



## Evaluation of ecotoxicity and inactivation of bacteria during ozonation of treated wastewater

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### ABSTRACT

Treated wastewater may be a valuable source of water and/or nutrients for crop production and fish farming. Disinfection of treated wastewater should be performed to protect water resources against pathogenic and opportunistic microorganisms, as well as micropollutants contained in effluents. However, in the case of ozonation of treated wastewater, a large portion of contaminants do not undergo complete mineralization and can be transformed into by-products of unknown toxicity. The research performed in this study by culture-dependent and independent methods showed that the inactivation of bacteria in treated wastewater by ozonation does not take place effectively and may depend on the presence of other contaminants that may first react with the disinfectant. Some bacterial cells proved to be damaged by a disinfectant to the extent that they were unable to grow on nutrient media, but they were still viable and potentially posing a sanitary threat. Possible reasons for the disinfection failure were investigated and discussed. Ecotoxicity tests with algae *Desmodesmus quadricauda*, crustacean *Daphnia magna*, and bacteria *Aliivibrio fischeri* showed that by-products may be formed during ozonation of treated wastewater which is toxic to aquatic organisms. The toxicity class of treated wastewater may change from the completely non-toxic to very high hazard category, and there is a clear relationship between the time of ozonation and the increase in ecotoxicity.

*Keywords:* Treated wastewater; Ozonation; Wastewater ecotoxicity; Disinfection by-products (DBPs)

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### 1. Introduction

In the past centuries, water was considered as a renewable, unlimited resource. During the last decades, however, the awareness that high-quality water is limited has started to arise in both people and government organizations around the world. Moreover, the increase of the world population and climate changes suggest the need for more rational use of water resources [1].

The results of the European Environment Agency [2] survey showed that in the year 2010 about 50% of European countries were characterized by a water stress index higher than 10%, indicating that water availability is becoming a constraint for the development of countries. In 2025 two-thirds of the world's population will feel a shortage of water in degree from moderate to significant, and more than half will suffer real limitations in water supply [3]. In view of these circumstances it is fundamental to protect the quality

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of water resources, which are the receivers of treated sewage. This can be achieved, among others, through effective wastewater treatment followed by disinfection, which will provide both the physicochemical quality of effluents, as well as their safety in terms of sanitary status.

Environmental protection is a policy goal in most countries, from the viewpoints of both conservations of natural resources as well as ecosystem services and public health protection. A narrow view of wastewater in this context would consider it to be a costly by-product of the urbanization process, requiring substantial investments in wastewater treatment plants (WWTPs) and disposal mechanisms. Yet such a view overlooks its value as a source of water and/or nutrients for crop production and fish farming [4]. Reuse of wastewater contributes significantly to efficient and sustainable water usage. Treated wastewater could provide an effective alternative for meeting agriculture's demands and also increase freshwater resources for other needs [1]. The practice is growing within Europe and is particularly well established in Spain, Italy, Cyprus, and Greece [2]. In 2006, more than 10% of the world's population consumed food produced by irrigation with wastewater [4]. The percentage was considerably higher among populations in low-income countries with arid and semi-arid climates. Both treated and untreated wastewater were used directly and indirectly (i.e. as faecally contaminated surface water) for irrigation.

Secondary treated effluent contains a range of pathogens that pose a potential risk to the health of humans and livestock [5]. Although during the treatment of wastewater, most bacteria are inactivated (in the case of application of highly effective biological wastewater treatment methods the reduction of bacterial indicators is over 99%), due to their exceptionally high number in raw sewage, their elimination is definitely inadequate [3]. Many pathogens, which are present in secondary effluents, can survive for long enough periods in soil or on crop surfaces to be transmitted to humans or animals. It is especially important in the case of antibiotic-resistant bacteria and free DNA containing antibiotic resistance genes (ARGs) [6]. Furthermore, secondary treated effluent is the main source of micropollutants and contaminants of emerging concern (CECs), including pharmaceuticals, personal care products, synthetic hormones, and endocrine-disrupting chemicals (EDCs). CECs are released into receiving water bodies because they are not eliminated efficiently in WWTPs [7–11].

Disinfection of secondary treated wastewater is the only measure to keep the sanitary state of water, soil, and crops on appropriate levels. It is expected that in the near future this process cannot be ignored during the planning and designing new and modernized WWTPs. A wide range of disinfection strategies currently exists, including chlorination, ultraviolet irradiation (UV), ozonation, and membrane filtration. Each disinfection technology has advantages and drawbacks, including the formation of various disinfection by-products (DBPs) [12]. Ozonation seems to be an effective technology not only in the inactivation of microorganisms but also in the removal of many CECs [13].

