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OZONIZATION OF WATER, RETENTION OF OZONE AND DEVITALIZATION OF *ESCHERICHIA COLI* IN WATER BY OZONE

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ABSTRACT

The aim of this study was to observe the efficiency of ozone transferred by an airstone bubble diffuser, using two ozone generators with different output of ozone (5 g.h^{-1} – G1; 15 g.h^{-1} – G2). The retention of ozone in ozonised distilled and potable water and the devitalisation effects on *E. coli* in the water were also noted. Ozone was introduced to two types of potable water of different composition intended for mass consumption, (MC)^{a} and (MC)^{b}, distilled water, and well water intended for individual consumption. The devitalisation effect of ozone on *E. coli* in well water (WW) and added to potable and distilled water was observed. The results of our study showed that under the conditions used, the level of ozone during ozonisation with G1 increased more rapidly in distilled water and after termination of ozonisation, the retention of ozone in distilled water was a little lower in comparison with the potable water. The devitalisation of *E. coli* either naturally present in the water or added to it required the level of ozone close to or equal to 0.25 mg.l^{-1}.

Key words: *E. coli*; ozone; ozone retention; ozonised water

INTRODUCTION

Ozone, an allotropic form of oxygen, is an inorganic gas (CAS n. 10028-15-6) constituted by three oxygen atoms (O_3) arranged in a bent structure, where the distance among oxygen atoms is 1.26 Å. It easily decomposes into oxygen (O_2) and one single, very reactive oxygen atom. Ozone is present in nature and its concentration in the atmosphere is approximately 0.04 parts per million (ppm: 1 ppm ~ 2 mg.m^{-3}). The solubility of ozone in water is 49.0 ml.100^{-1} ml (at 0 °C), tenfold than oxygen, thus causing an immediate reaction with any biomolecule in biological fluids. Its density (2.14 kg.m^{-3}) is higher than that of air, letting it concentrate close to the ground in indoor environments. Being a powerful oxidant, it is able to react with organic molecules containing double or triple bonds. This is the origin of its bactericidal, virucidal, and fungi-

cidal actions, which are used in water treatment, odour control and medicinal applications [10].

The applications of ozone are wide, varied and still expanding. They include disinfection of drinking water, groundwater, waste water, air purification, disinfection of surfaces, medical applications (dentistry, disinfection of external wound, possible treatment of diseases), grain and feed remediation [32], disinfection of stored foods, disinfection in dairy industry (milking equipment, udder, rear legs) and others. [15].

Ozone therapy has been utilized and extensively studied for many decades. Its effects are proven, consistent and with minimal side effects. Used to treat infections, wounds and multiple diseases, O_3's effectiveness has been well-documented. Ozone has been known to be used to treat as many as 114 different diseases [4].

The inhibitory and lethal effects of ozone on pathogenic microorganisms have been observed since the 19th century, and the most cited explanation of these effects is based on the disruption of their envelopes through peroxidation of phospholipids and the interaction with proteins [31]. As mentioned by Hudson et al. [8], the gas state allows ozone to get to areas that are difficult to reach and to disinfect much more than just surfaces.

Ozone has a broad antimicrobial spectrum and each microorganism species has inherent sensitivity to the gas [3]. Present at low concentration levels, dissolved O_3 is effective at destroying a wide variety of pathogenic bacteria including *E. coli*, salmonella, *Legionella pneumophila*, *Staphylococcus* spp., *Streptococcus faecalis* and *Mycobacterium tuberculosis*. However, contact time varies from one bacteria to another; *Mycobacterium tuberculosis* requires a contact time six times as long as *E. coli* in order to achieve the same level of microorganisms inactivation. Gram positive bacteria and spore forming bacteria have been found to be more resistant but are still sensitive to the disinfectant effect of O_3 [25].

