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Original Article

Disinfection of Medical Solid Waste through the Application Ozone Technology: Feasibility and Effectiveness Study

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Abstract

Background: We aimed to determine the feasibility of ozone for disinfection of infectious solid waste in hospital.
Methods: Spores of *Bacillus atrophaeus* were used to monitor the process of inactivating microbial agents using ozone in medical solid waste in the hospitals of Tabriz City, Iran. For this purpose, culture medium containing the mentioned bacteria was placed in the bags containing medical wastes. The ozone generator was equipped with a constant dose of 5 grams per liter, with a discharge of 1 and 3 liters per minute and contact time of 10 to 120 min. Then the ozone exposure indicators were incubated for 24-48h at 36 ± 1 °C and, finally, the absence of colony growth in the culture medium was considered as the success of ozone in disinfection of infectious solid waste. This process was performed with 4-time replications.
Results: The complete removal of *B. atrophaeus* was obtained for non-compacted and compacted infectious solid waste, at contact time of 15 and 50 min, respectively. The efficiency of removal of *B. atrophaeus* by the process of wet ozone injection through a glass column was 100% in 30 minutes and by separate injection of water vapor into the contact tank was 100% in 50 minutes. The results of this study showed that the use of ozone technology was effective in the inactivation and destruction of microbial agents in medical solid waste.
Conclusion: Employing different advanced technology of oxidization especially ozone in order to decrease the environmental pollution is considered as one of management approaches.

Keywords: Infectious waste; Medical solid waste; Ozone; Disinfection; *Bacillus atrophaeus*

Introduction

The production of infectious waste in medical centers, including hospitals and clinics are considered as a serious hygienic and environmental problem (1, 2). Management of medical wastes is considered as one the main challenges especially in developing countries. Because, inefficient management of infectious wastes can effect on environmental health and leads to environmental pollution such as pollution of water sources, soil, air. On the other hand, improper methods of medical waste disposal resulted in substantial risks and dangers for human health such as transmission of hepatitis b and c, AIDS, etc. (3, 4). Therefore, proper waste management at the

production site can create a safe and healthy environment (5, 6).
In medical solid wastes management, there are various methods such as incineration, chemical disinfection, wet thermal operation, dry thermal operation, using the waves of microwave, etc. Although mentioned methods can reduce the risk of infectious wastes considerably, they can cause some other environmental problems as spreading of some toxic and harmful substances such as dioxin, outbursts, and mercury in the air. According to the latest published methods, the best way to disinfect hospital waste is to use non-incineration technology at the waste generation site (7-9). Regarding the use of an autoclave, since the use of shredders is necessary (in some countries) and sometimes they do not work well, which interrupts the process (10). Chemical disinfection is not able to sterilize completely waste. One of the disadvantages of this method is the need to crush the waste. Moreover, there is a need for stronger disinfection, which, in addition to environmental threats, can be hazardous in use for the user's skin and mucous tissue (11). Therefore, it is very important to use modern and standardized methods to disinfect waste in hospitals (12).
One of the advanced technologies in dealing with disinfection is the use of ozone. Ozone is used in order to disinfection in medical centers and clinics (13). The general reaction of ozone might be due to the direct reaction of O_3 molecule with several other elements in wastes. Besides, it might be because of decomposition of ozone to reactive hydroxyl radicals. Ozone disinfection mechanism occurs in two ways: 1) Ozone causes sulfhydryl group, amino acids, enzymes, peptide, and proteins to oxidize. 2) Ozone reacts with unsaturated lipid with dual link, which leads to leakage in cells and eventually to shifty microbes. In addition, ozone is a strong disinfectant which can eliminate substances such as cyanide, aromatic compounds, and aliphatic. Generally, strong oxidants are able to attack the chemical compounds of microorganisms i.e. proteins, unsaturated fat, lipopolysaccharides (LPS) membrane of bacteria, nucleic acids, intracellular enzymes, proteins, peptides in spore, and virus cache (14).
Recently, ozone has been introduced as technology that is compatible with environment for the refinery of hospital wastes (5). Ozone is a safe, fast and inexpensive replacement with other sterilizers in low temperature. It is used for sterilizing medical tools (15, 16). In 2003, a sterilization Canadian company uses ozone as a sterilizer (17). Dick Zoutman and et al. conducted a research in which 80 ppm ozone with 1% hydrogen peroxide steam, which is a very strong disinfector, is obtained by reducing 6 Logarithm on pathogen bacteria and *Bacillus subtilis* spores and *Clostridium Difficile* in 30 to 90 min for surfaces and air (18).
However, based on conducted studies, there are few studies related to effect of ozone on disinfection of hospital wastes. This study aimed to investigate the feasibility and effectiveness of using ozone technology for disinfection of medical solid waste. Therefore, the results of this study can be helpful in technical feasibility and the use of ozone in disinfection of infectious wastes.