The mechanism of bacterial inactivation by ozone is by damage to the cell membrane, nucleic acids, and certain enzymes. Microorganism reactivation after ozonation

is unlikely to occur. Ozone is particularly effective against viruses; the mechanism of viral inactivation involves coagulation of the protein and oxidation of the nucleobases forming the nucleic acid. Protozoan cysts and bacterial spores are more resistant to ozone than bacteria and viruses. The effectiveness of disinfection depends on the quality of the effluent, the ozone dose and demand, and the transfer efficiency of the ozone contact reactor [14]. Significant variations were reported in ozone doses (2 to 30 mg L<sup>-1</sup>) to reach 1 to 3 log inactivation of total coliforms or *Escherichia coli* [13].

The combination of microbial disinfection and effective oxidation of CECs makes ozonation an attractive alternative to membrane filtration, chlorine, and UV, given that more advanced ozonation technologies have recently been developed [15,16]. However, research on the toxicity of chemical substances formed in the ozonation process, such as brominated DBPs, aldehydes (formaldehyde, acetaldehyde, glyoxal, and methylglyoxal), ketones and carboxylic acids (formic, acetic, glyoxylic, pyruvic, and ketomalonic acids), is still in progress and there is a shortage of information on residual bioactivity of transformation products formed during ozonation of secondary treated wastewater [14]. Furthermore, in contrast to the numerous experiments carried out on ozone disinfection in clean matrices, only a few studies investigated the ozonation efficiency of real WWTP effluents, as discussed by Nasuhoglu et al. [13]. Additionally, data on the toxicity of by-products formed during ozonation is generally missing, investigations are focused usually on one bioindicator and acute toxic responses [13]. However, few available data are alarming and suggesting that both the disinfection efficiency as well as by-products formation potential should be considered when ozonation is used for wastewater effluents [17,18].

Therefore, the aim of this research was to investigate the efficiency of ozonation of treated wastewater from a full-scale WWTP using both culture-dependent and independent methods, in terms of inactivation of selected microorganisms, as well as to investigate the ecotoxicity of the disinfected wastewater to assess the overall hazard when being discharged into the aquatic environment.

## 2. Materials and methods

### 2.1. Samples of treated wastewater

Samples of treated wastewater were collected from a municipal WWTP (Biogradex<sup>®</sup> activated sludge technology), which is located in Stare Babice near Warsaw (Poland). The plant has A2O configuration (anaerobic-anoxic-aerobic), without primary settling but with an additional post-denitrification tank placed after the aerobic tank and the Biogradex<sup>®</sup> installation for degasification of mixed liquor in vacuum conditions between the bioreactor and the secondary clarifiers. The WWTP received typical domestic wastewater (6,000 m<sup>3</sup> d<sup>-1</sup>) and complied to the stringent effluent limits of 8 mg L<sup>-1</sup> BOD<sub>5</sub>, 70 mg L<sup>-1</sup> COD, 30 mg L<sup>-1</sup> total suspended solids, 10 mg L<sup>-1</sup> N<sub>tot</sub> and 0.25 mg L<sup>-1</sup> P<sub>tot</sub> due to discharge to the Kampinoski National Park. The effluent samples for the research were collected in 9 research series within the period from October 2017 to March 2018 directly from the outlet of DynaSand filters which are

installed as a final polishing step. The samples were characterized by low pollution parameters (0–2 mg L<sup>-1</sup> BOD<sub>5</sub>, 21–30 mg L<sup>-1</sup> COD, 4–8 mg L<sup>-1</sup> N<sub>tot</sub>, and 0.13–0.25 mg L<sup>-1</sup> P<sub>tot</sub>). All the above-presented data was provided by the plant operator.

## 2.2. Disinfection by ozonation

The experiments were carried out in a 12 L reactor (internal diameter of the base: 0.217 m, height of the wastewater column: 0.325 m) using the ozone generator Korona L 20 SPALAB (“Korona” Laboratory, Piotrków Trybunalski, Poland). The generator was supplied with atmospheric air (flow 3 L min<sup>-1</sup>) and the outlet from the reactor was connected to the destructor of ozone filled with zeolite. The efficiency of the ozone generator was measured by the iodometric method and was on average 7.4 ± 0.9 mg O<sub>3</sub> min<sup>-1</sup>.

Three variants of the experiment were performed, including (a) treated wastewater, (b) sterile tap water spiked with *Escherichia coli* suspension (180–300 CFU mL<sup>-1</sup>), and (c) treated wastewater additionally spiked with *E. coli* suspension (340–510 CFU mL<sup>-1</sup>). Experiments (b) and (c) were carried out to examine the influence of micropollutants present in wastewater on the inactivation of *E. coli*, as well as the effect of bacterial forms other than single cells in suspension.