Inactivation of viruses by ozone is the result of reactions between this oxidant and the biomolecules constituting the essential structures, external and internal, of the target organism. Other oxidant species generated through ozone reactions, such as hydroxyl radical and singlet oxygen may also contribute to some extent to the inactivation of viruses during this process [19]. Ozone can react with the nucleic acids of the viral genome, following molecular diffusion through the external structures towards the nucleic material. The external structures are the capsid proteins and, only for some species, the proteins and lipids of the viral envelope, such as in the case of SARS-CoV-2.

Although the existing scientific literature supports the effectiveness of ozone in the inactivation of viruses, there are very few studies about it on the SARS virus and still not a single study about its efficiency of inactivation of SARS-CoV-2. Therefore, in the absence of scientific literature, it is possible to assume that ozone is equally effective in inactivating SARS-CoV-2, however specific studies must be conducted to know also the ozone dose and effective exposure times [2].

Ozone is also effective against protozoan cysts present in water, unlike a number of other competing disinfectants. O_3 appears to more easily penetrate through protozoal cyst walls compared to other disinfectants such as chlorine. Some protozoal cysts are more resistant than others; for example, *Cryptosporidium* oocysts have been found to be ten times more resistant than *Giardia* cysts to ozonisation [16, 17, 25].

O_3 is highly effective at destroying a wide range of pathogenic organisms but the concentration of O_3 required to deactivate microorganisms will differ depending on the particular microorganism in question. In all case, a critical concentration threshold value of O_3 must be reached [25].

Ozone generators are currently being promoted as effective tools for removing pollution and odours from indoor air, but it must be remembered that ozone is associated not only with adverse health effects but also with safety issues. Ozone generators are high voltage electric machines, with all the safety implications involved. Moreover, the corona effect, whichever the frequency of the applied current, produce a broad spectrum of radio frequencies depending on many different parameters [13]. The European Chemical Agency (ECHA) safety guidance forbids the entire room where the ozone generator is deployed to pacemaker bearers. The actual extension of the area where the electromagnetic field exceeds the reference levels set for the protection of general public and workers should be addressed in the risk assessment.

Human activities in industry and agriculture have been scaling up in order to meet the needs of a growing population. Such activities are having drastic consequences on the environment, such as water pollution. The main sources of water pollution include domestic and industrial sewage and animal waste produced in agriculture. An important

strategy that has been put in place throughout the EU in order to protect water sources against contamination is the use of Zones of Hygiene Protection (ZHPs) [20].

However, protecting water sources alone does not ensure that these sources are free from contamination. The quality of drinking water must be monitored regularly. Establishing the microbiological content of drinking water is essential in determining the presence of waterborne bacteria, viruses, protozoa and helminths that may have the potential to cause disease, in particular Gastro-Intestinal Track (GIT) diseases [30].

However, the detection of all of the potential waterborne microorganisms is expensive and time consuming. Therefore, routine microbiological tests should involve the detection of the indicator bacteria, total coliform bacteria and *E. coli*, being the most common to assess the quality of drinking water [29].

All drinking water intended for mass domestic water supply undergoes treatment and disinfection. The most commonly used large scale strategies for drinking water disinfection include use of chlorine, UV radiation, and ozone. Each of them has some advantages and disadvantages.

Chlorine is highly effective at inactivating some of the most significant microorganisms present in water sources. However, chlorinated water contains higher concentrations of by-products such as chloroform and trihalomethanes (THMs) which have carcinogenic properties [25]. The advantage of UV radiation is that there are no remaining chemical residues and no production of toxic by-products, however, there is no residual effect and some previously inactivated microorganisms may recover due to photo-reactivation.

Ozone is more effective than chlorine in destroying viruses and bacteria. This method has many advantages: the ozonisation process utilizes a short contact time (approximately 10 to 30 minutes); there are no harmful residuals because ozone decomposes rapidly. Ozone is generated onsite, and thus, there are fewer safety problems associated with shipping and handling. However, low dosage may not effectively inactivate some viruses, spores and cysts; ozonisation is a more complex technology; and it has short-lasting residual effects and suspended solids significantly reduce viral inactivation by ozone [26].

