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Electrochemical oxidation of landfill leachate using boron-doped diamond anodes: pollution degradation rate, energy efficiency and toxicity assessment

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Abstract:

Electrochemical oxidation (EO), due to high efficiency and small carbon footprint, is regarded as an attractive option for on-site treatment of highly contaminated wastewater. This work shows the effectiveness of EO using three boron-doped diamond electrodes (BDDs) in sustainable management of landfill leachate (LL). The effect of the applied current density (25–100 mA cm^{-2}) and boron doping concentration (B/C ratio: 500 ppm, 10,000 ppm and 15,000 ppm) on the performance of EO was investigated. It was found that, of the electrodes used, the one most effective at COD, BOD_{20} and ammonia removal (97.1%, 98.8% and 62%, respectively) was the electrode with the lowest boron doping. Then, to better elucidate the ecological role of LLs, before and after EO, cultivation of faecal bacteria and microscopic analysis of total (prokaryotic) cell number, together with eco-toxicity assay (*Daphnia magna*, *Thamnocephalus platyurus* and *Artemia salina*) were combined for the two better-performing electrodes. The EO process was very effective at bacterial cell inactivation using each of the two anodes, even within 2 h of contact time. In a complex

matrix of LLs, this is probably a combined effect of electrogenerated oxidants (hydroxyl radicals, active chlorine and sulphate radicals), which may penetrate into the bacterial cells and/or react with cellular components. The toxicity of EO-treated LLs proved to be lower than that of raw ones. Since toxicity drops with increased boron doping, it is believed that appropriate electrolysis parameters can diminish the toxicity effect without compromising the nutrient-removal and disinfection capability, although salinity of LLs and related multistep-oxidation pathways needs to be further elucidated.

Keywords: landfill leachates; boron-doped diamond electrode (BDD); advanced oxidation process; multistep-oxidation pathways; ecotoxicology; biodegradability; degradation efficacy.

Abbreviations:

0.5k BDD – Boron-doped diamond electrode with B/C ratio of 500 ppm

10k BDD – Boron-doped diamond electrode with B/C ratio of 10,000 ppm

15k BDD – Boron-doped diamond electrode with B/C ratio of 15,000 ppm

***A. salina* –** *Artemia salina*

AOPs – Advanced oxidation processes

B/C – Boron-to-carbon ratio

BDD – Boron-doped diamond electrode

BOD – Biochemical oxygen demand

COD – Chemical oxygen demand

***D. magna* –** *Daphnia magna*

EO – electrochemical oxidation

LL – Landfill leachate

MSS – Mineral suspended solids

MSWP – Municipal solid-waste plant

TN – Total nitrogen

***T. platyurus* –** *Thamnocephalus platyurus*

TSS – Total suspended solids

VSS – Volatile suspended solids

WWTP – Wastewater treatment plant

1. Introduction

Landfill leachate (LL), a by-product of waste landfilling, poses a significant environmental problem worldwide (Fudala-Ksiazek et al. 2017; De Brito et al. 2019; Farsani et al. 2021). It contains such a large and complex load of pollutants that it has been included, among others, on the hazardous material list by the US EPA (items: 19 07 02 and 19 07 03) (EPA 2015). Retention and recirculation are often used as an inexpensive LL treatment process; especially in low-income countries (Mendoza et al. 2010; Oktiawan et al. 2020; Zhang et al. 2021). Another often-considered option is an end-of-pipe approach, whereby LLs are discharged into the sewage system and directed to wastewater treatment plant (WWTP). But ammonia-rich LLs, which also contain high loads of non- or slowly biodegradable organic compounds, may lead to a reduction in performance of municipal WWTPs (especially, if by volume LLs constitute more than 10% of the inflow) (Fudala-Ksiazek et al. 2014; Kamaruddin et al. 2015; Mojiri et al. 2021). Moreover, in recent years, the legal requirements for treated wastewater quality standards have become more demanding (Bogacki et al. 2019; Srivastava and Singh 2021). This change has limited the direct discharge of LLs to wastewater system and forced municipal solid-waste plant (MSWP) managers to consider effective LL treatment on site (Luo et al. 2020).