## 2.3. Microbiological analysis

Microbiological analyses of treated wastewater before and after the disinfection processes were carried out using both culture-dependent and independent methods. To exclude the effect of ozone residual on microorganisms, the reaction was stopped by adding a sterile 0.2 N sodium thiosulphate solution. Enumeration of culturable psychrophilic and mesophilic bacteria, as well as *E. coli* was performed in accordance with PN-EN ISO 6222 [19] and PN-EN ISO 9308-3 [20], respectively.

The assessment of total live biomass (both culturable and unculturable) in treated wastewater before and after the disinfection processes, expressed as ME mL<sup>-1</sup> (Microbial Equivalents), was carried out based on ATP determination in accordance with DeltaTox ATP manual (Modern Water, UK) and calculated from the Eq. (1):

$$cATP = tATP - fATP \quad (1)$$

where cATP – intracellular ATP, reflecting total live biomass; tATP – total ATP; fATP – free-available ATP.

## 2.4. Ecotoxicity tests

Enzymatic, growth, and survival tests were carried out using bacteria, algae, and crustaceans. Growth test with green algae *Desmodesmus quadricauda* (CCALA 463) was performed in accordance with PN-EN ISO 8692 [21]. Evaluation of growth inhibition of algae was made on the basis of the measurement of cell densities after 72 h contact with wastewater samples. Immobilization assay with *Daphnia magna* was performed in accordance with PN-EN ISO 6341 [22]. The immobilized organisms were counted after 48 h incubation with wastewater samples. Bioluminescence inhibition

test with *Aliivibrio fischeri* was performed using a portable device for the ecotoxicological monitoring of environmental samples (DeltaToxII, Modern Water, UK). The inhibition of bioluminescence of *A. fischeri* was assessed after 5 min of exposure to wastewater samples. All the tests were carried out on samples of wastewater stored for 24 h in 2–6°C and tested for residual ozone by the iodometric method.

Lethal and effect concentrations (LC(EC)<sub>50</sub>) were determined using the probit analysis with 95% confidence intervals [23] and were then used to calculate acute toxic units (TU<sub>a</sub>) as described by Persoone et al. [24]. The ecotoxicity assessment of the examined wastewater samples was based on the hazard classification system for waste discharged into the aquatic environment, developed by Persoone et al. [24].

## 3. Results and discussion

### 3.1. Bacteria inactivation efficiency

The most important issue concerning the disinfection process is its efficiency. In this study the inactivation of bacteria in treated wastewater was monitored using both culture-dependent and independent methods. The results are presented in Fig. 1 and Table 1, respectively.

Relatively high inactivation of psychrophilic and mesophilic bacteria (86% and 84%, resulting in 0.9 and 0.8 log inactivation, respectively) was observed after 1h of the process. However, when total colony counts of both bacterial groups are analyzed, a distinct slowdown in the disinfection is observed after the first hour of the process and, surprisingly, after 5 h of ozonation the expected disinfection efficiency was not obtained. Furthermore, the ATP analysis shows that the percentage inactivation of viable bacterial forms (both culturable and unculturable) can be even far lower (40%), resulting in 0.2 log inactivation after 1 h (Table 1). Detection of more microorganisms by the sensitive DeltaTox ATP test (ratio CFU/ME did not exceed 1%) results from the fact that this technique detects all viable cells present in the sample, in contrast to the standard colony count method [25]. A significantly lower decrease in cATP after 1 and 2 h (Table 1), in comparison to the respective changes in CFU mL<sup>-1</sup> (Fig. 1), suggests that some bacterial cells could be damaged by a disinfectant to the extent that they are unable to grow on nutrient media, but they are still viable and can pose a sanitary threat. On the other hand, Zheng et al. [26] demonstrated that the ozonation of treated wastewater can produce a large number of free DNA containing ARGs after the inactivation of bacterial cells. Removal of ARGs was not significantly enhanced by increasing the concentration of ozone, because ozone, as a strong oxidant, reacts with a variety of cell material, and does not target DNA or ARGs. The free DNA, which can be released into the environment during the ozonation of treated wastewater, should be under control, as the persistence of ARGs in the form of free DNA in aquatic environments may intensify the public health risk.

