. Thus, it is seen that increasing the number of cycles from 20 to 40 and reducing the initial bacterial concentration increased the efficiency of the disinfection process. The removal efficiency of pathogens at the initial concentration of HPC = 500, TTCs = 200, and TCs=300 and in the presence of residual chlorine after 10 cycles was 89, 91, and 89, respectively. Therefore, it is obvious that in the presence of both disinfecting agents, the microbial load of the samples was effectively reduced.

Table 1. Hydropad steam KLEAR S38 device results after each cycle

Cycle number	Bacteria name	Before disinfection	After disinfection	Log inactivation	Removal percent
20	TC	5.00E + 04	4.55E + 04	0.04	9.0
	TTC	3.30E + 04	2.90E + 04	0.06	12.0
	HPC	9.50E + 04	9.20E + 04	0.02	3.0
20	TC	2.20E + 04	2.00E + 04	0.04	9.0
	TTC	1.50E + 04	1.40E + 04	0.03	6.6
	HPC	4.10E + 04	4.00E + 04	0.01	2.5
20	TC	3.50E + 03	2.50E + 03	0.20	28.6
	TTC	2.30E + 03	1.35E + 03	0.20	41.3
	HPC	6.20E + 03	5.00E + 03	0.10	19.3
40	TC	3.50E + 03	4.90E + 02	0.85	86.0
	TTC	2.30E+ 03	2.20E + 02	1.02	9.0
	HPC	6.25E + 03	8.00E + 02	0.87	87.2
10	TC	3.00E + 02	3.30E+01	1.00	89.0
	TTC	2.00E + 02	1.80E + 01	1.11	91.0
	HPC	5.00E + 02	5.50E + 01	0.96	89.0

TC: Total coilforms, TTC: Thermotolerant coliforms, HPC: Heterotrophic plate count

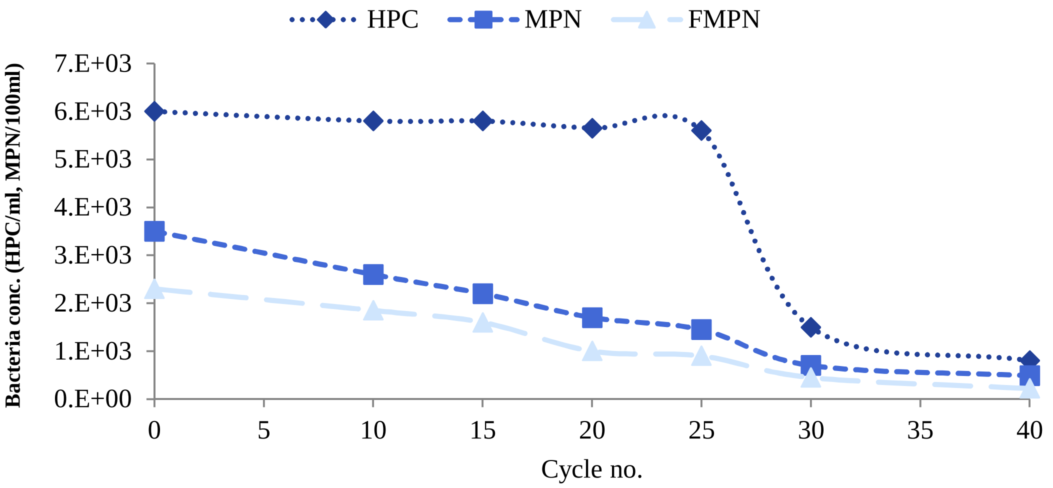

Figure 2. Total ciloforms, thermotolerant coliforms, and heterotrophic plate count removal trend after different cycles in absence of residual chlorine
HPC: Heterotrophic plate count; MPN: Most probable number; FMPN: fecal coliforms most probable number

