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Comparison of sulphonamides decomposition efficiency in ozonation and enzymatic oxidation processes

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Abstract: Sulphonamides (SAs) are one of the most frequently detected anthropogenic micropollutants in the aquatic environment and their presence in it may pose a threat to living organisms. The aim of the study was to determine susceptibility of selected sulphonamides, i.e. sulfadiazine (SDZ) and sulfamethazine (SMZ), to degradation in the ozonation process and in enzymatic oxidation by unspecific peroxygenase extracted from *Agrocybe aegerita* mushroom (*Aae*UPO). Moreover, the acute toxicity of the aqueous solution of the selected sulphonamides (SMZ and SDZ) before and after mentioned treatment processes were studied on the freshwater crustacean *Daphnia magna.* Initial concentrations were equal to 2×10^{-5} M for sulfadiazine and 1.8×10^{-5} M for sulfamethazine. The percentage of transformation for the O_3 process was at the level 95% for both SDZ and SMZ (after 10 s of the process), whilst enzymatic oxidation of SDZ and SMZ by *Aae*UPO caused transformation efficiencies at the levels of 97% and 94% (after 1 minute of the process), respectively. The second order rate constants of selected sulfonamides with molecular ozone and fungal peroxidase were also determined in the research. EC_{50} (median effective concentration) values from toxicity test on *D. magna* were found in the range from 14.6% to 37.2%, depending on the type of the process. The conducted oxidation processes were efficient in degradation of selected sulphonamides. The toxicity of the mixtures before and after treatment was comparable and did not change significantly. The research have shown that biological processes are not always safer for living organisms compared to the chemical processes.

Introduction

Conventional wastewater treatment techniques rely on processes such as: biological oxidation, membrane separation, adsorption, air stripping, etc. and they are designed to effectively remove organic and inorganic pollutants typical for wastewater. However, very often, they don't allow for complete degradation of anthropogenic micropollutants, which include, for example, human and veterinary pharmaceutical residues, including sulphonamides. Therefore, the micropollutants might be re-emitted to the environment again after treatment, which may pose a serious threat to organisms living in water reservoirs, and thus cause serious ecological threats. For that reason, there is an emergent demand for improving and developing technologies for water treatment, human consumption, re-use and wastewater discharge (Hester and Harrison 2015, Yang 2017). Although since the year 2006, the use of the antibiotics (include sulphonamides) as additives in animal nutrition is strictly prohibited, these types of substances are still used to treat or to prevent bacterial infections in animal husbandry, which make that manure, wastewater and other types of waste from agriculture constitute one of the sources of sulphonamides in the environment (Felis et al. 2020). From the chemical point of view, SAs are sulfanilic acid amides, which are used in human and veterinary medicine as components of bacteriostatic medicines or ingredients of disinfectants. In the environment, sulphonamides can remain bioactive and undergo various types of transformations, not losing their activity (Fletcher 2015, Hester and Harrison 2015, Feng 2020), which can cause toxic or harmful effects on the different organisms including aquatic species (Santos 2010). Even though they are not currently considered as priority substances under the

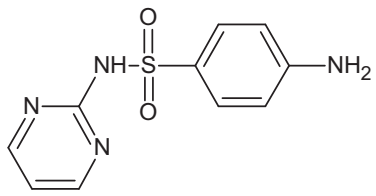
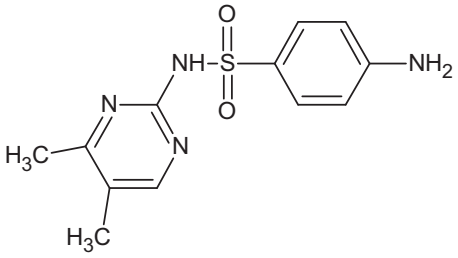
EU Water Framework Directive (WFD), the data suggests that there are concerns for aquatic and terrestrial ecosystems (Voulvoulis 2014, Hester and Harrison 2015). Some of the antibiotics were included in the first Watch List, and after its review, in the second Watch List under the Water Framework Directive, but sulphonamides were still not included in it, although their detection frequency in selected media of the aquatic environment reaches the level of 83% (Loos et al. 2018).

The selected sulphonamides (SAs) as: sulfamethazine (SMZ) and sulfadiazine (SDZ) (Table 1) are the oldest chemotherapeutic agents used for antimicrobial therapy mostly in veterinary treatment rather than in human medicine. Therefore, the residues of these compounds, alone or in combinations, have been repeatedly detected in the various compartment of the environment (De Liguoroa 2009, Hester and Harrison 2015, Tahergorabi 2019, Bílková 2019). For example, Zhang et al. (2016) observed that sulfadiazine (396 ngL^{-1}) and sulfamethazine (382 ngL^{-1}) were the dominating antibiotics in the raw wastewater and their removal during wastewater treatment was less than 50%.