Materials and Methods

An ozonation pilot system was used separately for disinfection of dry, wet and sharp infectious waste in Tabriz hospitals, Iran. For this purpose, ozone gas was used as a disinfectant. *B. atrophous* was used as a biological indicator to measure the efficiency of this disinfection process in removing various microbial forms. The ozone gas dose was varied between 1 to 3 liter per minute and the contact time was altered between 10 to 120 min. Pure oxygen was used as the feed gas for the ozone generator produced by oxygen maker machine (New Life Elite I Type CFS-concentrator, Airsep Corporation company, US).

Pilot setup

The configuration pilot setup for dry and wet gas injection is shown in Fig. 1. In order to check the effectiveness of ozone in removing microbial agents in infectious wastes, Tryptic Soy Agar (TSA) culture was first sterilized using an autoclave under 15 PSI (pound per square inch) pressure and 121 degrees Celsius. Then, 6 gram of Peptone Broth (PB) were carefully weighed and poured into one-liter water. It was slowly stirred and then poured into sterile tubes and sterilized in an autoclave. After sterilization, it was placed in the refrigerator. Bands of *B. atrophaeus*, which is a biological indicator, were placed inside the peptone water culture and again placed inside the incubator for 24 hours to grow spores. In the third step, a sample was taken from peptone water containing indicator and it was put into TSA. The plates containing indicators were put into infectious wastes bags and sharp wastes safety boxes in the worst condition of ozone penetration. Finally, ozone was injected by the ozone generator device in fixed doses of 5 grams per liter and with a flow rate of one liter per minute.

In order to inject ozone in wet form to infectious wastes tank and safety box, ozone gas was injected into wet form to the tanks containing infectious wastes and safety boxes. Ozone gas was injected into a glass column containing water up to 1.20 height. Then the bubbles containing ozone gas were injected into infectious wastes and safety boxes by silicon hoses in different time duration 0f 10, 15, 30, 50, and 80,120. Next, after reaching he optimal duration, infectious wastes were put into the tank in dense and non-condense form, ozone with fixed dose of 5 gram per hour with flow rate one liter per minute and once more 3 liter per minute in two dry and wet form. The effect of ozone penetration was investigated. Then the culture containing spores affected by ozone were incubated for 24-48 h in 36±1 degrees Celsius. Finally, no growth of clones in the culture displayed the rate of disinfection of infectious and sharp wastes which were being investigated by wet and dry ozone (Figs. 1,2).