Generation of O_3 for water or air treatment takes place in a number of steps. First of all a source of O_2 is required. This O_2 must then be fed into an O_3 generator to be converted into O_3 and O_3 must be transferred to the intended gas or aqueous environment. The final step may involve the destruction of any unreacted O_3 via an off-gas destruction system or alternatively recycling of O_3 back into the system [15]. O_3 gas transfer into water poses more of a challenge than transfer into air. The most widely used and efficient method of O_3, the bubble diffusion, involves the injection of O_3 gas into the water through porous diffusers which create small rising bubbles of O_3 within the water. O_3 that comes into contact with the water dissolves and reacts with organic and inorganic compounds within the water, as well as with microorganisms. The efficiency of ozonisation is related to the size of the diffuser pores, as well as the height of water vessel used (effect on contact time).

Once ozone enters into water, it becomes highly unstable and rapidly decomposes through a complex series of reactions [7, 24].

Contact time (CT) is crucial to the effectiveness of ozonisation as predicted by Chick's Law. For example, the standard CT value used in the bottled water industry is 1.6 $mg.l^{-1}$ min. CT value should increase with increasing pH [25].

Ozonised water is defined as the water obtained after the ozonisation (a process of infusing water with ozone) of water. During the ozonisation of water there are no chemical reactions between ozone and pure water. The only chemical reactions take place between ozone and organic, inorganic, or biological material present in the water. The solubility of ozone in water depends on different variables: pH, temperature, salts contents, and others. The half-life of ozone in water also depends on these variables [12].

The aim of this study was to observe the efficiency of ozone transfer by airstone bubble diffuser, retention of ozone in distilled and potable water and disinfectant effect of ozone in water naturally contaminated or spiked with *E. coli*.

MATERIALS AND METHODS

Materials

Ozone generators used in this study are depicted in Figures 1 and 2.

According to the manufacturer's recommendations, this generator is suitable for the disinfection of the inte-

Fig. 1. Ozone generator 1: Q-005 with output of 5 g.h^{-1}

Ozone unit: air cooling ceramic tube, 20 000 hours lifespan, power: 230 V AC, 80 W. Ozone source: ambient air. Manufacturer: FANDS Trade s.r.o., Valaliky, Slovakia. Source: http://www.technik.fands.eu/Generator-ozonu-Q-003B-3g-hod-d51.htm

Fig. 2. Ozone generator 2: FST-I-15 with output 15 g.h^{-1}

Ozone unit: air cooling ceramic tube, 10 000 hours lifespan, power: 230 V AC, 4000 W. Ozone source: ambient air. Manufacturer: FANDS Trade s.r.o., Valaliky, Slovakia. Source: http://www.technik.fands.eu/fotky35619/fotos/_vyr_6115g-vent.jpg, 2017

Fig. 3 Airstone bubble diffusers

riors of ambulances, pharmacies, use in households, small facilities etc. It is effective in rooms of size 60—300 m^3 at concentration 2—30 mg.m^{-3}. It can also be used for disinfection of water.

Manufacturer's recommendations: Suitable for premises up to 1200 m^3. This ozone generator can be used for disinfection of public facilities including restaurants, sports centres, playrooms, commercial canteens. Can also be used in pig and poultry housing and in food processing and food storage premises.

Ozone concentration in water was measured with the apparatus F-DOZ30 which allows one to read the levels of dissolved O_3 directly from the monitor. HACH Lange 2-canal multimeter (Germany) equipped with pH (PHC301 and conductivity (CDC401) electrodes was used for determination of water pH and conductivity.

Other materials required for our experiments included large glass cylinders (1000 ml), thermometers, plastic pipes with airstone bubble diffusers (Fig. 3, diffuser 1) relaying ozone from the generator to the water.

Endo agar (EA) was the culture medium used for cultivation of *E. coli*. On this agar, *E. coli* can be visualised as dark red colonies with a metallic shine.

Methods

Water pH and conductivity of water was determined using Hach Lange 2-canal multimeter and the relevant electrodes according to [21].