Lee et al. [15] compared different methods (ionizing radiation technology, chlorine, UV, and ozone) for disinfection of the effluent from a municipal WWTP in Korea. The expected disinfection efficiency was not obtained,

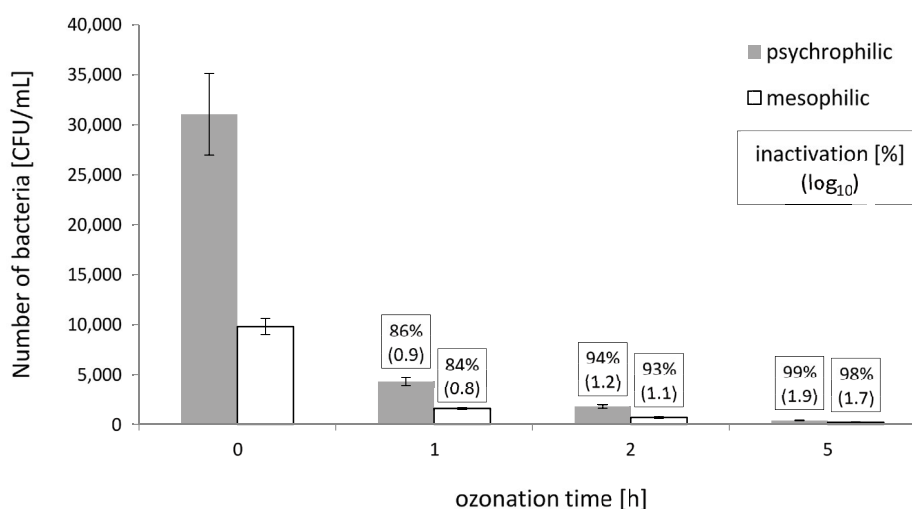


Fig. 1. Number of culturable bacteria [CFU mL<sup>-1</sup>] and inactivation (percentage and log in parenthesis) in treated wastewater during the ozonation process. Error bars illustrate standard errors.

Table 1

Total ATP (tATP), free-available ATP (fATP), intracellular ATP (cATP), reflecting total live biomass (both culturable and unculturable), inactivation (percentage and log in parenthesis) and percentage of bacterial cells detected by the colony count method (CFU/ME) in treated wastewater during ozonation process

Ozonation time [h]	tATP [ME mL <sup>-1</sup> ]	fATP [ME mL <sup>-1</sup> ]	cATP [ME mL <sup>-1</sup> ] (inactivation [%]; log)	CFU/ME [%]
0	$7.7 \times 10^6$	$0.05 \times 10^6$	$7.7 \times 10^6$	0.5
0.25	$7.6 \times 10^6$	$0.17 \times 10^6$	$7.4 \times 10^6$ (4%; 0.02)	n.d.
1	$4.8 \times 10^6$	$0.20 \times 10^6$	$4.6 \times 10^6$ (40%; 0.2)	0.1
2	$1.9 \times 10^6$	$0.27 \times 10^6$	$1.7 \times 10^6$ (78%; 0.7)	0.1

n.d. – not determine

showing even more than 90% of microorganisms remained in the post-disinfection effluent, depending on the environmental conditions and parameters of the treated wastewater. Additionally, Lee et al. [15] demonstrated that the UV method could not control the regrowth of the microbial cells after the disinfection process in a WWTP.

In this study, *E. coli* was used as a representative microorganism to examine the sanitary aspects of treated wastewater disinfection. Despite this bacterium does not produce spores, its inactivation was not efficient enough, either, and resulted in a significant number of viable bacterial cells present in the treated wastewater after 2 h of disinfection (Fig. 2a). Since much higher inactivation of *E. coli* was expected in a shorter period of time, additional experiments were carried out to examine the influence of micropollutants present in wastewater and the effect of bacterial forms other than single cells on the disinfection process. In sterile tap water spiked with *E. coli* suspension, complete inactivation of *E. coli* cells was observed after 1 h ozonation (Fig. 2b). However, similar efficiency of disinfection was not obtained for treated wastewater spiked with the same *E. coli* suspension (Fig. 3b).

Two aspects should be considered to explain the more efficient inactivation of *E. coli* in tap water than in treated wastewater: (i) dissolved organic matter in treated

wastewater can partly use ozone, which makes it insufficient to inactivate bacteria and (ii) different forms and properties of *E. coli* cells in the laboratory pure culture and in WWTPs effluent. Ozone undergoes reactive diffusion into bacterial cells to inactivate them. Cho et al. [27] suggested that the extent of reaction compared to diffusion determines the mechanism of bacterial cell inactivation by disinfectant. In the case of ozone, which is a highly reactive oxidant, its diffusion in treated wastewater into cell plasma is retarded by the presence of dissolved organic substances with which ozone reacts immediately. Therefore, the lower inactivation of bacteria than expected may be due to ozone consumption for the oxidation of chemical compounds contained in wastewater. With a relatively small dose of ozone used in the study, the concentration of disinfectant could be insufficient. Nasuhoglu et al. [13] demonstrated that higher ozone doses were required in the case of higher total and dissolved organic load in disinfected effluents.