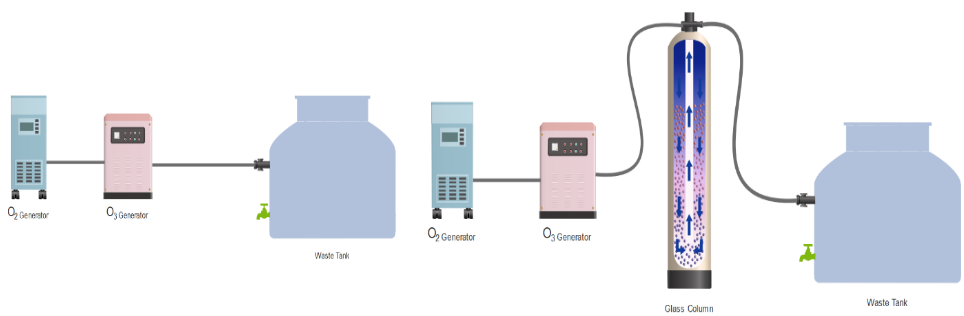
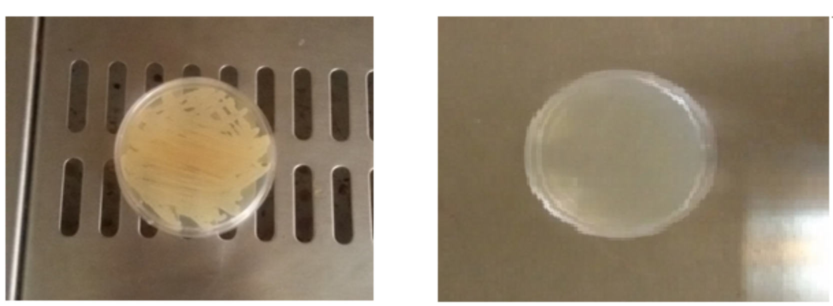


Fig. 1: The configuration pilot set up for dry and wet gas injection



a b

Fig. 2: *B. atrophaeus* media without any contact with ozone gas (a) and in contact with ozone gas (b)

Statistical methods

Excel software has been used to enter the data and extract the relevant graphs. Statistical analysis of data was performed using the SPSS v.23 package for Windows (IBM Corp., Armonk, NY, USA).

Besides utilizing descriptive analysis, K2 test was applied to determine and compare the efficiency of wet and dry ozonation in disinfection of hospital wastes and the efficiency differences of in different duration of time. Moreover, independent sample *t*-test was utilized to find out the relationship between the effect of volume and density and crushing of wastes in disinfection of infectious wastes.

Results

Dry ozone efficiency in bacteria deactivation

In Fig. 3a, in the first 10 to 15 min of the process, there was no efficiency elimination of bacteria in infectious and sharp wastes while after 30 min was eliminated *B. atrophaeaus*. By increasing ozone density from 1 liter to 3 liters per minute and after 15 min, the efficiency of elimination of *B. atrophaeaus* for both infectious and sharp wastes was 100%. By increasing the density from 1 to 3 L the efficiency elimination increased, which indicates the time duration for elimination of *B. atrophaeaus* was reduced.