Counts of *Escherichia coli* were determined by membrane filtration method and cultivation on Endo agar (EA) at 43 °C for 24 hours [22].

Ozonisation trials:

1. **Efficiency of ozone transfer by airstone bubble diffuser and retention of ozone in distilled and potable water**

   We investigated the efficiency of ozone transfer through an airstone bubble diffuser and the time duration required to reach 0.25 mg.l^{-1} dissolved ozone in water

while using ozone generators 1 (G1) and 2 (G2). Ozonisation was performed in duplicate (results are presented as an average) in a laboratory (water and air temperature 20 °C, 49—53 % relative humidity) using distilled water (D_1, D_2) and potable water intended for mass consumption $(MC)^a$, $(MC)^b$ [6].

Potable or distilled water was poured up to mark to glass cylinders (1 litre volume, height of water column 36 cm) and ozone was introduced to the bottom of each cylinder through a bubble diffuser attached to the end of a plastic pipe, which extended from the respective ozone generator. The top of the cylinder was covered during ozonisation in order to minimize the escape of ozone. Small rising bubbles of ozone gas within the water could be observed. Generator G1: after the G1 was switched on, we measured the concentration of ozone in distilled (D_1) and potable water $(MC)^a$ in the intervals of 30, 60, 90, 120 and 150 minutes. After 150 min of ozonisation, the generator was switched off, the covers were removed and concentration of ozone was measured in 30-minute intervals up to 270 min from the beginning of the experiment.

Generator G2: Concentration of the dissolved ozone in potable water $(MC)^b$ was measured in the intervals of 30, 40 and 50 min from the beginning of ozonisation. In addition, retention of ozone in this water was observed after the termination of ozonisation upon reaching the effective level of ozone (0.25 $mg.l^{-1}$) in intervals of 10, 30, 60 and 120 min.

2. **Devitalisation effect of ozone in two types of potable water $(MC)^a$ and $(MC)^b$, in distilled water (D_2) spiked with *E. coli*, and in well water naturally contaminated with *E. coli***

Experiments were carried out in glass cylinders (1 litre volume, 36 cm water column). Cultivation of samples were done on Endo agar [22].

Generator G1: Potable water $(MC)^a$ previously ozonised to the level of 0.25 $mg.l^{-1}$ of O_3 was spiked with 1 ml of *E. coli* culture and samples for cultivation of *E. coli* were collected in intervals of 10, 20, 30 and 40 minutes. Counts of *E. coli* were determined in well water (IC) naturally contaminated with *E. coli* (2100 $CFU.l^{-1}$) in intervals of 1, 2, 5 and 10 min.

Generator G2: in trial one. counts of *E. coli* were determined in distilled water (D_2) previously ozonised to the level of 0.25 $mg.l^{-1}$ of O_3 and spiked with *E. coli* in intervals of 10, 30, 50 min and 2 and 24 h. In trial two, after spiking the ozonised distilled water with *E. coli* we continued with ozonisation and examination in intervals of 10, 30, 50 and 120 min.

RESULTS

Results of pH and conductivity of two types of potable water, distilled water and well water used in the study are presented in Table 1.

Efficiency of ozone transfer and retention of ozone

Ozone generator G1

The concentration of the dissolved ozone introduced continuously to potable water intended for mass consumption $(MC)^a$ and to distilled water (D_1) by G1 is presented in Fig. 4 and the level of residual ozone measured after the ozonisation was switched off is shown in Fig. 5.

Supported by previous studies, a concentration of 0.25 $mg.l^{-1}$ of dissolved ozone in water has a disinfecting effect of 99.99 % particularly on *E. coli* [14]. This level was reached after 120 min of ozonisation in distilled water, but not in potable water that required more than 120 min of ozonisation with G1, and was slightly exceeded after 150 min (0.26 $mg.l^{-1}$). The efficiency of ozonisation of distilled water was slightly higher throughout the process (Fig. 3).

