

EFFECT OF OZONE TREATMENT ON THREE BACTERIAL STRAINS OF DRINKING WATER

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Abstract. Because of its excellent disinfection and oxidation qualities, ozone is widely used for drinking water treatment. Ozone is frequently used to destroy and inhibit the growth of microorganisms from contaminated water. In this paper, ozone efficiency was tested for water treatment containing cell suspensions of *Escherichia coli* K12-MG 1655, *Enterococcus faecalis* ATCC 29212 and *Pseudomonas aeruginosa* ATCC 9027 using an ozone generator OZONFIX 8G. Water samples were subjected to ozone treatment for different periods, ranging from 15 seconds to 15 minutes. Ozone was generated by the corona discharge method. The use of electrical power to generate ozone by corona discharge is the most commercially viable method. Analyses were performed both for determining the cell viability by the total plate count technique, and for determination of the cell growth curves after the applied ozone treatment. The bacteria were cultured in liquid media on an orbital shaker at 150 rpm and 37° C. The bacterial multiplication was measured by recording the absorbance $A_{600\text{nm}}$ on a UV-VIS spectrophotometer. Following the experiments, it was found that ozone showed a strong effect of inhibiting the growth of the three bacterial strains, both in terms of viability and multiplication capacity. The most resistant was the bacterial strain of *Pseudomonas aeruginosa* ATCC 9027.

Keywords: ozone treatment, bacterial strains, growth.

Introduction

Human exposure to wastewater discharged into the environment has increased within the past 15 to 20 years with the rise in population and the greater demand for water resources for recreation and other purposes. The organisms of concern in domestic wastewater include enteric bacteria, viruses, and protozoan cysts [1]. Disinfection is considered to be the primary mechanism for the inactivation/destruction of pathogenic organisms to prevent the spread of waterborne diseases to downstream users and the environment. The disinfecting process has been considered as one of the most important steps in drinking water treatment and improper attention to this process can cause a social disaster [2].

An excellent disinfectant of drinking water is ozone. This is able to inactivate even more resistant pathogenic microorganisms such as *Escherichia coli*, *Enterococcus faecalis* and *Pseudomonas aeruginosa*, along with the conventional disinfectants (chlorine, chlorine dioxide). Ozone has been applied for water disinfection purposes for almost a century. A recent survey in Switzerland among water works that apply ozone has shown that for 90 % of these plants the main reason for its application is disinfection [3].

Ozonation represents an alternative to chlorination in drinking-water disinfection, which was done first a century ago and has become an established means of disinfection [4; 5]. Bacteria, including *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus megaterium*, *Salmonella typhimurium*, *Shigella flexneri*, and *Vibrio cholerae* are sensitive to ozonated water under various conditions [6].

It was found that the cost of ozone disinfection systems is dependent on the manufacturer, the site, the capacity of the plant, and the characteristics of the wastewater to be disinfected. Ozonation costs are generally high in comparison with other disinfection techniques. In 2005, Nabi Bidhendi et al. have developed a study that explains the opportunities and threats to the drinking water ozonation process [2]. Prabakaran M. et colab. have analyzed ozone treatment by applying to human pathogenic bacteria such as *Escherichia coli* and *Pseudomonas fluorescens*, *Salmonella typhi* and *Klebsiella pneumoniae*. Among the treated bacterial species, *E.coli* revealed high sensitivity to ozone treatment compared to other bacterial strains [7].

The aim of this paper was to evaluate the ozone efficiency for water treatment containing cell suspensions of *Escherichia coli* K12-MG 1655, *Enterococcus faecalis* ATCC 29212 and *Pseudomonas aeruginosa* ATCC 9027 using an ozone generator OZONFIX 8G.

Materials and methods

Bacterial strains

Three strains of bacterial species commonly found in water were used: *Escherichia coli* K12-MG 1655, *Pseudomonas aeruginosa* ATCC 9027 and *Enterococcus faecalis* ATCC 29212. The bacterial strains were cultivated in tubes on Nutrient Agar medium (Merck) with the following composition: peptone from meat 5.0 g L⁻¹, meat extract 3.0 g L⁻¹, agar-agar 12.0 g L⁻¹, pH 7.0, and kept at 4 °C until use. The viability of bacteria after ozone treatment was evaluated by the conventional total plate count method on Plate Count Agar medium. The bacterial growth behaviour was assessed by cultivation in liquid media on a rotary incubator (Thermoshake, Gerhardt), at 150 rpm, 37 °C, for 26 hours. The absorbances of bacterial cultures were measured at 600 nm using the T92 + UV VIS spectrophotometer, PG Instruments.