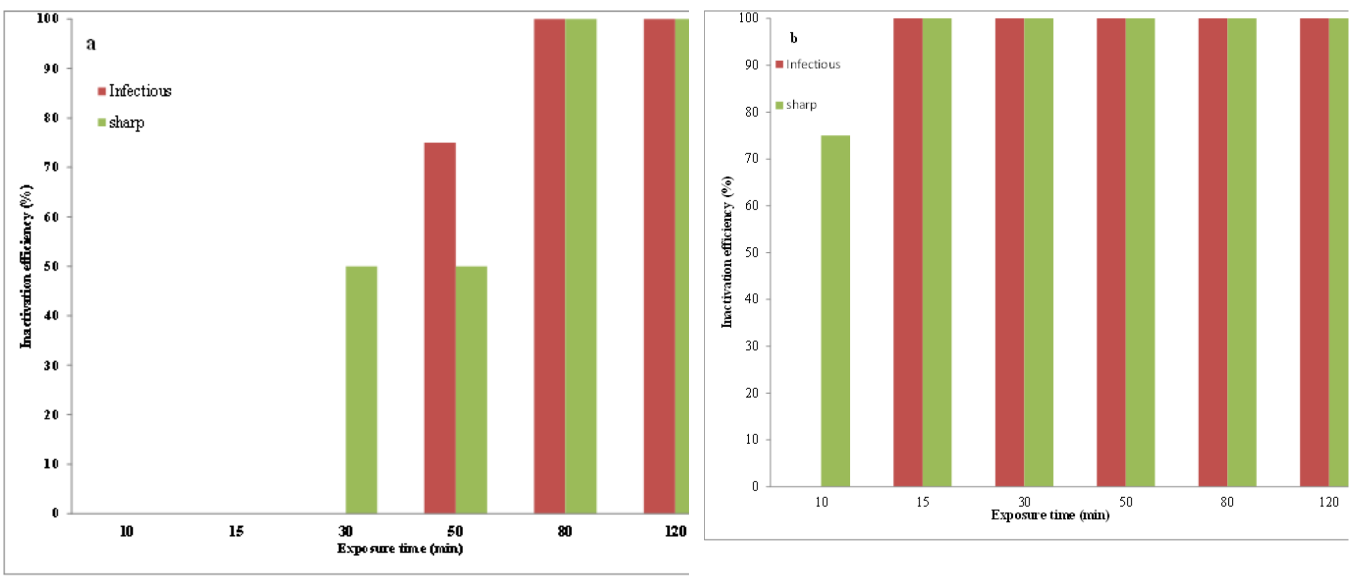
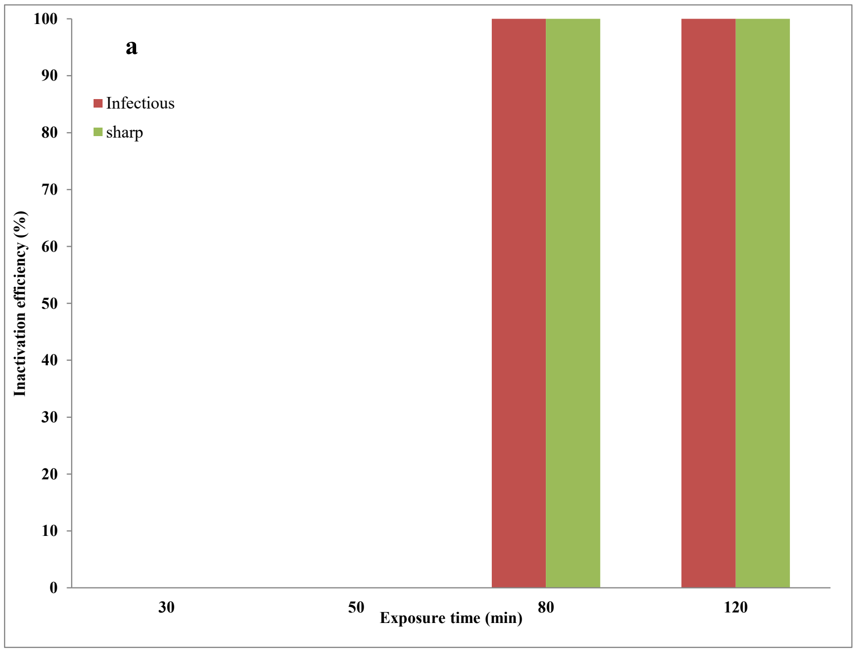


Fig. 3: Comparing the efficiency of dry ozone gas in bacteria inactivation at two ozone gas flowrates: a) 1 l/min b) 3 l/min

Effectiveness of dry ozone on disinfection of infectious and sharp solid waste

As Fig. 3 (a, b) and Fig.4 (a, b) illustrate, disinfection in non-condense from was done in 30 and 50 min and elimination occurred. In dense disinfection, ozonation with density of 1 liter per minute was conducted, but no elimination was observed. In addition, the time duration in dry ozonation with density of 3 liter per minute was 15 min, while in dense from the time duration was 50 min.

Crushing and mixing the solid waste can promote the effect of disinfection of ozone in infectious waste because crushing and mixing of solid waste increases the penetration and contact between ozone and solid waste (5). However, density of wastes is an influential factor in eliminating the Bacillus bacteria because the increase in density reduces the transfer of ozone to different layers of wastes and the contact between Bacillus bacteria and O_3 and the duration of contact for disinfection increases.