Table 1. Results of pH and conductivity of water used in the experiments

Parameter	Range/Limit for potable water	$(MC)^a$	$(MC)^b$	WW	D_1	D_2
pH	6.5—9.5	7.59	6.8	7.43	7.06	7.26
Conductivity [$mS.m^{-1}$]	125	43.9	32.2	131.7	2.4	8.72

$(MC)^a$, $(MC)^b$—water suitable for mass consumption [6]; WW—well water; D_1—distilled water used in ozonisation trial 1; D_2—distilled water used in ozonisation trial 2

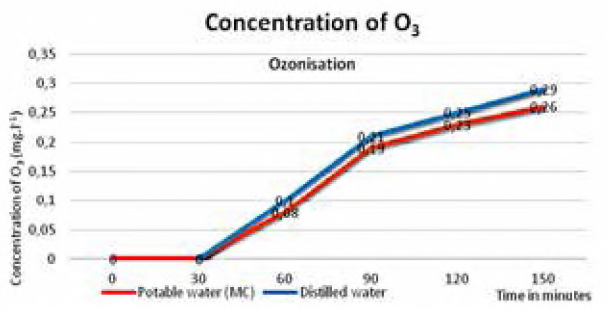

Fig. 4. Concentration of ozone in potable (MC)[a] and distilled water (D_1) during ozonisation with G1

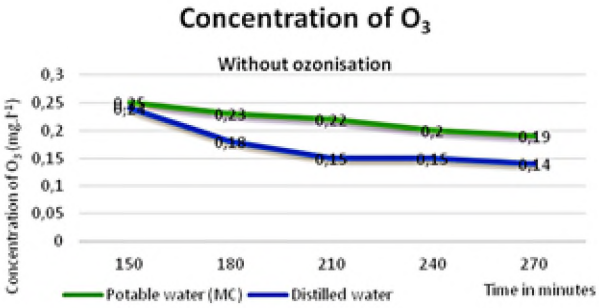

Fig. 5. Retention of ozone in potable (MC)[a] and distilled water (D_1) after termination of ozonisation

Table 2. Concentration of ozone in potable water (MC)[b] during ozonisation with G2

Duration of ozonisation [min]	Concentration of O_3 [mg.l^{-1}]	pH	Conductivity [mS.m^{-1}]
0	0	6.8	32.2
30	0.14	6.58	34.6
40	0.25	6.15	37.5
50	0.31	6.0	40.7

After termination of ozonisation (150 min), the level of ozone decreased more rapidly in distilled water but the decrease was surprisingly slow and after 270 min from the beginning of ozonisation both potable and distilled water still contained 0.19 and 0.14 mg.l^{-1} of dissolved ozone, respectively (Fig. 5).

Ozone generator G2

This generator was used for ozonisation of potable water (MC)[b] with lower initial pH and conductivity compared to water ozonised with G1 (MC)[a]. Results of the relevant parameters during ozonisation of this water and after termination of ozonisation are presented in Tables 2 and 3.

According to expectation, when using ozone generator with higher output the target concentration of 0.25 mg.l^{-1} dissolved ozone for the disinfection of 99.99 % *E. coli* was reached after 40 minutes of ozone exposure. This appears to be the optimal time needed to achieve the target concentration of ozone; however, the composition of ozonised water may play some role in this respect. The pH of water decreased and conductivity increased during ozonisation.

The decrease in concentration of dissolved ozone was more rapid in comparison with the decrease observed after ozonisation of potable water (MC)[a] with G1.

Table 3. Retention of ozone in potable water (MC)[b] after termination of ozonisation

Time intervals* [min]	Concentration of O_3 in potable water [mg.l^{-1}]
0	0.25
10	0.20
30	0.11
60	0.04
120	0

*Time intervals after reaching the effective level of ozone, starting at the termination of ozonisation

Devitalisation effect of ozone on *E. coli*

Results of ozonised potable water (MC)[a] spiked with 1 ml of *E. coli* culture in comparison with the control are presented in Table 4.

The effect of ozone was evident as no *E. coli* were detected in ozonised water after 10 minutes of exposure to ozone.