Ozonation is a well-known technology, which has proven to be an option for water treatment and disinfection. Ozone is doubly beneficial in water treatment because it is not only a disinfectant, but also a powerful oxidant. It can directly and/or indirectly oxidize various classes of recalcitrant compounds (Huber 2005, Bourgin 2017, Paździor et al. 2019), therefore this substance was selected for the research as a chemical oxidant. Ozone can be used separately or in combination with e.g. ultraviolet or gamma radiation, ultrasounds, Fenton reagent for hydroxyl radical production. The highly oxidative hydroxyl radical can ultimately lead to complete mineralization of organic substances through formation of CO_2, H_2O and mineral acids (von Sonntag 2012, Hester and Harrison 2015, Yang 2017). However, it should be noted that every change in the composition of the medium (e.g. water, wastewater) in which the ozonation is conducted may impact on the final efficiency of the process (von Sonntag 2012). Despite the relatively high costs of applying ozone for tertiary treatment, it is already successfully used in highly developed countries, i.e. Switzerland, USA and Germany (Rakness 1996, von Sonntag 2012, Bourgin 2018). Nonetheless, special attention should be paid to the risk of by-products formation, which may cause potential toxic effect on the living organisms (Bourgin 2017). In example, during ozonation relatively high bromide concentrations in the raw medium may lead to the formation of bromate, which is a potential human carcinogen (Huber 2005, Bourgin 2017).

For this reason, an alternative might be the enzymatic oxidation. It was observed that the selected fungal strains can effectively degrade selected pharmaceuticals (Aracagök et al. 2018). Fungal enzymes also appear to be particularly promising in this issue, for example heme-thiolate peroxidase, which is unspecific peroxygenase extracted from the Black poplar mushroom *Agrocybe aegerita* (*Aae*UPO EC 1.11.2.1) discovered over decade ago (Ullrich 2004). Unspecific peroxygenases (UPOs) have remarkable catalytic properties and might be a powerful biocatalytic tool for biotechnological applications. These enzymes catalyse various reactions such as peroxygenation, brominations or one-electron abstraction in the oxidation process (Anh 2007, Pecyna 2010, Hofrichter 2010). Additionally, *Aae*UPO mimics a number of P450 cythochrome monoxygenase reactions (inactivation of toxicants and carcinogens) and it is active over a wide pH range (3–10), with maximum activity at pH equal to 5.5 and 7 (Hofrichter 2010). Thanks to these features, peroxygenase isolated from *A. aegerita* may play an important role in degradation of intractable compounds from the environment, such as phenol, toluene, naphthalene, polycyclic aromatic hydrocarbons

Table 1. Basic data on sulfonamides selected for research

Name	Sulfadiazine	Sulfamethazine
Chemical formula	$C_{10}H_{10}N_4O_2S$	$C_{12}H_{14}N_4O_2S$
Structure		
CAS number	68-35-9	57-68-1
Molecular weight (g/mol)	250.278	278.33
Bacteria spectrum	most Gram+ and many Gram- (PABA synthesis inhibitor)	most Gram+ and many Gram- (PABA synthesis inhibitor)
Use	Human medicine: rheumatic fever; meningococcal meningitis; toxoplasmosis; burn wound infections Veterinary: prevent & treat diarrhoea, growth promoters	Human medicine: treatment bacterial infections causing bronchitis, prostatitis and urinary tract infections Veterinary: treatment of bacterial pneumonia, metritis, foot-rot, diphtheria, and other large animals infections

(PAHs) or many pharmaceuticals (Poraj-Kobielska 2011). UPOs are extracellular highly glycosylated and stable proteins and they do not need any other cofactors to be active than hydrogen peroxide. Due to this fact, they might find application in many fields related to biotechnology, environmental engineering and others (Kinne 2009, Hofrichter 2010). In the case of the oxidation by fungal enzyme obtained from *Agrocybe aegerita* one of disadvantages is that it has relatively narrow catalytic cleft. Another one is the high general activity of UPO enzymes, which need the inclusion of a radical scavenger (Poraj-Kobielska 2011). In addition, the purification of the enzyme demands the usage of an advanced instrumental techniques and apparatus that generate costs of the entire process (Ulrich 2004). For this reason, it is important to find possibilities to apply the enzymes in a more economical way, either by using crude preparations or extending their "life-span", for example, by immobilization and/or re-use over several cycles (Poraj-Kobielska 2011).