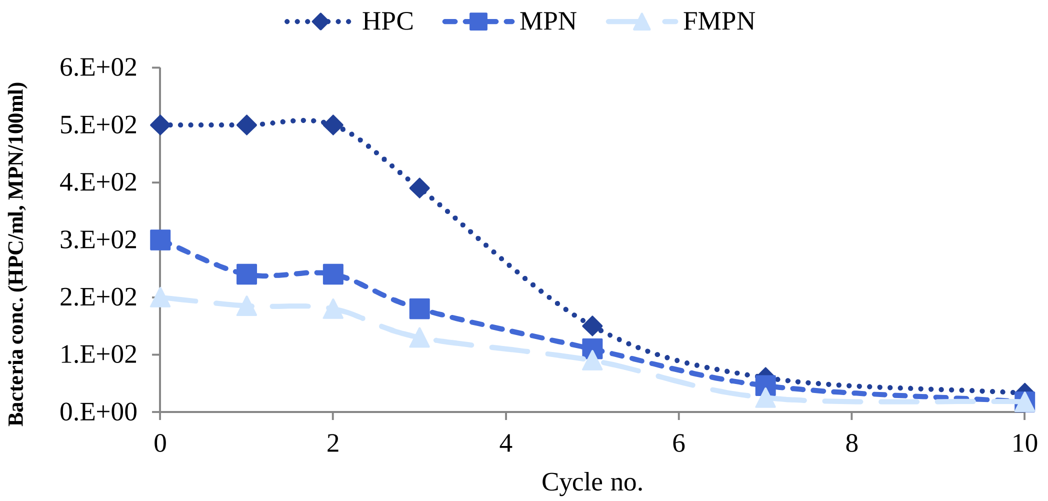

Figure 3. Total ciloforms, thermotolerant coliforms, and heterotrophic plate count removal trend after different cycles in the presence of residual chlorine
HPC: Heterotrophic plate count; MPN: Most probable number; FMPN: fecal coliforms most probable number

Figure 2 shows the efficiency of radio waves at the initial microbial concentrations during 40 cycles and in the absence of residual chlorine. As can be observed in the figure, the removal efficiency was constant until the 25th cycle, while it was highly increased by passing the cycles from 25 to 35.

Figure 3 shows the number of TCs, TTCs, and HPC after disinfection by radio waves and in the presence of residual chlorine over 10 cycles. Efficiency of removing pathogens at the initial concentration of HPC = 500, TTCs = 200, and TCs = 300 was constant until the third cycle and was effectively increased after the 7th cycle. Efficiency of disinfection improved when RF was used along with residual chlorine (Figures 2 and

3). From this point of view, the results were similar to those of Ukuku et al. which the twin method of RF and ultraviolet (UV) was applied for inactivation of E. coli in juice.[21] In the presence of both RF and UV, RF destroyed cell structure first and chlorine inactivated the cells with its expected mechanisms such as affecting nucleic acid, breathing system, and electron exchange system.[22] Thus, observing an increase in bacterial inactivation is expected with the presence of these two disinfecting agents. Simultaneous use of chlorine and RF is important because, in addition to improving RF effects, chlorine can provide a residual which may prevent secondary pollutions (as RF leaves no residual after disinfecting the samples, similar to UV). In addition, the application of these disinfectants can be discussed in pool water samples. As with a proper operation system, RF can be used in pool disinfection and chlorine can be useful with its residual. Indeed, the power needed for bacterial inactivation will reduce in the presence of chlorine; i.e., the power and retention time of RF can be reduced. In this case, the effective factors such as chlorine dose, RF power, thermal effects, pH, and the costs should be carefully studied.

Furthermore, efficiency of hydropad device is shown in table 2 during cycles 1-6 (at different microbial concentrations) and in 1 and 2 h of contact time. As shown by the results, at different microbial concentrations, there was no important change in removal efficiency by increasing the contact time from 1 to 2 h.

Results showed that at the frequency between 120 kHz and 200 kHz and flow intensity of 1.2 W/cm, microbial contamination of the samples was reduced by 50-80%. However, this frequency and the related power were unable to reduce total and fecal coliforms to zero per 100 mL and HPC to 250 CFU/mL in drinking water (standard of Iranian Environmental Protection Organization) (Tables 1 and 2). Therefore, more studies are needed to modify the system and improve the efficiencies for using in disinfection process. On the other side, in these systems, efficiency of disinfection is related to the used power and wave frequency. Some studies have reported that the capacity of bacterial inactivation by RF is related to the field intensity used in the system.[13,14]

Table 2. Hydropad steam KLEAR S38 (Hydropad Co. UK)device results after 1 and 2 h contact time