Ozone was also introduced into well water (WW) intended for individual consumption (Table 5). However, this particular water did not comply with the requirements on potable water [6].

Table 4. Results of ozonised potable water (MC)[a] containing 0.25 mg.l^{-1} of dissolved ozone, spiked with *E. coli*

Time [min]	Concentration of ozone [mg.l^{-1}]	Ozonised potable water + *E. coli* [CFU.l^{-1}]	Non-ozonised potable water + *E. coli* [CFU.l^{-1}]
0	0.25	3.2×10^5	3.2×10^5
10	0.17	0	9.8×10^3
20	0.20	0	6.0×10^3
30	0.22	0	5.6×10^3
40	0.16	0	4.6×10^3

Table 5. Counts of *E. coli* in well water (WW) during ozonisation with G1

Time [min]	Concentration of ozone [mg.l^{-1}]	Well water [CFU.l^{-1}]
0	0.00	2100
1	0.07	1600
2	0.17	25
5	0.26	0
10	0.36	0

Table. 6. Counts of *E. coli* in distilled water (D_2) initially containing 0.25 mg.l^{-1} of dissolved ozone, spiked with *E. coli*

Sample collection intervals	Dissolved O_3 [mg.l^{-1}]	*E. coli* [CFU.l^{-1}]
0 min	0.25	1.5×10^8
10 min	0.21	0
30 min	0.11	0
50 min	0.05	0
2 hours	0	0
24 hours	0	0

Table 7. Counts of *E. coli* in distilled water (D_2) initially containing 0.25 mg.l^{-1} of dissolved ozone and undergoing continual ozonisation after spiked with *E. coli*

Sample collection intervals [CFU.l^{-1}]	Dissolved O_3 [mg.l^{-1}]	*E. coli* [CFU.l^{-1}]
0 min	0.25	1.5×10^8
10 min	0.31	0
30 min	0.35	0
50 min	0.41	0
120 min	1.0	0

E. coli present in the well water were devitalised within 5 min of ozonisation with the less efficient generator G1. By this time dissolved ozone reached the level considered effective against *E. coli*.

In trial one, 1 ml of *E. coli* culture was added to previously ozonised distilled water (D_2) containing an initial concentration of 0.25 mg.l^{-1} of dissolved ozone. Samples of distilled water were taken for cultivation at 10, 30 and 50 minutes and 120 min after ozonisation. The results can be seen in Table 6.

The concentration of dissolved ozone decreased with time up to the 50 min of observation when it dropped to the lowest detected level. *E. coli* added to this water did not survive the exposure to the effective level of ozone.

In trial two, 1 ml of *E. coli* culture was added to previously ozonised distilled water (D_2) containing 0.25 mg.l^{-1} dissolved ozone and undergoing continual ozonisation via an airstone bubble diffuser. Samples of water spiked with *E. coli* were taken immediately after addition of *E. coli* (0 min) and at 10, 30, 50 and 120 min of continuous ozonisation. The results can be seen in Table 7.

Our results showed that within 10 minutes of ozonisation, the level of dissolved ozone in the distilled water had increased from 0.25 mg.l^{-1} to 0.31 mg.l^{-1} and continued to increase up to 1.0 mg.l^{-1} at 120 min. It was observed that *E. coli* could not be detected when concentration of dissolved ozone was 0.25 mg.l^{-1} or higher.

DISCUSSION

The use of ozonised water is gaining on importance in many segments of human activities, such as treatment of water, food and agricultural industries and medicine. As a result of this there is available a wide range of different types of ozone generators that differ in their characteristics and thus also in their output. Thus, the customers should be able to select the optimum configuration for their specific use. However, the extensive offer produces some difficulties, as the scientific studies present results obtained under different conditions and affected by various factors which makes it sometimes very difficult to arrive to definite conclusion regarding the suitability of a particular generator.

The aim of this study was to contribute to the available information about ozonisation of water and the effect of this treatment on *E. coli* in water. Two types of ozone generators with different output of ozone (G1: 5 g O_3 per h; G2: 15 g O_3 per h) were used.