While for ozonation numerous of publications show it usefulness in decomposition of pharmaceuticals, little is known on processes incorporating enzymes obtained from *Agrocybe aegerita* mushroom. No less, the enzymatic treatment arouses an increasing interest, especially in case of enzymes that belong to laccases group (Rodríguez-Rodríguez 2012, Schwarz 2010, Cruz-Moratóa 2014). The novelty of this study relies on direct application of peroxygenase *Aea*UPO isolated from *Agrocybe aegerita* mushroom for degradation of selected sulphonamides as an example of the anthropogenic micropollutants. Therefore, the main goal of this study is to compare the oxidation efficiency of sulfadiazine (SDZ) and sulfamethazine (SMZ) by ozone and unspecific peroxygenase *Aea*UPO. Furthermore, the usefulness of the tested enzyme (*Aae*UPO) for treatment aqueous media containing the selected sulphonamides was examined as well. The acute toxicity tests of the raw aqueous media containing antibiotics (SMZ and SDZ), and the media after ozonation and enzymatic processes were performed on the freshwater crustacean *Daphnia magna.* Admittedly, the ecotoxicity tests of selected sulphonamides have already been carried out on aquatic organisms such as crustaceans, but they mainly focused on the acute effects of selected pharmaceutical solution in MilliQ or distilled water, not a post-reaction mixture. Only a few studies determined the toxic effect of sulphonamides when released into the environment (Dalla Bona 2014, De Liguoroa 2009, Anskjær 2013). The standard toxicity tests are needed to indicate if the substances and their by-products have a potential to cause adverse effect on the aquatic environment. Therefore, an additional aim of this study was to compare toxicity for a single antibiotic compound in two different mediums: a pure solution in MilliQ (De Liguoroa 2009) and the pharmaceutical suspension in aqueous solution.

Materials and methods

Materials and analytical procedures

The studies were carried out in the aqueous solutions, at fixed conditions that are consistent with general environmental parameters: pH ~7.0 stabilised by potassium phosphate buffer (50×10^{-3} M) and at a temperature of 18–20°C. Initial concentrations of each compounds used in the experiments were equal to 2×10^{-5} M for sulfadiazine and 1.8×10^{-5} M for sulfamethazine. All standards were purchased from Sigma Aldrich. The SAs concentrations were selected based on literature and previous experiments (Kümmerer 2010, Lemańska-Malinowska 2013, Hester and Harrison 2015, Yang 2017).

The concentration of the investigated compounds was measured by high performance liquid chromatograph (HPLC) equipped with diode array detector (DAD) (Agilent 1200 Infinity Series, Agilent Technologies, US). For the enzymatic oxidation and kinetic studies, a Nucleodur Phenyl--Hexyl column (4.6 mm diameter by 150 mm length, 3 µm particle size) was used for analysis. The column was run at 40°C and a flow rate of 0.5 $mLmin^{-1}$, with a mixture of ammonium formate (0.1×10^{-3} M, pH 9) and acetonitrile, 95:5, for 5 min, followed by a 10-min linear gradient to 100% acetonitrile. After the ozonation process the studied substances were analysed on column Betasil Phenyl-Hexyl (4.5×150; 4.6), which was eluted at 0.95 $mL\times min^{-1}$ and 20°C with aqueous vol/vol ammonium formate (0.1×10^{-3} M, pH 9)/acetonitrile, 95:5, for 2 min then followed by a 1-min linear gradient to 1.5 $mL\times min^{-1}$ flow rate and 10% acetonitrile for 5 min. For determination of studied compound after ozone kinetic investigation, the column was eluted with a mixture of ammonium formate (0.1×10^{-3} M, pH 9) and acetonitrile, 80:20, at isocratic flow of 1 $mL\times min^{-1}$. The limit of quantification (LOQ) for all standards in all used chromatographic methods was equal to 250 μgL^{-1}.

Ozonation

Ozone stock solution was produced by bubbling O_3-containing oxygen through MilliQ water (pH~3) that was placed in the cooling bath. The bath steady temperature (~1°C) was maintained in order to achieve a concentration of ozone at the level of 4×10^{-4} M, which is a typical concentration as used in stock solutions. (Bertanza et al. 2011). Determination of the ozone concentration in the stock solution was monitored spectrophotometrically at 260 nm (von Sonntag 2012) and by the indigo method (Bader 1982). Due to the studied compound spectral interference, the spectrophotometric measurements of the tested mixtures were not possible. The reaction mixture contained the investigated substance (SDZ or SMZ), the potassium phosphate buffer mentioned previously (section 2.1) and bicarbonate in concentration of 5.7×10^{-3} M_{HCO3}-. The bicarbonate was used here as a radical scavenger. In the studies, three ozone doses were investigated, this is: 2×10^{-5} M_{O3}, 6×10^{-5} M_{O3} and 1×10^{-4} M_{O3}. The composition of the reaction mixtures in ozonation experiments is presented in Table 2.