The second aspect of the higher efficiency of disinfection in tap water is related to the use of suspension of laboratory cultured *E. coli* in testing. Single cells are more susceptible to attack by ozone because no shielding of bacteria from the disinfectant is possible, as is the case in WWTPs effluent containing activated sludge flocs

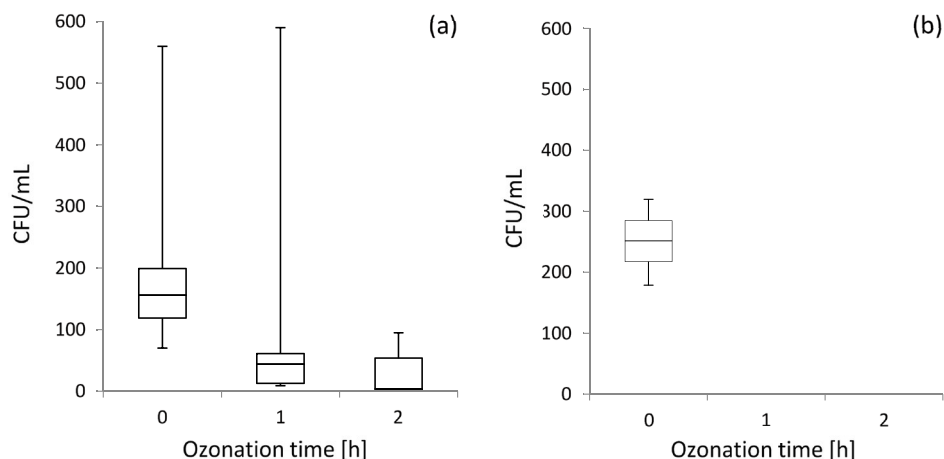


Fig. 2. (a) Inactivation of *E. coli* cells in treated wastewater and (b) sterile wastewater spiked with *E. coli* suspension. The bottom and top of each box are the first and third quartiles, the band inside the box is the median, the whiskers represent the minimum and maximum values of each data set.

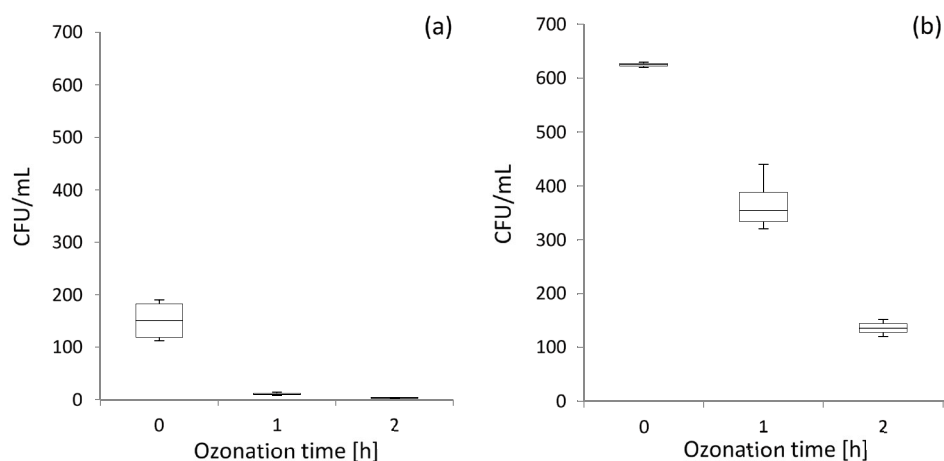


Fig. 3. (a) Inactivation of *E. coli* cells in treated wastewater and (b) treated wastewater spiked with *E. coli* suspension. The bottom and top of each box are the first and third quartiles, the band inside the box is the median, the whiskers represent the minimum and maximum values of each data set.