Results of ozonisation of potable and distilled water at 20 °C showed that the increase in O_3 level during ozonisation with G1 was more rapid in distilled water where the concentration of 0.25 mg.l^{-1}, that should be effective against *E. coli* [14], was reached after 120 min while in potable water after 150 min. Initial pH of this potable water (MC)[a] was 7.59 and of distilled water 7.06 and initial conductivity of potable water was 43.9 mS.m^{-1} and of distilled water 2.4 mS.m^{-1}.

When different potable water (MC)[b] with pH = 6.8 and conductivity equal to 32.2 mS.m^{-1} was ozonised with the higher output generator (G2), the effective concentration of ozone in the distilled water was reached after 40 min and ozone was retained for 60 min (0.04 mg.l^{-1}).

After termination of ozonisation with G1 (150 min), the level of ozone decreased more rapidly in distilled water. The decrease was surprisingly slow and after 270 min from the beginning of ozonisation both potable and distilled water still contained 0.19 and 0.14 mg.l^{-1} of dissolved ozone (Fig. 5). This is in contrast with the study by Andoyo et al. [1] who explored the stability of ozone in distilled, double distilled and tap water at different temperature (5 °C, 25 °C, 40 °C), pH, water type and several variations of mineral content. According to their results, the retention time of ozone in acid pH was longer than that in the alkaline pH, and high level of minerals has a shorter retention time compared to low minerals samples. However, in this study there was no mention about the ozone output of the ozone generator used. The authors reported that after 5 and 10 min of ozonisation the level of ozone reached 4.3 and 5.8 mg.l^{-1}, respectively, which is a much higher level than that investigated in our study. The authors also noted that ozone is a reactive compound that readily binds to other compounds when ozone concentrations are high; nevertheless, at high concentrations ozone tends to be unstable. Mineral content affects the diffusion of the ozone bubbles into the water, the higher the mineral content, and the easier the ozone to be decomposed and thus causes the retention time of ozone to be shorter. [13]. According to Von Gunten [18], ozone firstly decomposed inorganic compound then organic compounds will be decomposed afterwards. In the condition of water that contains many minerals such as tap water, ozone more easily decompose into O_2 (oxygen) and O* (free oxygen ions), while in condition of water with less minerals such as distilled water, ozone was decomposed longer. The mineral content of the types of water in the study by Andoyo et al. [1] was characterised by the content of Ca while in our study we reported conductivity of water. Conductivity is a measure of the ability of water to pass an electrical current. It is affected by the presence of inorganic dissolved solids such as chloride, nitrate, sulfate, and phosphate anions (ions that carry a negative charge) or sodium, magnesium, calcium, iron, and aluminium cations (ions that carry a positive charge). Organic compounds like oil, phenol, alcohol, and sugar do not conduct electrical current very well [27].

In our study we used two generators with different output of ozone. In water ozonized at lower rate the ozone was retained for longer time despite its higher pH (7.59 versus 6.8) and higher conductivity (43.9 versus 32.2). Thus, the retention of ozone may be affected by the rate of its relaying into water and, potentially by the use and type of bubble diffuser.

Changes in pH and conductivity during ozonisation of potable water (MC)[a] with G1 were minimal but observed a decrease in pH from 6.8 to 6.0 and rise in conductivity from 32.2 to 40.7 mS.m^{-1} in potable water (MC)[b] during ozonisation with generator G2 (Table 2). This differed from the results of Subedi et al. [23] who reported no remarkable effect of ozonisation on pH and conductivity but observed a significant effect on chemical properties (nitrate, hardness, iron) when using dielectric barrier dis-

charge unit to produce highly oxidising ozone molecules. These authors also reported an effective removal of faecal coliforms from water from 3 different sources.

During ozonisation, oxidation of organics with ozone gives rise to intermediate compounds. Carbon dioxide as a product of oxidation plus water increase conductivity of water. Also, impurities present in air from which ozone water was generated can increase the conductivity of water [28].