Enzymatic experiments

Enzymatic oxidation of selected sulphonamides was carried in the mixture containing the substrate (SDZ or SMZ), potassium phosphate buffer mentioned above, *A. aegerita* peroxygenase (8.52×10^{-6} M) purified according to the procedure described by Ulrich (2004), ascorbic acid (4×10^{-3} M_{C6H8O6}) as radical scavenger and hydrogen peroxide (2×10^{-3} M) as a cofactor. The composition of the reaction mixtures in enzymatic experiments is presented in Table 2.

Table 2. The composition of the medium in the research on the decomposition of selected sulphonamides in the process of ozonolysis and enzymatic oxidation

Component	Ozonation		Enzymatic oxidation	
	SDZ	SMZ	SDZ	SMZ
Substrate, M	2×10^{-5}	1.8×10^{-5}	2×10^{-5}	1.8×10^{-5}
KP buffer	maintaining pH = 7	maintaining pH = 7	maintaining pH = 7	maintaining pH = 7
O_3 stock solution, M	1×10^{-4}	1×10^{-4}	–	–
HCO_3^-, M	5.7×10^{-3}	5.7×10^{-3}	–	–
Vit C, M	–	–	2×10^{-2}	2×10^{-2}
H_2O_2, M	–	–	1×10^{-2}	1×10^{-2}
*Aae*UPO, M	–	–	0.8×10^{-3}	0.8×10^{-3}
Water	adjustment	adjustment	adjustment	adjustment

The extracellular peroxygenase of *Agrocybe aegerita* (*Aae*UPO, isoform III, 46 kDa) specific activity was equal to 48 Umg^{-1}, where 1 U represents the oxidation of 1µmol of veratryl alcohol to veratrylaldehyde in 1 min in 23°C (Ulrich 2004). The specific activity of the *Aae*UPO was determined spectrophotometrically coupled with Bradford assay (Ulrich 2004). Amounts of the reactants were used in accordance to the procedure proceed by the International Institute in Zittau and the US 2010/0279366 patent (Pecyna 2010).

Determination of kinetic constants in ozonation process

For determination second-order rate constants of studied SAs (i.e. SDZ and SMZ) with molecular ozone, the rules of competition kinetic were exerted. For SDZ and SMZ, sulfamethoxazole (SMX) was selected as a reference compound because of its structure, similar reaction mechanism and known rate constant with a molecular ozone ($k_{O3} = 2.5\times10^6$ $M^{-1}s^{-1}$) (Huber 2004). The experiments were carried out at pH = 7, with solution containing equal concentrations of test substance (SDZ or SMZ) and SMX as the reference compound (2×10^{-5} M). Different understechiometric concentration levels of ozone, from 20.8×10^{-6} M to 10.4×10^{-4} M were used in the experiment. During ozone insertion to the reaction vials, the solutions were vigorously stirred at 350 rpm. Data obtained from HPLC measurements were evaluated based on Eq. (1), where $k_{O3}(R)$ and $k_{O3}(M)$ are the rate constants for the reference (R) and target compound (M), respectively. The different ozone doses are represented as n.

$$ln\left(\frac{[M_{(n)}]}{[M_{(0)}]}\right) = ln\left(\frac{[R_{(n)}]}{[R_{(0)}]}\right)\frac{k_{O3}(M)}{k_{O3}(R)} \quad (1)$$

The apparent rate constant $k_{O3}(M)$ was determined from a plot of $ln([M(n)/[M(0)])$ versus $ln([R(n)]/[R(0)])$ with $k_{O3}(M)/k_{O3}(R)$ as the slope of the linear function.

Determination of kinetic constants in the enzymatic process

The experiments, in order to determine the parameters of the enzymatic process were performed at stirred microscale reactors (0.2 mL, 23°C). To the microreactors were added: 0.8×10^{-3} M peroxygenase (*Aae*UPO), potassium phosphate buffer (50×10^{-3} M, pH 7, ascorbic acid (4×10^{-3} M) and respectively from 0.025×10^{-3} M to 1.6×10^{-3} M of SDZ or from 0.025×10^{-3} M to 2×10^{-3} M of SMZ. Enzymatic reactions were initiated by adding of H_2O_2 (2×10^{-3} M) and stopped by adding 0.02 mL of 50% TCA (trichloroacetic acid). The reactions with sulfadiazine were stopped after 10 s whilst reactions with sulfamethazine were stopped after 5 s. The termination time with *Aae*UPO was set based on the substrates concentrations decrease time curves. The kinetic parameters were determined by nonlinear regression using the Michaelis-Menten model in ANEMONA program (Hernanndez and Ruiz 1998).