or other agglomerates of bacterial cells. This hypothesis is confirmed by a linear decrease in the number of CFU during the disinfection of treated wastewater, spiked with the same *E. coli* suspension (Fig. 3b). The laboratory cultured *E. coli* cells, artificially added to wastewater, have the same sensitivity to ozone throughout the duration of the experiment. In turn, a clear decrease in the rate of disinfection is observed for treated wastewater not spiked with *E. coli* lab culture (Fig. 3a), which can be attributed to the lower efficiency of the inactivation of bacterial cells in aggregates, shielded from ozone. The laboratory strain did not seem to possess such properties and showed similar sensitivity to the disinfectant over time. In order to confirm this hypothesis, however, a longer experiment should be carried out to determine whether the rate of bacterial inactivation in wastewater spiked with *E. coli* suspension remains constant until all single cells are completely inactivated. Furthermore, individual bacterial cells can be

released from activated sludge flocs during the oxidizing attack of ozone, leading to positive responses when enumerating microorganisms by the colony count method. Additionally, indigenous bacteria in wastewater can have different resistance to disinfectants when compared to laboratory strains as bacterial inactivation efficiency has been shown to be influenced by cell size, shape, and membrane composition of the bacterial community [28].

Due to the high oxidation-reduction potential of ozone, cell surface damage is more pronounced with ozone, where as damage in inner cell components is more apparent with weaker oxidants which have limited reactions with cell surface components and relatively effectively reach the cell plasma. Solubility and stability, and therefore oxidant action of ozone in water, correlate with a lower diameter of gas bubbles [29] and there is an ongoing survey on ozone micro and nanobubble technologies [30,31], which indicates that there are more factors that may affect the process.

### 3.2. Ecotoxicity of disinfected wastewater

Non-disinfected wastewater and wastewater ozonated for 1 h stimulated growth of green algae in concentrations up to 25%, whereas toxic effects (up to 22% and 27% growth inhibition, respectively) were observed only for higher concentrations. A sharp increase in toxicity towards *D. quadricauda* was observed for 2 and 5 h ozonation times, resulting in at least acute toxicity (Table 2) and complete inhibition of algal growth within the tested ranges of wastewater concentrations (Fig. 4). This indicates the formation of toxic DBPs. Typical ozonation by-products include formaldehyde, acetaldehyde, glyoxal, and methylglyoxal. Silva et al. [32] demonstrated that the formation of DBPs changed with the dose of ozone, however, it did not depend to a large extent on contact time. The concentrations of DBPs in their study did not exceed the permissible levels proposed in the WHO guidelines, but the ecotoxicity was not investigated.

The growing toxic effect along with the increase in wastewater concentration in relation to *Daphnia* was clearly observed for 5 h ozonation time (Fig. 5).  $LC_{50}$  which was calculated after 48 h of contact with disinfected wastewater samples (Table 2), was 78%, resulting in 1.3  $TU_d$  which reflects acute toxicity in accordance to hazard classification system for waste discharged into the aquatic environment [24]. However, no significant increase in ecotoxicity in comparison to non-disinfected wastewater was observed for 1 h and 2 h ozonation times – the samples showed no acute toxicity or slight toxicity (Table 2). Park et al. [17] demonstrated that wastewater effluent exposed for 15 min to much higher ozone dose ( $0.8 \text{ mg O}_3\text{-min L}^{-1}$ ) was not toxic to *D. magna*. This suggests that the formation of toxic DBPs depends not only on the ozone dose but also on contact time and/or effluent composition.

Non-disinfected treated wastewater significantly stimulated the bioluminescence of *A. fischeri* and the effect was the stronger the higher the concentration of the effluent was (Fig. 6). However, 2 and 5 h ozonation times triggered

ecotoxicity towards *A. fischeri* – the calculated  $EC_{50}$  after 5 min exposition of bacteria to disinfected wastewater was 30% and 17%, respectively, resulting in Class III ecotoxicity (Table 2). Bioluminescence inhibition of *A. fischeri* (MicroTox test) increased with increasing ozone reaction time also in the laboratory study of Tang et al. [16]. However, the authors claimed that these observations contradicted the results observed in the pilot study and assumed that the effluent properties had been changed during the freezing and thawing process before the ozone experiments were carried out in the laboratory. On the other hand, Li et al. [18] used similar tests (LUMIStox) to show that first by-products of antibiotic oxytetracycline after partial ozonation (5–30 min,  $11 \text{ mg O}_3 \text{ L}^{-1}$  in the gas phase) were more toxic than the parent compound. Nasuhoglu et al. [13] demonstrated that in order to reduce the effluent toxicity to less than the target inhibition of 20%, ozone doses in the range of  $0.7\text{--}1.8 \text{ g O}_3 \text{ g}^{-1}$  DOC were required, which were slightly larger than the ozone doses required only for disinfection.