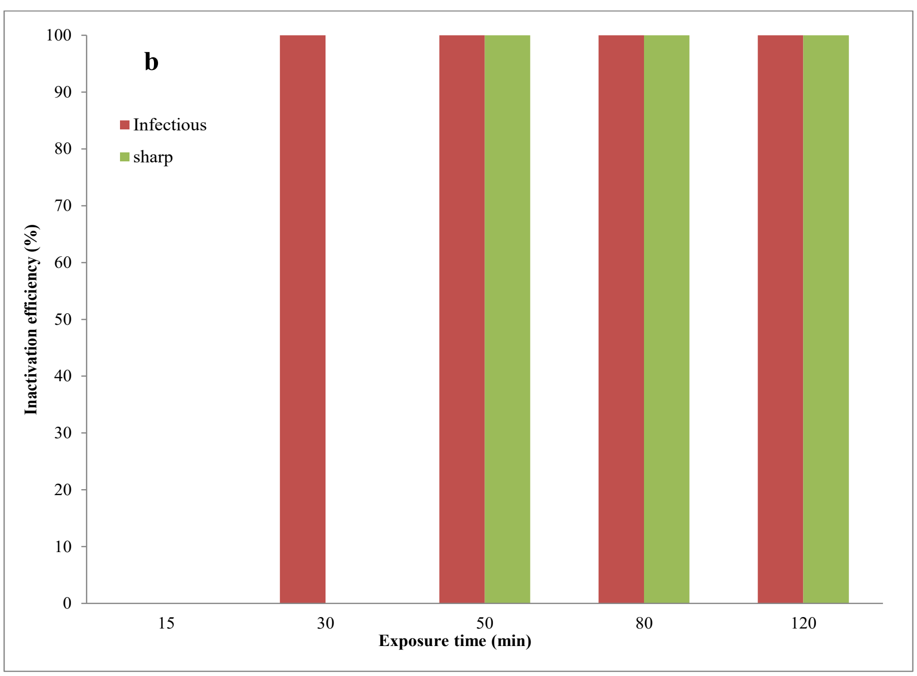
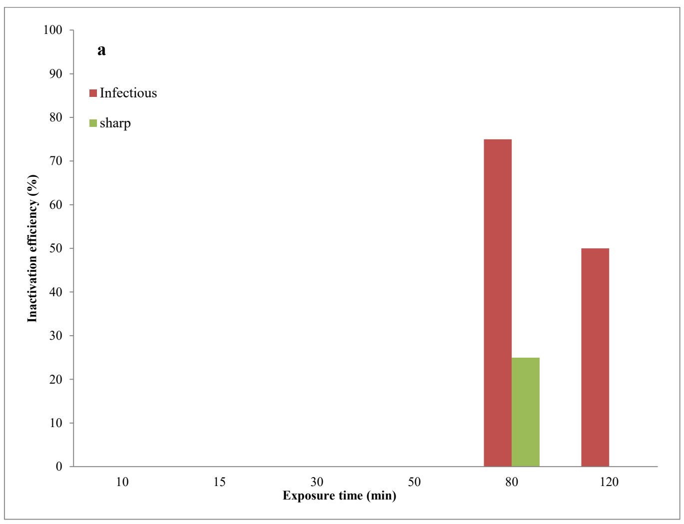


Fig. 4: Comparing the efficiency of dry ozone gas in bacteria inactivation for compacted waste at two ozone gas flowrates: a) 1 l/min b) 3 l/min.

