Inhibition of staphylococci and *S. aureus* in wastewater by ferrates and electrochemical methods

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Abstract: Increasing concentration of antibiotics in environment and their subinhibitory concentrations in wastewater may result in increased antibiotic resistance of present bacteria. Therefore, this study was aimed to analyze the efficiency of coagulase-positive staphylococci and *Staphylococcus aureus* inhibition in wastewater by electrochemical methods and addition of ferrates. Advanced electrochemical oxidation by boron doped diamond electrodes in anode; cathode and anode-cathode connection were used for wastewater disinfection. Results showed that the most effective connection was the anodic one, as complete inhibition of coagulase-positive staphylococci as well as of *S. aureus* was observed after 40 min. Energy consumption was 3.69 kWh/m³ for effluent wastewater disinfection. The second studied method of wastewater disinfection was the application of powdered ferrates. Addition of 100 mg of ferrates resulted in the inhibition of 84–96 % of coagulase-positive staphylococci and 97–99 % of *S. aureus* in influent water, while the inhibition of coagulase-positive staphylococci and *S. aureus* was 61–83 % and 83–86 %, respectively, in effluent wastewater.

Keywords: BDD electrode, coagulase-positive staphylococci, disinfection, ferrates, municipal wastewater, *Staphylococcus aureus*

Introduction

The presence of pharmaceuticals, illicit drugs, hormones or other micropollutants and their metabolites can have negative effect on water animals, plants and microorganisms (Brodin et al., 2013; Le et al., 2014; Mackulák et al., 2015a, 2015b; Nuel et al., 2018; Wilkinson et al., 2018). Besides these micropollutants, the presence of antibiotic resistant bacteria and genes encoding this resistance is monitored in wastewater (Novo et al., 2013; Birošová et al., 2014). Elimination efficiency of bacteria and micropollutants at Wastewater Treatment Plants (WWTPs) is limited and therefore many recent works are focused on the removal of these contaminants (Kümmerer, 2009; Mackulák et al., 2015b; Michael-Kordatou et al., 2018; Nuel et al., 2018). The presence of pathogenic and potentially pathogenic bacteria is of particular concern. While some bacteria are mainly nosocomial, some are community-acquired, including coagulase-positive staphylococci and *Staphylococcus aureus*. Staphylococci frequently colonize the nasal vestibule and skin of about 10–50 % of asymptomatic humans (ECDC, 2017). Moreover, pathogenic *S. aureus* is

regarded as a “superbug” due to its huge capacity to be resistant to a wide range of antibiotics, e.g. *S. aureus* strains resistant to methicillin (MRSA) or vancomycin (VISA/VRSA) and to many other antibiotics. These strains represent an urgent global problem since the mortality of *S. aureus* bacteremia remains approximately 20–40 % despite the availability of effective antimicrobials (Mylotte et al., 1987; ECDC, 2017). In community, *S. aureus* can be easily transferred from carriers or infected persons via direct contact and specific carriers (Stepanović et al., 2008) as it can survive in different environments, including wastewater.

Nowadays, many scientific studies deal with chemical and biological elimination of micropollutants such as X-ray substances, antibiotics, psychoactive pharmaceuticals, drugs and resistant types of microorganisms in municipal wastewater (Golovko et al., 2014; Mackulák et al., 2015a; Verlicchi and Zambello, 2015; Iakovides et al., 2019; Kulandaivelu et al., 2019). Wastewater from point sources, e.g. health care institutions, are intensively studied to find biological and chemical methods for wastewater treatment such as membrane bioreactors, Fenton reaction, ozonization, heteroge-

neous catalysis, boron-doped diamond electrodes (BDDE), application of ferrates etc. (Verlicchi and Zambello, 2015; Mackuľak et al., 2015a, 2015b, 2016; Michael-Kordatou et al., 2018; Kulandaivelu et al., 2019). These techniques show significant degradation of various groups of pharmaceuticals and illegal drugs. These processes produce radicals efficiently eliminating multidrug-resistant microorganisms (Michael-Kordatou et al., 2018; Yuan et al., 2013). Furthermore, it is possible to combine these methods with the original ones. A good example is shown in the study by Kajitvichyanukul and Suntronvipart (2006), where the photoFenton reaction was combined with biological treatment. Application of BDDE is one of the most promising techniques; however, the research is still insufficient. Therefore, this study deals with monitoring of coagulase-positive staphylococci and *S. aureus* in wastewater from Slovak WWTPs and the possibility of using ferrates and boron doped diamond electrodes application as disinfection methods.