Results of ecotoxicological studies showed that ozonation significantly increased the ecotoxicity of treated wastewater. This suggests that DBPs were formed during the disinfection, which changed the toxicity class of the tested treated wastewater from the completely non-toxic for bacteria (containing virtually no toxic chemicals) or slight toxic for algae and crustaceans, to the acute toxicity hazard category or, in the case of algae, even to the high or the very high toxicity class (Table 2). Furthermore, there was a clear relationship between the time of ozonation and the increase in DBPs ecotoxicity – the most harmful effects were observed for treated wastewater which was ozonated for 5 h. The most sensitive bioindicator to toxic DBPs after ozonation was bacteria *A. fischeri* used in DeltaToxII.

### 4. Conclusions

There is scientific evidence that ozonated treated wastewater may be toxic to organisms. The results obtained in

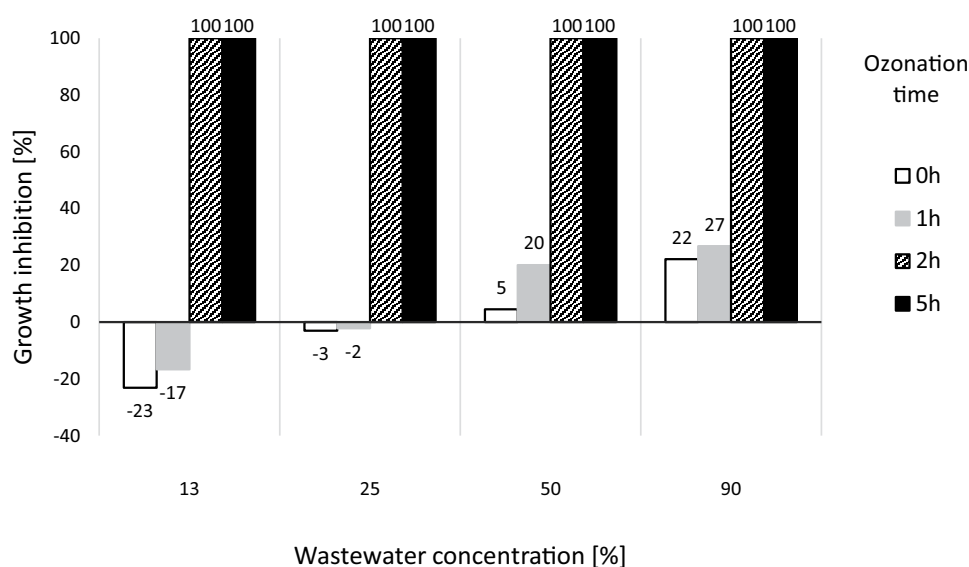


Fig. 4. Growth inhibition of *Desmodesmus quadricauda* after 72 h contact with disinfected treated wastewater, depending on wastewater concentration and ozonation time. Negative values of inhibition represent growth stimulation.

Table 2

Ecotoxicity assessment of treated wastewater before and after ozonation, based on the hazard classification system for waste discharged into the aquatic environment [24]

Ozonation time [h]	Parameter	<i>Desmodemus quadricauda</i>	<i>Daphnia magna</i>	<i>Aliivibrio fischeri</i>
0	LC(EC) <sub>50</sub> -t [%]	>89.6	>100	not defined
	TU <sub>a</sub>	<1.1	<1	0
	Toxicity class	I or II	I or II	I
	Acute toxicity assessment	no toxicity or slight toxicity	no toxicity or slight toxicity	no toxicity
1	LC(EC) <sub>50</sub> -t [%]	>89.6	>100	>81.9
	TU <sub>a</sub>	<1.1	<1	<1.2
	Toxicity class	I or II	I or II	I or II
	Acute toxicity assessment	no toxicity or slight toxicity	no toxicity or slight toxicity	no toxicity or slight toxicity
2	LC(EC) <sub>50</sub> -t [%]	<12.5	>100	30
	TU <sub>a</sub>	>8	<1	3.3
	Toxicity class	III or IV or V	I or II	III
	Acute toxicity assessment	toxicity, high or very high toxicity	no toxicity or slight toxicity	toxicity
5	LC(EC) <sub>50</sub> -t [%]	<12.5	78	17
	TU <sub>a</sub>	>8	1.3	5.9
	Toxicity class	III or IV or V	III	III
	Acute toxicity assessment	toxicity, high or very high toxicity	toxicity	toxicity