Effectiveness humid ozone gas on disinfection of infectious and sharp solid waste

Figures 5 and 6 display the ozonation in two different conditions (glass column and tank) for disinfection of infectious and sharp wastes. In wet ozonation with glass column with one-liter volumetric flow rate per minute the amount of bacillus bacteria elimination with 80 and 120 min time duration increased. After 10, 15, 30, and 50 min of ozonation, live bacteria was observed. However, the process of O_3 led to the elimination of indicator bacteria in which by the increase of ozone dose for, 1 liter to 3 l/min in 30 min the efficiency of elimination increased up to 100%. Moreover, the efficiency of optimal elimination by wet ozone in deactivation of bacteria in separated tank in 50 min indicated that in comparison with glass column in order to deactivate *B. atrophaeaus* more time duration and more ozone dose are needed in equal circumstances.



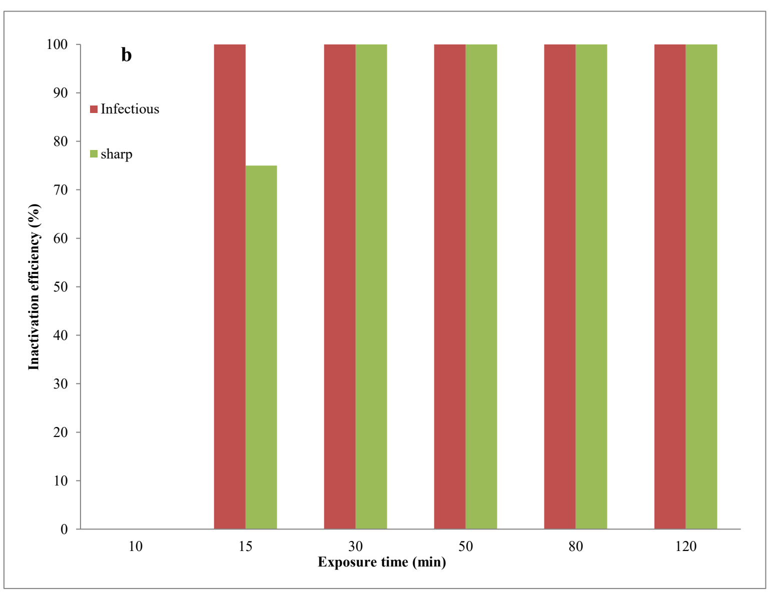


Fig. 5: Determining the efficiency of bacteria inactivation by humidified ozone gas at two ozone gas flowrates: a) 1 l/min b) 3 l/min

Comparing the efficiency of humid and dry ozone

The efficiency of optimal elimination in wet condition (glass column Fig. 5b) with density of 3 liters per minute for infectious wastes is 15 min whereas in dry and non-condensed condition (Fig. 3b) the efficiency of elimination of Bacillus in both infectious and sharp wastes is 15 min, but optimal disinfection in wet ozone in separated tank is roughly 50 min.

Table 1: The effects of ozonation parameters on the infectious and sharp solid waste disinfection

Variables		*number (percent)*		*P-value*
		no growth	**growth**	
Time (min)	10	(3.7)7	33(27.7)	< 0.001
	15	(7.9)15	(27.7)33	
	30	(14.7)28	(23.5)28	
	50	(18.4)35	(14.3)17	
	80	(28.4)54	(1.7)2	
	120	(26.8)51	(5)6	
Solid waste	infectious	(52.6)100	(47.9)57	0.418
	sharp	(47.4)90	(52.1)62	
Ozone gas flow rate (l.min)	1	(24.2)46	(66.4)79	<0.001
	3	(75.8)144	(33.6)40	
Waste form	compacted	(23.2)44	(23.5)28	0.940
	non-compacted	(76.8)146	(76.5)91	
Ozone gas humidity	dry	(56.8)108	(47.1)56	0.011
	wet bubble column	(25.3)48	(41.2)49	
	vapor injection	(17.9)34	(11.8)14	

As Table 1 displays there is a significant relationship between growth and not-grown bacteria, in a way that by passing time in 80 min by 28.4% efficiency there is the highest elimination rate (P<0.001). According to chi2, there is a significant relationship between dry ozonation and wet ozonation (P<0.001). There is no significant difference infectious and sharp wastes (P=0.418) and dense and non-condense wastes (P=0.940). Duration of contact and density of ozone are the most important factor in elimination of bacteria.