Material and methods

Wastewater sampling

In this study, wastewater from Petržalka WWTP was used. Samples from influent and effluent of Petržalka WWTP were taken on 14. 9. 2015 and 14. 1. 2016 during 24 hours by an automatic sampler at the entry into the WWTP mechanical stage (Mackuľak et al., 2015a, 2015b). WWTP Petržalka treats wastewater from the largest housing estate in Bratislava as well as wastewater from health care facilities with about 2 000 hospital beds. The WWTP is designed to treat wastewater from 200 000 p.e. (population equivalent) and contains a mechanical pre-treatment stage for raw wastewater and a biological treatment stage, which is designed as RADN (based on the ongoing processes – regeneration, anaerobia, denitrification and nitrification). The produced sludge is digested and produced biogas energy is recovered.

Determination of *S. aureus* in wastewater

Presumptive counts of coagulase-positive staphylococci and *S. aureus* were determined after cultivation on Baird-Parker agar (Sigma-Aldrich, St. Louis, USA) according to EN ISO 6888-1 (2003) with 48 h incubation at 37 ± 0.5 °C under aerobic conditions. The suspected colonies of coagulase-positive staphylococci and *S. aureus* were distinguished based on their typical appearance as it is mentioned in ISO 6888:1 (2003) and were further confirmed by tube coagulase test, microscopic examination, the presence of catalase using a 3 % solution of hydrogen peroxide and by the MALDI-TOF biotyper.

Production and use of BDD electrode for disinfection

The used BDD thin films were grown for 120 min (thickness of about 500 nm) in a HFCVD (Hot filament chemical vapor deposition) reactor on both sides of an Si (10 9 10 mm) substrate. Boron deposition was achieved by adding trimethylboron (TMB) into the 2 % CH₄ in H₂ gas mixture to obtain the B/C ratio of 10 000 ppm. The deposition pressure was 3 kPa and substrate temperature was 650 °C. Gases were activated by five tungsten filaments (0.7 mm diameter) heated up to 2100 °C. Surface of the BDD thin film was observed by SEM JEOL 7500F (Figure 1).

Fig. 1. SEM image of BDD electrode surface.

In Fig. 1, fine polycrystalline diamond surface with sub-micron crystal size can be seen. For testing purposes, a prototype electrode system was prepared consisting of 10 BDD thin film overgrown silicon substrates positioned in two rows by five as the anode (16 cm²) and a graphite rod cathode (10 mm diameter) located above (10 cm²) the anode. Stabilized DC power supply GPS 3005 (Goldsun Electronics Co., Ltd., Suzhou City, China) was used for anodic disinfection (Mackuľak et al., 2016). Boron-doped diamond electrode connected in three different ways was inserted into 300 ml sample of wastewater. In the first two cases, the BDD electrode was connected as the anode or cathode while the graphite electrode was used as the second electrode. In the third case, the BDD electrode served concurrently as anode and cathode. The distance between electrodes was 1 cm and DC power supply set to 30 V was used to create the potential difference. Wastewater disinfection time was 60 min while samples for treatment efficiency analysis were taken at 5 and 60 min. In case of BDD electrode connected as anode, the samples were taken after 2, 5, 10, 20, 40 and 60 min of disinfection. Temperature of the samples during the

disinfection was monitored as increased temperature was expected with this type of treatment.

Production and use of Fe (VI) for disinfection

Potassium ferrate was prepared as follows: electrolysis was performed in an oil thermostat with a calibrated sensor, stainless steel box and PTFE open crucible with the sample (KOH). Reference end of the thermocouple was immersed in a Dewar flask with ice water. Anode was composed of steel class 11 (Valsteel, Zubří, Czech Republic) comprising five interconnected rods with the diameter of 8.5 mm and length of 46 mm. The counter electrode was composed of the same material as the anode. The cathodic and the anodic compartments were divided by a diaphragm. Mass fraction of KOH was below 70 % (w/w). Based on the phase diagram, the temperature for this experiment was chosen to be 40 °C. The working anodic current density was optimized to be 20 mA/cm². The final concentration of the prepared potassium ferrate was approximately 80 % (w/w) (Czölderová et al., 2018).