Ozone equipment

Ozone was produced by an ozone generator type OZONFIX 8G commercially available, with a production of 8 grams of pure ozone per hour and a flow rate of 2.5 L min⁻¹. The equipment produces ozone by corona discharge. The gas was sterilized with a sterile air filter and was bubbled through a ceramic frit (3 cm x 1.8 cm) in a volume of 200 mL of bacterial cells suspension in sterile water.

Methods

10 mL of bacterial cells suspension from 48 hours old cultures on Nutrient Agar medium in tubes were used to inoculate 200 mL liquid media (mentioned below) in 500 mL Erlenmeyer flasks. *E. coli* was grown in Luria Bertani (Merck) liquid medium (triptone 10 g L⁻¹, yeast extract 5 g L⁻¹, NaCl 5 g L⁻¹), *E. faecalis* and *P. aeruginosa* in Nutrient Broth (Merck) medium (peptone from meat 5.0 g L⁻¹, meat extract 3.0 g L⁻¹). The cultures were incubated at 37 °C, for 24 hours on an orbital incubator at 150 rpm. 0.1 mL of each bacterial culture was used to prepare the cell suspension to be treated with ozone. At time intervals of 15 sec, 30 sec, 1 min, 1min 30 sec, 2 min, 2 min 30 sec, 3 min, 5 min, 10 min, 15 min, samples of 1 mL were taken for total plate count determination. The dilution of each sample and the addition of agar medium have been made as quickly as possible, because the interaction of RONS (reactive oxygen and nitrogen species) produced in the ozone generator with bacterial cells was very strong. At the same time, another volume of 1 mL of ozonized cell suspension was taken for inoculation of liquid media in Erlenmeyer flasks. Petri dishes were incubated at 37 °C for 48 hours and the number of viable cells was determined. The samples from Erlenmeyer cultures were analyzed during 26 hours of incubation. All samples were performed in triplicate.

Results and discussion

The three tested bacteria, known as potentially dangerous for health, if they are present in large number in water, can be destroyed through ozone treatment. The results of sensitivity of *E.coli* K12-MG 1655, *E. faecalis* ATCC 29212 and *P. aeruginosa* ATCC 9027, evaluated by plate count technique are presented in Table 1. The values of ufc mL⁻¹ for all bacterial species after ozonation at different times, compared to the untreated reference sample, showed a very large difference. After only 15 seconds the viability decreased by about three orders of magnitude for all species. After 30 seconds, only a few colonies have grown on culture media. Keeping the bacterial cells in ozone bubbling water for more than 1 minute, caused irreversible damage and cell death.

Table 1

Results of sensitivity evaluated by plate count technique

Strain/Time	<i>E. coli</i> K12-MG 1655, ufc·ml ⁻¹	<i>E. faecalis</i> ATCC 29212, ufc·ml ⁻¹	<i>P. aeruginosa</i> ATCC 9027, ufc·ml ⁻¹
0	62x10*4	22x10*4	31x10*4
15 sec	280	120	650
30 sec	24	5	59
1 min	0	0	0
1 min 30 sec	0	0	0

For ozonation times longer than 1 minute, the number of ufc mL⁻¹ was zero.

The growth kinetics of the tested bacterial strains have been examined through measurement of absorbance at 600 nm in cultures incubated on an orbital shaker, for 26 hours. The obtained results are shown in Figure 1.