Toxicity tests

The effects of the pharmaceutical aqueous solutions were tested in accordance to the OECD 202 standard procedure (OECD 2004). The OECD standard methods are commonly used to determine the safety of chemicals and their by-products produced in WWT (wastewater treatment) processes. *Daphnia magna* were continuously cultured in laboratory at School of Life Science (Heriot-Watt University, UK). The culture medium (M7) containing the macro-nutrients and vitamin was prepared in accordance with the OECD guideline and it was renewed two times each week. The test organisms were fed daily with algae *Chlorella vulgaris*. The culture was maintained at a constant temperature (21±1°C) with 8/16 hour's light-dark cycle.

Semi static acute toxicity test of samples were conducted based on the OECD standard procedure (OECD 202 2004). Seven test concentrations (from 5–51%; volume/volume) plus a blank control (0%; v/v) were used in the experiment. Test solutions of pharmaceuticals were prepared immediately in prior to use by diluting the stock solution with *daphnia* culture medium (so-called M7 medium), reconstituted according to standard guideline of OECD (OECD 202 2004). As an endpoint, concentration causing 50% immobilization of tested *D. magna* population has been set (EC_{50}). For each concentration, ten neonates (6–24 h old), from a designated brood, were placed in polypropylene tubes containing 40 mL of the test solution. *D. magna* organisms were not fed during the testing period. After an exposure for 24 h and 48 h, the immobilization rate for individuals in each container were calculated. The freshwater crustaceans, which were unable to swim after 15 s of a gentle

agitation of the test container, were considered as immobile. All experiments were replicated at least three times. The 24 h and 48 h EC50 (immobilization) values were calculated using SigmaPlot® software.

Results and discussion

Comparison of the efficiencies of SDZ and SMZ degradation in the ozonation and the enzymatic oxidation processes

Several authors have confirmed that the ozonation may be an effective method in the degradation of selected sulfonamides (Garoma 2010, von Sonntag 2012, Tahergorabi 2019, Pelalak et al. 2020). Research confirms that molecular ozone can react first with the amine group that is characteristic of sulfonamides. The decomposition of sulfonamides is possible in the absence of hydroxyl radicals, which was confirmed by studies conducted in the presence of bicarbonates as hydroxyl radical scavengers (Nanaboina 2010; von Sonntag 2012).

A similar observation can be made when analyzing the obtained results – the dose of 1×10^{-4} M ozone, in the presence of bicarbonates, gave the most satisfactory results in removal of both SDZ and SMZ. In such condition, after 10 s of the process, the removal efficiencies of SDZ and SMZ were equal to 95% and 96%, respectively (Figure 1). In experiments conducted without addition of bicarbonates, the removal efficiencies of these compounds after the same time of reaction was lower and they were equal to respectively for SDZ and SMZ (Figure 1). It is worth mentioning that the highest concentration of ozone used in the experiment fall within the lower range of economical doses used in water and wastewater treatment plants (Lee 2012; Reungoat 2012).

For enzymatic oxidation, the maximum percentage of degradation was at the level 97% in case of SDZ and 94% in the case of SMZ. However, it was reached after about a minute of the process. It can therefore be seen that the ozonation process is a process in which the degradation of selected SAs occurs almost instantaneously, while enzymatic oxidation requires a little more time. In terms of the selected compounds degradation efficiency, can be said, that both oxidation processes brought satisfactory results (~90%) (Figure 2), however, decomposition of SAs in the case of chemical oxidation takes place after 10 s.

In case of unspecific peroxygenase extracted from the *Black poplar mushroom* (*Agrocybe aegerita*) there is a data shortage showing the benefits of that kind of enzymatic treatment. To the best of our knowledge, there are lack of the publications in whose this type of enzyme to oxidize other anthropogenic pollutants was used. Therefore, it is very difficult to discuss the results obtained. Most publications are focused on the enzymes, which belong to the laccases group. Removal efficiency of different pharmaceuticals (i.e. tetracycline, endocrine disrupting compounds, and erythromycin) by the laccases enzymes in those cases is in the range from 30% to 83% (Rodríguez-Rodríguez 2012, Cruz-Moratóa 2014). For sulfadiazine, the removal efficiency by means of laccase enzyme obtained from *Trametes versicolor* was equal 80% (Cruz-Moratóa 2014), whereas in the studies presented here the investigated compound (SDZ) was transformed by *Aae*UPO almost completely. In case of sulfamethazine the complete removal of this pharmaceutical by the same laccase was achieved after 20 h of exposure (Garciá-Galán 2011), while enzyme *Aae*UPO needed less than 1 min for 94% transformation. Therefore, it can be assumed that the isolated enzyme is very promising when it comes to its use in the decomposition of SAs, and compared to other described enzymes is very effective, and the oxidation process takes place in a significantly shorter time.