Three different amounts of ferrates – 10, 50 and 100 mg (obtained from Palacký University in Olomouc, Czech Republic) were added into 300 ml of wastewater samples from influent and effluent of the WWTP. The solution was subsequently intensively mixed for one hour on a shaker at laboratory temperature. OH⁻ ions are released into wastewater during this method of disinfection and thus the pH change was monitored for the ferrates samples of 10 and 100 mg.

Results and discussion

Two possible types of wastewater disinfection methods: application of boron doped diamond

electrode and addition of ferrates, were compared in this study. Efficiency of these methods in reducing the counts of coagulase-positive staphylococci and *S. aureus* was studied in influent and effluent wastewater of WWTP Petržalka, collecting 24 h wastewater samples on 9. 14. 2015 and 1. 14. 2016. A BDD electrode was used in three different connections, as anode or cathode with a counterpart carbon electrode and concurrently as anode and cathode. Ferrates powder was added in three different doses – 10, 50 and 100 mg, to 300 ml of wastewater samples.

The obtained results, summarized in Table 1 and Table 2, showed that the most effective application of the BDD electrode against coagulase-positive staphylococci and *S. aureus* in wastewater was obtained using the anodic connection as the inhibition up to 100 % (decrease about 2 log CFU/ml) was reached. These results correspond well with those of other studies where the anodic connection was marked as a method with the highest efficiency (Panizza and Cerisalo, 2009; Rajab et al., 2015). The highest efficiency of anodic connection can be explained by the production of hydroxide radicals HO[•], which belong to the strongest oxidizing agents (Kraft, 2008; Michael-Kordatou et al., 2018). In case of cathodic connection, hydrogen peroxide (H₂O₂) is generated as the reactive agent (Kraft, 2008). However, as staphylococci are catalase-positive (Halpin-Dohnalek and Marth, 1989), they can degrade a certain amount of H₂O₂ to water and free oxygen by enzyme catalase. Therefore, the cathodic connection has lower efficiency compared to the anodic one. Surprisingly, the lowest inhibitory efficiency was observed using the BDD in simultaneous anode-cathode connection.

Tab. 1. Efficiency of coagulase-positive staphylococci and *S. aureus* inhibition in wastewater samples from 9. 14. 2015.

Disinfection method	Disinfection application	Inhibition efficiency [%] INFLUENT water		Inhibition efficiency [%] EFFLUENT water	
		coagulase-positive staphylococci	<i>S. aureus</i>	coagulase-positive staphylococci	<i>S. aureus</i>
BDD electrode	5 min	83.0	96.8	56.5	100.0
Anode	60 min	100.0	100.0	100.0	100.0
BDD electrode	5 min	74.8	94.8	21.7	42.9
Cathode	60 min	100.0	100.0	69.6	85.7
BDD electrode	5 min	54.1	90.7	*	*
Anode+cathode	60 min	96.1	92.3	30.4	85.7
Ferrates	10 mg	74.0	94.3	*	*
	50 mg	80.6	96.2	*	*
	100 mg	84.3	97.5	82.6	85.7

*increase in bacteria number

Tab. 2. Efficiency of coagulase-positive staphylococci and *S. aureus* inhibition in wastewater samples from 1. 14. 2016.

Disinfection method	Disinfection application	Inhibition efficiency [%] INFLUENT water		Inhibition efficiency [%] EFFLUENT water	
		coagulase-positive staphylococci	<i>S. aureus</i>	coagulase-positive staphylococci	<i>S. aureus</i>
BDD electrode	5 min	73.2	93.8	17.8	50.0
Anode	60 min	99.9	99.7	100.0	100.0
BDD electrode	5 min	74.3	93.6	16.8	75.0
Cathode	60 min	97.4	99.4	71.0	58.3
BDD electrode	5 min	65.2	86.7	*	*
Anode+cathode	60 min	81.2	92.9	16.8	33.3
Ferrates	10 mg	50.9	95.9	*	*
	50 mg	66.4	95.1	*	*
	100 mg	96.3	98.8	60.8	83.3

*increase in bacteria number

The addition of ferrates led to the inhibition of both coagulase-positive staphylococci and *S. aureus* in influent wastewater in all cases. Moreover, the higher the addition of ferrates, the more intensive staphylococci inhibition, ranging from 74 % to 97.5 % in the September sample and from 50.9 % to 98.8 % in the January sample.