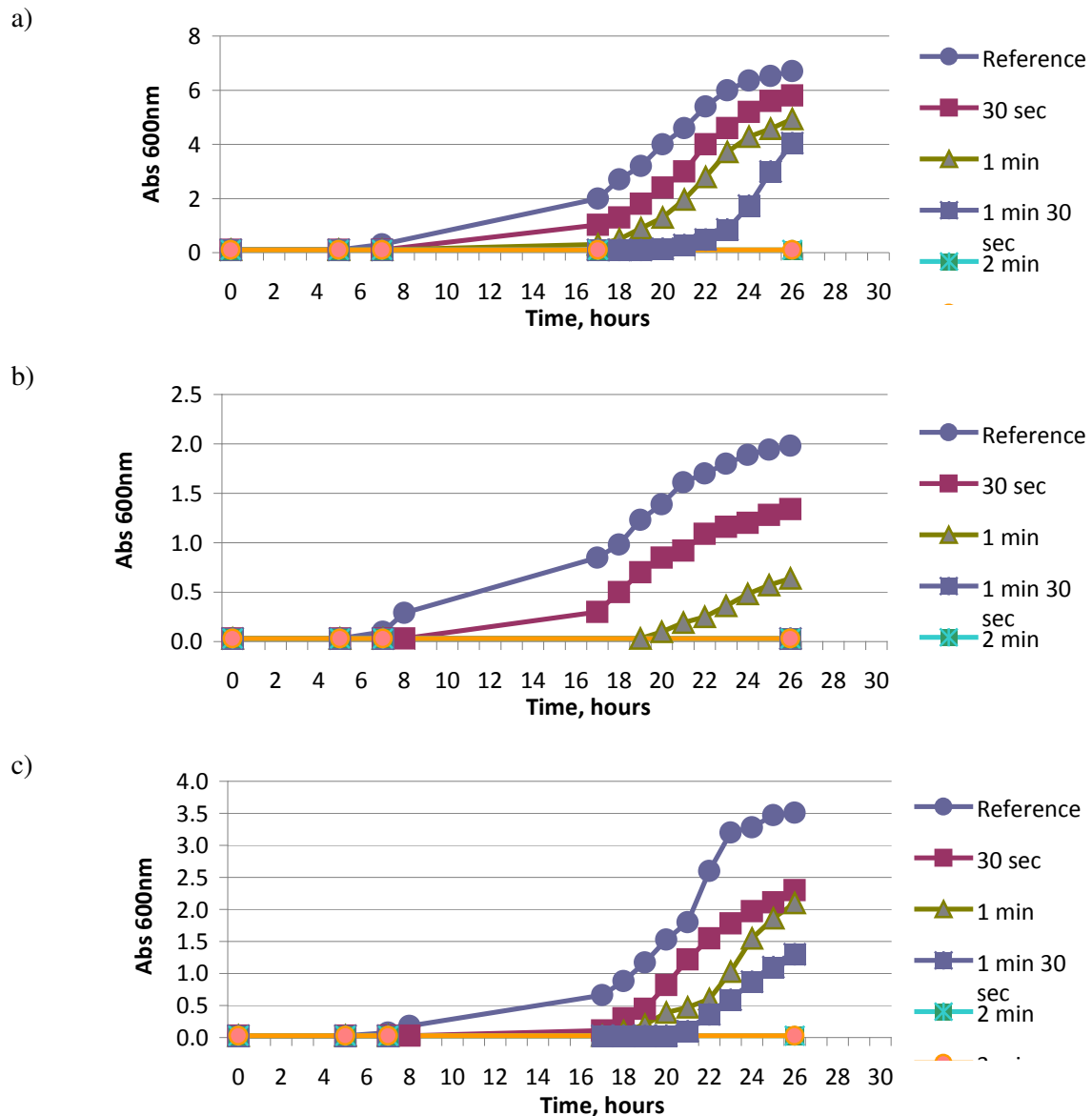


Fig. 1. Absorbance at 600 nm values in a) *E. coli* K12-MG 1655, b) *E. faecalis* ATCC 29212, c) *P. aeruginosa* ATCC 9027 cultures on orbital shaker after ozone treatment compared to untreated reference

The growth curves showed a characteristic appearance, with a lag phase of 6-8 hours for reference (untreated cultures) and longer lag phases, up to 16-18 hours for the cultures derived from the treated cells. This can be explained both by the lower number of survivors in ozonated samples and also by the cellular lesions that occur and are repaired in a longer time. For an ozonation time of more than 2 minutes, the cell damage was too great and could not be repaired. For all cases, the absorbance values were dependent of the ozonation time, and the lag phase was as long as the duration of treatment was greater.

Comparable results were obtained in the previous research on ozone use in water disinfection. Significant antimicrobial action was observed for *E. coli* and *S. aureus* after current air-liquid discharge plasma [8]. Xu et al., showed damaged bacterial outer structures, decreased concentration of cellular protein and nucleic acid after ROS treatment. In 2018, Wang H. et al. studied the effect of O_3/Cl_2 disinfection on opportunistic pathogens in drinking water distribution systems by the most

probable number (MPN) method and demonstrated that ozone disinfection inactivated or damaged the bacterial cells due to its strong oxidizing properties [9]. In 2009, Lee Y. et al measured the total cell count (TCC) and intact cell count (ICT) by flow cytometry in order to evaluate the inactivation kinetics of bacteria in ozonated water, showing that the bacterial communities may have high and low ozone sensitivity [10].

Patil S. et al. studied the inactivation of *E. coli* in orange juice and showed that the applied gaseous ozone treatment resulted in a population reduction of 5 log cycles [11].

Conclusions

1. The ozone treatment of water has considerable antimicrobial effect due to the ozone strong oxidizing properties.
2. The bacterial strains of *E. coli* K12-MG 1655, *E. faecalis* ATCC 29212 and *P. aeruginosa* ATCC 9027 were sensitive to ozone treatment; the viability of cells decreased with 3 orders of magnitude after 30 seconds of ozonation and the effect became lethal after 1 minute.
3. The growth curves showed larger lag phases and lower numbers of cells, depending on the ozone treatment time.

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