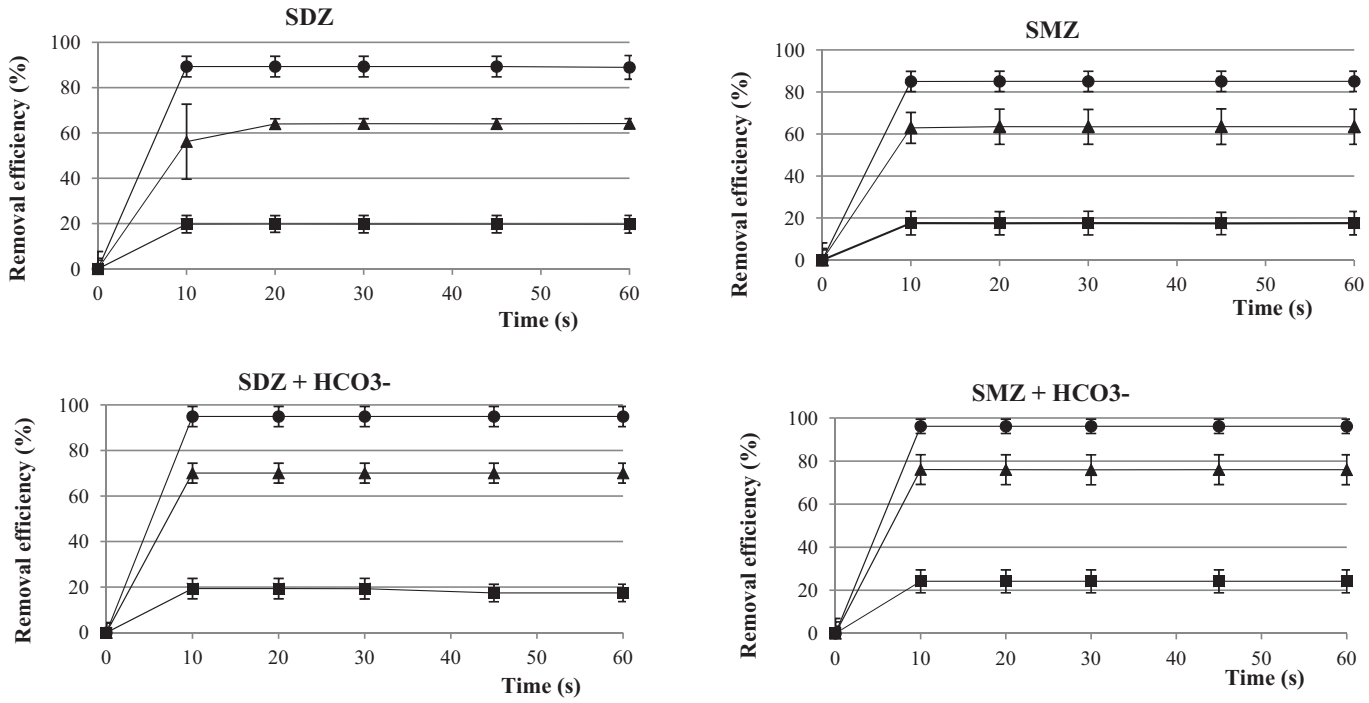


Fig. 1. Removal efficiencies of selected sulphonamides in the presence and absence of bicarbonates ($5.7\cdot10^{-3}$ M_{HCO3}-) in the ozonation process by various ozone doses ("■" – 2×10^{-5} M_{O3}/L; "▲" – 6×10^{-5} M_{O3}/L; "●" – 1×10^{-4} M_{O3}/L)

The research also allowed to determine the second order rate constants of selected sulfonamides in relation to molecular ozone and the enzyme (Table 3). Higher values of these constants, i.e. in the order of 1×10^6 M^{-1} s^{-1}, are observed for the ozonation reaction for both sulfonamides. In the case of enzymatic reactions, the rate constants remain at the level of 1×10^5 M^{-1} s^{-1}. It should be noted that the differences in the values of these constants in the ozonation process for both sulfonamides are almost the same. In the case of the enzymatic reaction, it can be seen that sulfamethazine is prone to enzymatic degradation more than sulfadiazine.