On the other hand, the increase of coagulase-positive staphylococci including *S. aureus* counts in effluent samples was observed in case of 10 and 50 mg ferrates addition. The addition of 10 mg of ferrates led to an average increase of about 1.47 ± 0.15 log CFU/ml of studied bacteria, while the addition of 50 mg of ferrates led to the average increase of about 0.61 ± 0.21 log CFU/ml. Only

the highest ferrates addition caused staphylococci inhibition of about 61–86 %.

It is important to remark that added ferrates do not react only with organic substances but also cause water decomposition (see Eq. 1), and the released OH^- increases wastewater pH (Fig. 2), mainly in case of addition of 100 mg of ferrates. The initial wastewater pH was 7.5 and after ferrates addition it increased to pH 9.27 and 9.55 in influent and effluent water, respectively. Such high pH values may show inhibition effect on staphylococci as they optimal growth is observed in the pH range of 6–7 (Halpin-Dohnalek and Marth, 1989).

Fig. 2. Changes of pH after adding 10 and 100 mg of powdered ferrates into 300 ml of influent and effluent wastewater.

These results suggest the following question to be further investigated: Is the disinfecting effect of ferrates due to their oxidizing properties or the high pH of the sample?

Considering the obtained results it can be concluded that the most effective disinfection method against coagulase-positive staphylococci is the application of BDD electrode in anode connection. However, it is necessary to determine the WWTP stage where it is useful to place the electrodes. Therefore, disinfection efficiency was monitored in dependence on time as it is shown in Fig. 3. In influent wastewater, the coagulase-positive staphylococci were not eliminated even after 60 min of disinfection (their final count was 2.44 log CFU/ml). However, in effluent wastewater, complete inhibition of staphylococci was observed after 40 min. So, it can be stated that the rate of coagulase-positive staphylococci elimination in early minutes is significantly higher in influent than in effluent water. For example, after 10 min of disinfection, inhibition efficiency of 70.4 % was observed for staphylococci in influent but it was only 16.8 % in effluent wastewater, suggesting that the rate of disinfection depends on the concentration of bacteria in the sample as the initial staphylococci counts were 4.17 log CFU/ml and 1.03 log CFU/ml in influent and effluent wastewater, respectively. Another important information for practical application of the BDD electrode is the energy consumption estimated using Eq. 2 (Rajab et al., 2015).

$$E\left(\frac{\text{kWh}}{\text{m}^3}\right) = \frac{UIT}{V_{\text{vz}}} \quad (2)$$

where U is the electrical voltage (V), I the electrical current (A), T time (h) and V_{vz} is the sample volume (m^3). The initial concentration of coagulase-positive staphylococci in effluent wastewater sample was 1.03 log CFU/ml. Complete disinfection was reached after 40 min at 29.7 V and 60 mA, i.e. energy consumption was 3.96 kWh for complete disinfection of 1 m^3 of the effluent sample. Usual energy consumption in a WWTP is about 0.4 kWh/ m^3 ; hence, the energy consumption of treatment using the BDD electrode is approximately ten times higher than that of the standard biological method.

Conclusions

Results of staphylococci inhibition by BDD electrode in three different connections showed that the anodic connection with a graphite electrode counterpart is the most effective one. Using this type of connection, the efficiency of coagulase-positive staphylococci and *S. aureus* elimination reached 100 % (decrease of about 2 log CFU/ml). Considering the disinfection process efficiency, the recommended location of the BDD electrode is the tertiary treatment in the WWTP. A disadvantage of this process is the relatively high energy consumption (3.96 kWh/ m^3) required to complete coagulase-positive staphylococci elimination. The addition of ferrates as a disinfection method showed the addition of 100 mg of powdered ferrates to be the most efficient. Using lower doses of ferrates (10 and 50 mg) resulted in an increase of contaminants concentration. Further research on ferrates

Fig. 3. Time dependence of disinfection efficiency against coagulase-positive staphylococci in influent and effluent wastewater using BDD electrode in anodic connection.

application is necessary because it is not clear whether the disinfecting effect of ferrates is due to their oxidizing properties or their ability to increase pH (up to 9.5). Further research on advanced oxidation processes is also needed for intensification of methods to remove micropollutants and resistant types of bacteria from wastewater because these substances can cause health problems.

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