Ersoy et al. [5] comprehensively evaluated the inactivation mechanism of ozone on *E. coli* and *E. faecalis*. The results supported the idea that ozone targeted mainly cell walls of these bacteria. According to the TTC dehydrogenase relative activity and flow cytometry results, ozone was more effective for Gram-positive cells inactivation. *E. faecalis* cells were inactivated totally in a very short time, whereas much more time was required for the total inactivation of *E. coli* cells. In their study, conductivity increased during first 5 min followed by decrease after treatment with 1 $mg.l^{-1}$ ozone and contact times of 30 s, 1, 5, 10 and 20 min. Treatment for 30 s resulted in the release of intracellular components (i. e. DNA and protein) for both types of cells. Cell wall integrity was disturbed as indicated by leakage of K^+ ions and cellular organic molecules.

According to Manousaridis et al. [11], ozone is very unstable both in the gaseous phase and in solution, decomposing into hydroxy (·OH), hydroperoxy ($\cdot HO_2$) and superoxide ($\cdot O_2^-$) radicals. The reactivity of ozone is attributed to the great oxidizing power of these free radicals, making ozone a potent disinfectant in water treatment and the food industry. The above authors evaluated the effect of ozone gas on *Escherichia coli* O157:H7 inoculated on an organic substrate, and the efficacy of ozonised water in controlling the pathogen. Water was ozonised at 45 $mg.l^{-1}$ for 15 min and *E. coli* O157:H7 was exposed for 5 min to the ozonised water immediately after ozonisation, and after storage for 0.5, 1.0, 1.5, 3.0, and 24 h at 8 °C. Ozonised water was effective in inactivating of *E. coli* O157:H7 in all treatments. Furthermore, refrigerated ozonised water stored for up to 24 h was effective in the control of *E. coli* O157:H7.

The effect of ozonation on the rate of disinfection of *Escherichia coli* was investigated as a function of ozone concentration, ozonation duration and flow rates. Ozone was generated *in situ* using corona discharge method using compressed oxygen stream and depending on the oxygen flux the ozone concentrations ranged from 0.91–4.72 $mg.l^{-1}$. The inactivation was faster at lower pH than at basic pH. Molecular ozone was more effective in disinfection than hydroxyl radicals [33].

Among the factors that affect the action of ozone on microorganisms, highlighted are pH, temperature, and composition of the medium. In addition, organic compounds can compete with microorganisms for ozone [9]. This justifies the greater efficiency of microorganism inactivation by ozone in substrates with less organic matter.

One of the goals of our study was to examine devitalization effect of ozone in potable and distilled water spiked with *E. coli* and in well water containing *E. coli* bacteria. Exposure of *E. coli* added to potable (MC)[a] and distilled water previously ozonised to the level of 0.25 $mg.l^{-1}$ of ozone caused complete devitalization of these bacteria within 10 minutes. Generator G1 was used to ozonise well water (WW) containing 2100 $CFU.l^{-1}$ of *E. coli*. Within 2 min of action of very low concentrations of ozone (0.07—0.17 $mg.l^{-1}$ *E. coli* counts decreased to 25 $CFU.l^{-1}$ and within 5 min when the level of 0.26 $mg.l^{-1}$ ozone was reached, these bacteria were devitalised completely. These results confirmed the high effectiveness of ozonised water against *E. coli*, an important indicator of contamination of water with animal and human feces.

CONCLUSIONS

Results of our study conducted under laboratory conditions showed that the level of ozone during ozonisation with G1 increased more rapidly in distilled water compared to the concentration of ozone in potable water and after termination of ozonisation, retention of ozone in distilled water was a little lower in comparison with potable water with higher pH and conductivity. At higher rate of ozonisation, pH of distilled water decreased and conductivity increased during ozonisation. Complete devitalisation of *E. coli* that was either present in water or added to it required the level of ozone close to or equal to 0.25 $mg.l^{-1}$ considered as effective against *E. coli*.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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