Toxicity assessment

In spite of relatively promising outcome from chemical and enzymatic oxidation processes, in concern of the transformation by-products effect on living organisms, the mixtures before and after oxidation processes were tested. On that account, the freshwater crustacean *Daphnia magna* were exposed to the sulphonamides mixtures. Effective concentration values (EC_{50}) were determined for all process options after 48 hours of exposure. The determined values of the EC_{50} parameters are shown in Figure 3. The values of estimated EC_{50} parameters were found in the range from

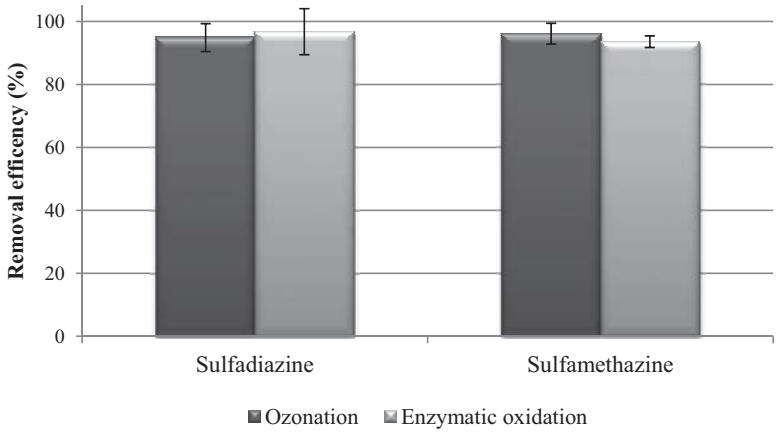


Fig. 2. Comparison of SDZ and SMZ removal efficiency in the enzymatic oxidation (0.8×10^{-3} M_{AaeUPO}, 4×10^{-3} M_{C6H8O6}) and ozonation processes (1×10^{-4} M_{O3}, 5.7×10^{-3} M_{HCO3}-)

Table 3. Second order rate constants of selected sulphonamides with molecular ozone (k_{O3}) and fungal peroxidase (k_{cat}) at pH=7

Compound	k_{O3} (M^{-1} s^{-1})	k_{cat} (M^{-1} s^{-1})
sulfadiazine	2.27×10^6	1.56×10^5
sulfamethazine	2.38×10^6	2.97×10^5

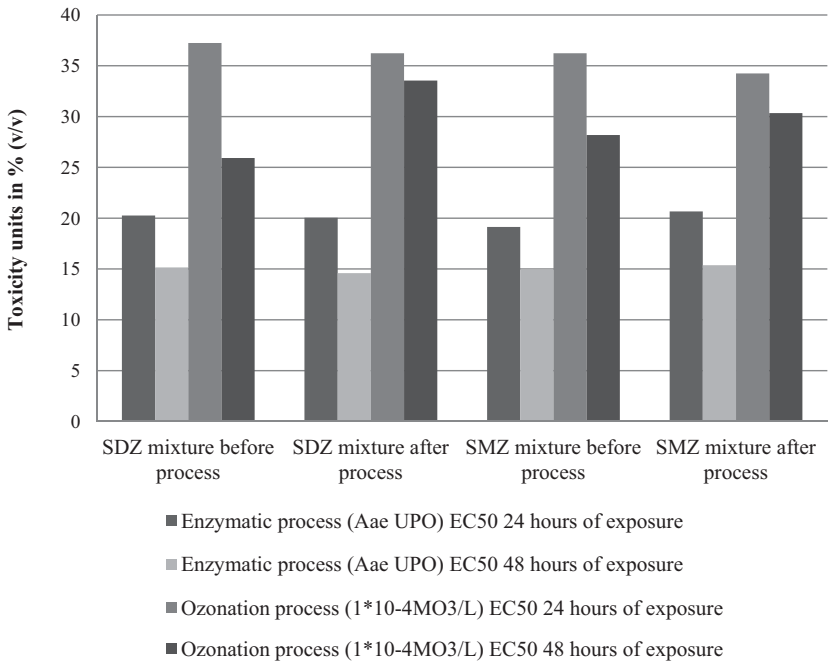


Fig. 3. *Daphnia magna* acute test: comparison of the determined EC_{50} parameters after 24 h and 48 h of exposure on the reaction mixtures before and after treatment processes

14.6% to 37.2% (volume/volume). No statistically significant changes in the toxicity of the mixtures before and after the processes were observed, although it can be seen that the mixtures used for the enzyme tests were more toxic compared to the mixtures after the ozonation process. Probably it is caused by the presence of the residues of hydrogen peroxide and ascorbic acid in the reaction mixtures. Perhaps it is also dependent on the fact that peroxidases isolated from *Agrocybe aegerita* mushroom have been used, and in some cases the products of transformations catalysed by fungal strains are more toxic than the substrate itself. However, this aspect of the work requires further research. Some data on acute toxicity of selected sulphonamides have already been published (De Liguoro 2009). The authors reported EC_{50} values after 48 hours of exposure to SMZ and SDZ as 202 mgL^{-1} and 212 mgL^{-1}, respectively. However, they relate to single substances dissolved in water, not to pre- and post--reaction mixtures. Taking into account initial concentrations of selected sulphonamides 2×10^{-5} M for SDZ and 1.8×10^{-5} M for SMZ that correspond to 5 mgL^{-1}, it may be concluded that obtained EC_{50} values were significantly lower than values presented in publication of DeLiguoro (2009).