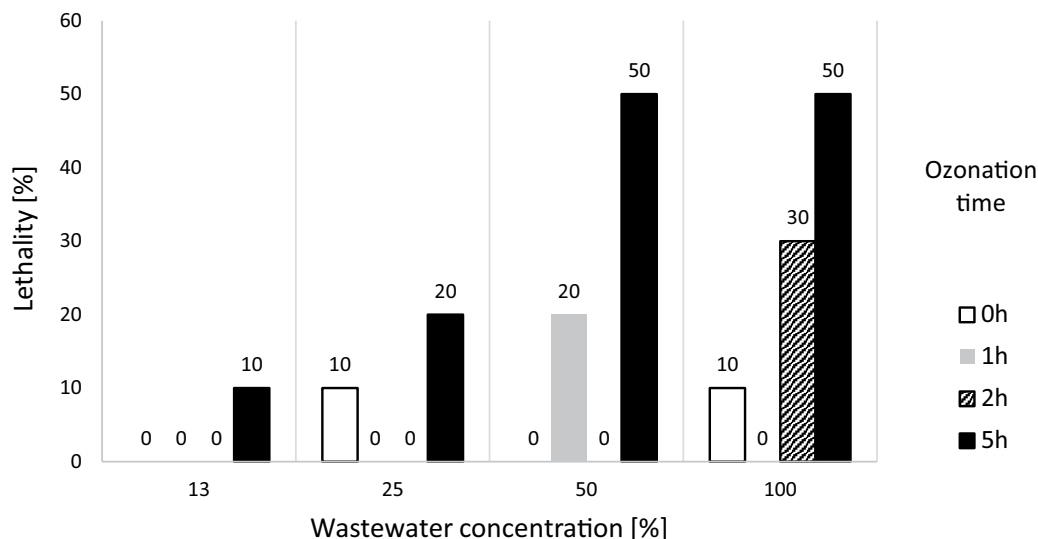


Fig. 5. Lethality of *Daphnia magna* after 48 h contact with disinfected treated wastewater, depending on wastewater concentration and ozonation time.

this study clearly imply that, under the conditions considered in the tests, toxicity increased with increasing ozonation time. This suggests that during the process of ozonation, by-products were formed which were toxic to bacteria, algae, and crustaceans. Further studies, including the use of a wider range of bioindicators in a larger number of testing series, as well as parallel detailed analyses of

the chemical composition of treated wastewater before and after disinfection, are needed to determine in detail which DBPs cause a significant increase in toxicity.

The effectiveness of the disinfection of treated wastewater by ozonation depends on the presence of other contaminants that may first react with the disinfectant. Cells that form colonies in agglomerates, sludge flocs, or other



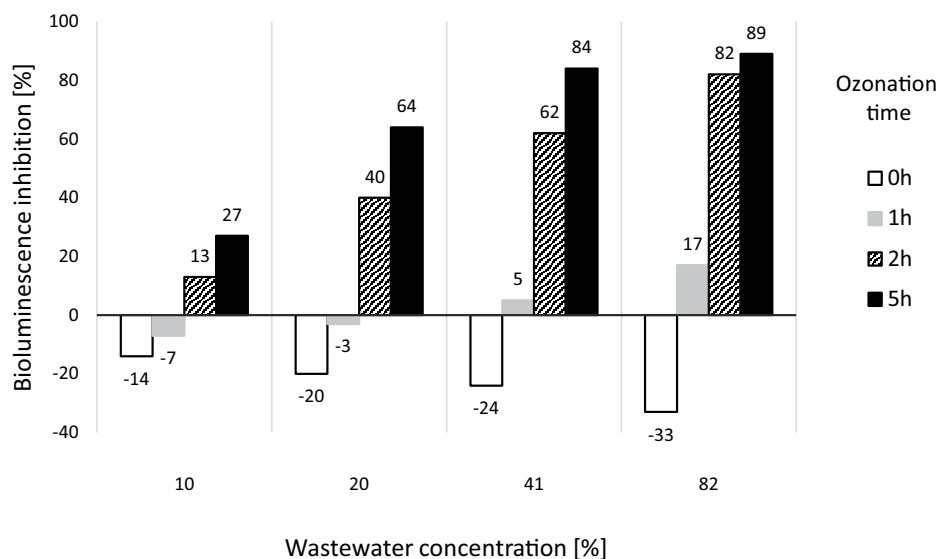


Fig. 6. Bioluminescence inhibition of *Aliivibrio fischeri* after 5 min contact with disinfected treated wastewater, depending on wastewater concentration and ozonation time. Negative values of inhibition represent bioluminescence stimulation.

particles of suspension, may be shielded and protected against the action of a disinfectant, and tests using higher doses of ozone should be performed.

Current lack of regulations on microbiological parameters of effluents from WWTPs in many countries is not conducive to investing in the purchase of disinfection equipment by municipal enterprises. Still, the primary goal of wastewater disinfection remains the same: protecting human health against microbial infections and reducing human and environmental exposure to DBPs.

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