Cycle number	Bacteria name	Before disinfection	After disinfection		Log inactivation		removal percent	
			1 h	2 h	1 h	2 h	1 h	2 h
1	TC	5.40E + 02	2.20E + 02	1.30E + 02	0.39	0.39	59.3	75.9
	TTC	4.00E + 02	1.75E + 02	1.75E + 02	0.36	0.36	56.0	56.0
	HPC	8.20E + 03	7.50E + 03	5.00E + 03	0.04	0.20	8.5	40.0
2	TC	5.00E + 02	2.30E + 02	1.70E + 02	0.34	0.47	54.0	66.0
	TTC	3.80E + 02	1.85E+ 02	1.20E+ 02	0.31	0.50	51.3	68.4
	HPC	8.10E+03	7.50E + 03	5.00E + 03	0.03	0.20	7.5	38.3
3	TC	2.40E + 03	4.90E + 02	4.90E + 02	0.69	0.69	79.6	79.6
	TTC	1.50E + 03	3.50E + 02	3.50E + 02	0.63	0.63	76.0	76.0
	HPC	5.00E + 03	2.00E + 03	2.00E + 03	0.40	0.40	60.0	60.0
4	TC	2.30E + 03	4.50E + 01	4.50E + 01	0.71	0.71	80.4	80.4
	TTC	1.50E + 02	3.00E + 01	3.00E + 01	0.69	0.79	80.0	80.0
	HPC	5.00E + 03	1.90E + 03	1.90E + 03	0.40	0.50	62.0	62.0
5	TC	7.80E + 01	2.00E + 01	1.70E + 01	0.59	0.66	47.4	78.2
	TTC	5.00E + 01	1.40E + 01	1.00E + 01	0.55	0.69	72.0	80.0
	HPC	1.50E + 03	6.50E + 02	4.80E + 02	0.36	0.50	56.6	68.0
6	TC	2.30E + 01	1.30E + 01	5.00E + 00	0.25	0.66	43.5	78.3
	TTC	1.50E + 01	8.00E + 00	3.00E + 00	0.27	0.69	46.6	80.0
	HPC	2.50E + 03	1.00E + 03	1.00E + 03	0.40	0.40	60.0	60.0

TC: Total ciloforms, TTC: Thermotolerant coliforms, HPC: Heterotrophic plate count

In fact, these two factors are the main reasons for increasing temperature in the environment where exposure to RF is occurred,[16] and the high temperature is the main reason of inactivation by RF, which is called thermal effect. Ukuku et al. showed that by increasing temperature from 25 to 40°C, E. coli survival was reduced by more than 1.5 log.[21] Awuah et al. studied RF efficiency for the E. coli removal in milk, and declared that the main reason of bacterial inactivation by RF was temperature increase by radio waves. It was also mentioned that using RF depended on its contact time and radiation power and the results showed that using RF was a suitable method for inactivating L. innocua and E. coli in milk for a short retention time i.e., 1 min, and 1200 W energy used 5-7 log inactivation was observed. Therefore, they concluded that it can be an effective method for milk pasteurization.[16] Besides thermal effects, it is believed that other mechanisms like nonthermal effects are effective in the efficiency of RF in biological systems. In fact, temperature is the only factor that might lead to cell proliferation damage. Changes which occur in cell proliferation from exposure to RF are not solely the result of heat production although high temperature can lead to such a kind of changes. In sum, it has been proven that temperature changes cannot lead to biological changes in the normal range of RF.[21] In the present study, RF in the used range made no changes in the solution temperature; hence, it seems that the microbial changes were related to nonthermal effects of RF. Velizarov et al.[23] reported that stress induction was one of the most important non-thermal effects of RF and such an effect can increase the release of stress proteins like heat-shock proteins (HSP), which are not produced only by temperature increase. It has been proven that biological cells in exposure to stresses like hyperthermia, chemical substances, etc., would excrete the HSPs. One of the factors which cause increasing such proteins is electromagnetic flow with low frequency (such as RF). In the case of inactivation increase over time (Figures 2 and 3), the present findings were in good agreement with Velizarov et al., since they stated that longer contact times did not improve the removal efficiency owing to the improved cell adaptation to the new condition.[23]

As shown in table 2, the removal efficiency of TCs, TTCs, and HPC with contact time did not increase significantly. Although in the Velizarov et al. study, longer contact time was ranged from several hours to some days; it seems that increasing contact time from 1 to 2 h was enough for cell adaptation.[23]

For RF application in water and wastewater disinfection, it should be considered that no similar mechanism such as decreasing microbial load in milk and other food product is expected. For example, in studies related to E. coli and Listeria inactivation in milk, there are two factors which distinct the findings from water and wastewater samples; first, by irradiation with RF, as mentioned above, the main reason of bacterial inactivation in the milk samples is related to heating mechanisms and the second is that even the RF energy and frequency used for water samples would be the same with the amounts used for milk samples due to the fact that milk samples on average are heated 16% faster than water samples; this issue can be attributed to milk proteins, minerals, and vitamins. It is not possible to claim the same effects in both samples. It has been claimed that milk warming rate has a linear relationship with RF energy.[16] In water samples, there is also no reason for extra heating and RF power to inactivate microorganisms with thermal mechanisms, because there are far better methods for heating up to pasteurization point both practically and operationally.

Conclusion

Although RF role for water and wastewater disinfection was not investigated completely, the results showed some possibilities for its use in disinfection process. RF was able to eliminate some percentage of the microorganisms in this study; but, it was not sufficient for reaching the environmental standards. Hence, more studies should be done for determining the power, frequency, and suitable contact time and also the method should be modified.

Conflict of Interests

Authors have no conflict of interests.

Acknowledgements

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