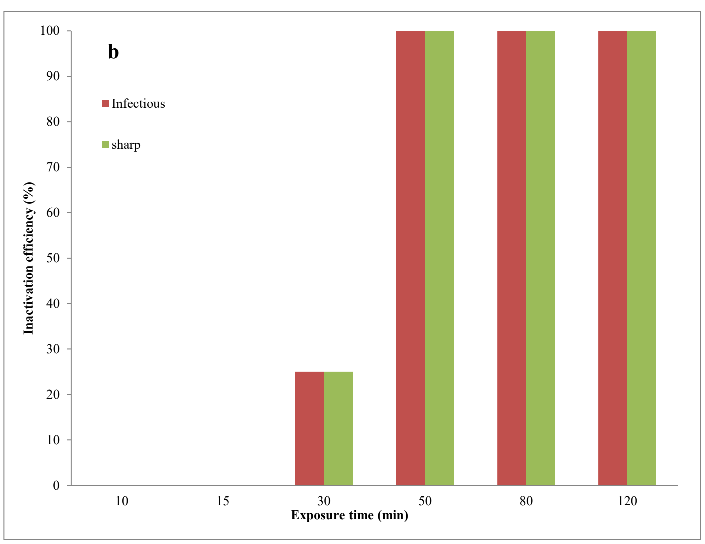


Fig. 6: The effect of wet ozone process (injection of water vapor into the contact tank) on deactivation of biological indicator with a flow rate 3 l/min

Discussion

Dry ozone efficiency in bacteria deactivation

By increasing the density from 1 to 3 liter the efficiency elimination increased, which indicates the time duration for elimination of bacteria was reduced and the contact rate between ozone and *B. atrophaeaus* was increased. The findings of this study are in line with the findings that found by using ozonation system and ozone/peroxide hydrogen for refining the wastes in landfills (19).

Coronel et al. studied the effect of ozone on disinfection of infectious hospital wastes with the density of 40 mg /l. They managed to decrease the *Staphylococcus aureus, Pseudomonas aeruginosa, Aspergillus niger fungus, Candida albicans* to 105 cfu/g (20). The damages to lipopolysaccharides layers of gram-negative bacteria was more outstanding than lipopolysaccharides layers of gram-positive bacteria (21). MURATA, ozone was used for the disinfection of medical waste of Kyushu-university hospital by BOX-O_3 apparatus. Ability of the BOX-O_3 apparatus to disinfect contaminated medical and laboratory waste that included a variety of bacterial strains (22). Ozone gas able to penetrate the cell and their reactions such as proteins, enzymes involved in controlling DNA structure and as a result causing false folding in DNA. Bacteria spores are the most resistant part of microorganisms and the most important for evaluation of sterilization process. Bacteria spores are able to resist to inconvenient conditions such as heat, sun shine, chemicals and dryness. Bacillus bacteria spores are resistant to ozone (14). The ozone gas process is effective in inactivating bacteria and their spores in water such *as B. subtilis* and *B. globigii*. When density of ozone gas rises, penetration and destruction of spores increases (23).

In the present study, the effectiveness of ozonation on *B. atrophaeus* in dense and dry form was investigated (Fig. 3, and 4). As Fig. 3a, and Fig. 4a displays ozone with the dose of one liter per minute between 30 to 80 min has no effect on eliminating the microbial factor, but in 80 to 120 min was able to eliminate *B. atrophaeus* totally which is due to duration and increase of the time of contact. Fig. 3b, and Fig. 4b shows that ozonation with density of 3 liter per minute can eliminate *B. atrophaeus* in infectious wastes at 30 min. By 50 min, the bacterial factor was eliminated from both types of wastes (infectious, sharp). Most of deactivation occurred in 50 min and the least deactivation happened in 10 min which the difference between them is statistically significant ($P<0.001$). Therefore, the contact time with ozone plays a more effective role in killing bacteria. These findings are in line with other researchers' findings about the length of contact time and elimination of bacteria. The rate of inoculation of *Sallemona bacteria* with 30 min contact time with density 2.8 and 5.3 mg/l reduced from 5.8 log CFU to 0.2 and 0.6, respectively. The antimicrobial activity of ozone against spores of bacillus after 10 and 30 min can lead to a reduction of 90.15% and 98.74%, respectively. The deactivation of *B. atrophaeus* was reported up to 99.9% after being exposed to 10 mg/l in 10 min (24, 25).