The toxic effect data of SMZ and SDZ on *Daphnia magna* found in the literature is generally between 150–250 mgL^{-1} (Felis et al. 2020).

Unfortunately, there are no data that would make it possible to compare the toxicity of not individual drugs, but reaction mixtures, especially after processes using isolated fungal enzymes as oxidants. The present study showed that in the case of enzymatic processes, the mixture in which the process is carried out is over 200 times more toxic than aqueous solutions of the tested drugs, although the toxicity of this mixture does not change statistically significantly during the entire process. The toxicity of post-process mixtures cannot be compared. This observation proves how important it is from the environmental point of view, testing the ecotoxicity of mixtures (before and after the reaction), and not only of aqueous solutions of individual drugs. This also shows how important it is to compare the toxicity of the media before and after the process with each other, and not to relate the toxicity results obtained after the process to the toxicity results obtained in tests where the toxicity of aqueous solutions of the test drugs is tested.

Conclusions

The results show that two oxidation processes, i.e. ozonation and enzymatic oxidation, are efficient in elimination of selected sulphonamides from the aqueous solution. Other conclusions drawn on the basis of the tests carried out are as follows:

- The calculated values of the kinetic constant rates prove susceptibility of investigated sulphonamides to degradation as a result of both oxidation processes.
- Both conducted processes, ozonation and oxidation by *Aae*UPO, gave the elimination effectiveness of SDZ and SMZ in the range of 90%.
- Based on obtained results, it seems sufficiently precautionary to consider the toxicity of antibiotics in environmental matrix as additional tool to evaluate possible adverse effects on the living organism.
- The studies showed that the ecotoxicity of the reaction mixture (before and after the enzymatic oxidation process) is about 200 times higher compared to the toxicity of aqueous solutions of the tested drugs.
- In spite of the fact that both processes might be applicable to the decomposition of micropollutants from the aquatic environment, more studies containing investigation of by-products should be borne in mind.
- Based on the obtained results, it can be concluded that the methods used to eliminate micropollutants from the aquatic environment should be assessed not only in terms of their effectiveness, but also be assessed in terms of ecotoxicity.

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Porównanie efektywności rozkładu sulfonamidów w procesach ozonowania i utleniania enzymatycznego

Streszczenie: Sulfonamidy są jednymi z najczęściej wykrywanych mikrozanieczyszczeń antropogenicznych w środowisku wodnym, a ich obecność w nim może stanowić zagrożenie dla organizmów żywych. Celem badań było określenie podatności wybranych sulfonamidów, tj. sulfadiazyny i sulfametazyny na degradację w procesie ozonowania i utleniania enzymatycznego przez nieswoistą peroksygenazę ekstrahowaną z grzyba *Agrocybe aegerita* (*Aae*UPO). Ponadto badano toksyczność ostrą wodnego roztworu wybranych sulfonamidów przed i po wspomnianych procesach na słodkowodnym skorupiaku *Daphnia magna*. Początkowe stężenia wynosiły odpowiednio: 2×10^{-5} M (sulfadiazyna) i $1{,}8\times10^{-5}$ M (sulfametazyna). Procent transformacji dla procesu O_3 kształtował się na poziomie 95%, zarówno dla sulfadiazyny, jak i sulfametazyny, natomiast enzymatyczne utlenianie przez *Aae*UPO spowodowało odpowiednio transformacje badanych leków na poziomie 97% (sulfadiazyna) i 94% (sulfametazyna). W badaniach wyznaczono drugorzędowe stałe szybkości reakcji badanych sulfonamidów z ozonem cząsteczkowym i peroksydazą grzybową. Wartości EC_{50} z badań ekotoksyczności na *D. magna* wahały się od 14,6% do 37,2%, w zależności od rodzaju procesu. Wybrane do badań procesy utleniania okazały się być skuteczne w degradacji (transformacji) badanych sulfonamidów. Toksyczność mieszanin przed i po procesach była porównywalna, i nie zmieniała się znacząco, jednak to mieszaniny przed i po procesach enzymatycznej oksydacji charakteryzowały się wyższą toksycznością w porównaniu do mieszanin przed i po procesie ozonowania. Badania wykazały, że procesy biologiczne nie zawsze są bezpieczniejsze dla organizmów żywych w porównaniu z procesami chemicznymi.