Comparing the efficiency of humid and dry ozone

The best condition for deactivating bacillus bacteria is using dry non-condensed system because the main influential factor on efficiency of high penetration of O_3 among wastes is dry wastes. The results of the present study indicated that the efficiency of gas ozone in deactivating bacteria is better than wet ozonation. The use of ozone as a germicide by raising humidity is increased which can be due to more reaction of ozone radicals with steam of water. It makes ozone to have more efficiency and is a reliable factor for disinfection of surfaces. The effect of germicide of ozone depends on microorganism characteristics, density of ozone, time duration, and surface of material (26). Two conditions for relative humidity 88% to 90% and 55% to 60% were considered in mentioned study. The effect of relative humidity for the left microorganism (*Escherichia* and *B. subtilis*) in humidity of 85% was less than 50%.

This study confirms that the rate of deactivation of microorganisms in reaction with ozone in relatively high humidity can be more due to production of more free radical, which can react more, with steam of water. Moreover, the study indicates that ozone is a safe and reliable factor for refinery and disinfection of superficial pollution of microorganisms (26).

Furthermore, in another study, relatively high humidity for deactivation of microorganisms by ozone is needed and optimal humidity is about 90% to 95 % (27). The increase in humidity leads to the increase in deactivation by ozone. The same effect was observed in barely seeds for deactivation fungus. In the similar study, the increase in the temperature of water and the increase of heat from 10 degrees to 40 degrees contributes to deactivation of most of the spores of fungus. In order to help increase the deactivation of spores of bacteria the relative humidity can increase (28). The presence of humid in spores for the speeding up the penetration of ozone is necessary, or it is necessary for ozone to react with superficial structure of spores. Currier et al. found out that *B. globiggi* spores with relative humidity of 80%-85% with density of 18 g/l could decrease significantly. They used cluster spores in their study in which a significant difference was observed in high humidity. The researchers of the study suggested that subtle circumstance among spores higher than 80% must be created. The water at this space creates a volume, which is resistant against transfer of gas. Therefore, by the increase of humidity the rate of death reduces. Researchers believe that if spores are not clustered, the space between spores can be so big that can create capillary density. As a result, increasing the relative humidity may not have any effect on transfer (29).

Pascual et al. studied the duration for the function of ozone gas is between 1 to 4 h in which the confrontation is 1 to 10 min for wet ozone. Moreover, germicide is increased by relatively high humidity, which occurs because of reaction between free radicals of ozone with steam of water (27).

Based on the findings of the present study, duration of contact and density of ozone are the most important factor in elimination of bacteria. Cesar et al. obtained the same results in an investigation of clearing the polluted dentistry tools to *B. atrophaeus* spores and reached a significant relationship between contact time and efficiency of elimination (P<0.05) (24). However, there is not sufficient information about the rate deactivation of spores, different levels of ozone density, kind of surface, and physical effect of ozone on different materials and more research is need to be done.

Conclusion

Employing different advanced technology of oxidization especially ozone in order to decrease the environmental pollution is considered as one of management approaches. One way in which this kind of technology can be applied is in dealing with hospital wastes. Ozone technology can be used as an effective and helpful method for disinfection of infectious waste. By considering the challenges, it is of utmost importance to pay sufficient attention to it. The consequences of microbial pollution contribute to better management and control of medical solid wastes. This study was an attempt to employ the advanced oxidation technology to step forward a clean and without any pathogenic factors.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflicts of interest

There is no conflict of interest for the authors.

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