LLs are generated by landfill cells for more than 30 years, therefore their quantity and quality result from many factors – mainly the age of the landfill cell and the decomposition stage (initial aerobic, acid anaerobic, initial and stable methanogenic), climatic conditions and operating procedures (waste stream dedicated to disposal, aeration procedures, LLs recirculation, etc.)(Abiriga et al. 2021; Farsani et al. 2021; Wilk et al. 2021). In terms of LL treatment, it is suggested that young and intermediate cells generate LLs that are more susceptible to biological treatment as compared to old ones (Aziz et al. 2010; Khoo et al. 2020; Siracusa et al. 2020; Tałałaj et al. 2021), which contain an essential amount of refractory organic matter (expressed, e.g. by $BOD_5/COD < 0.5$) as well as an inadequate C/N ratio (Fudala-Ksiazek et al., 2018a, b; Tałałaj et al., 2019). But the effective treatment of LLs has also to consider their potential toxicity, due to the possible presence of xenobiotic compounds and heavy metals (Aziz et al. 2010; Bandala et al. 2021). Thus, it is generally suggested that high efficacy of LL treatment cannot be achieved via the activated sludge process (Ren et al. 2017; Grosser et al. 2018; Bandala et al. 2021), making it imperative to develop and implement more suitable treatment technologies to replace or support conventional ones.

Although many technologies have been tested, developed and implemented (Ying et al. 2013; Song et al. 2020; Wai et al. 2020), proper LL treatment is still an open issue, mainly in the former Eastern Bloc and developing countries, and is a very urgent topic in environmental policy (Žgajnar Gotvajn and Pavko 2015; Ahmad et al. 2020; Deng et al. 2020; Pisharody et al. 2022). Nowadays, chemical and physical methods (adsorption, air stripping, chemical oxidation and precipitation, sedimentation/flotation, coagulation/flocculation, membrane technologies and biological methods (aerobic, anaerobic, or mixed) are used for LL treatment (Pereira et al. 2016; Amor et al. 2019). Unfortunately, these technologies have many disadvantages. In case of biological treatment, the changes of LLs quality and quantity cause serious operational problems (e.g. foaming or difficulties in biomass acclimation) (Payandeh et al. 2017; Cossu 2018; Ehrig et al. 2018; Teng et al. 2021). Biological methods are also not effective for the removal of toxic and recalcitrant compounds (polyaromatic hydrocarbons, polychlorinated biphenyls, etc.) and produces a remarkable amount of excess sludge (Torretta et al. 2017; Teng et al. 2021). Other, commonly used technology is membrane treatment, which

besides being very expensive, also causes the formation of retentate (a highly concentrated by-product that needs to be further treated by some means) (Huang et al. 2018; Suo and Ren 2021). Moreover, membanes are prone to fouling, have a generally short lifetime (Saleh and Gupta 2016; Rahmawati et al. 2021; Zhang and Hao 2021). Thus, usually the combination of different techniques (e.g., activated sludge system combined with membrane filtration) tends to be implemented as the most effective (Fudala-Ksiazek et al. 2016; Dubey 2019; Wilk et al. 2021), however, it significantly increases the investment and exploitation costs (Costa et al. 2019; Bandala et al. 2021; Mojiri et al. 2021).

Additional implementation possibilities are noted for AOP technologies, whose treatment effectiveness for highly contaminated wastewater has been studied with promising results (Didier et al. 2006; Tejera et al. 2019; Pierpaoli et al. 2020). AOPs have been reported as effective in removing organic matter, including recalcitrant pollutants, and in eliminating microorganisms and pathogens (Ike et al. 2019; Sánchez-Montes et al. 2020). Among AOP processes, electrochemical oxidation (EO) was found to be a promising technology for treating highly concentrated wastewater (Cheng et al. 2020; Solomon et al. 2020). Another important aspect of EO is disinfecting capability, already tested in wastewater, ballast and surface water (Kraft 2008; Lacasa et al. 2012; Cho et al. 2014; Ghernaout 2019). Operated without the addition of any chemical, EO seems to be a promising environmentally friendly technology, as compared to other disinfection methods (e.g., chlorination, photo-catalytic oxidation, ozonation) (Ferreira et al. 2020; Qiao and Xiong 2021). But strongly oxidising conditions, which allow disinfection and nutrient removal to be combined, may also result in the formation of toxic by-products, especially in a complex matrix of LLs (Jasper et al. 2017; Ambauen et al. 2020; Liu and He 2020). Moreover, a crucial point (though often overlooked) is hazard assessment of by-products generated during LL treatment. In the work of Silva et al. (2004), acute LL toxicity was assessed by *Vibrio fisheri*, *Daphnia similes*, *Artemia salina* and *Brachydanio rerio* before and after ozonation, coagulation/flocculation, and membrane fractionation treatments. It was found that acute toxicity was almost the same in all the fractionated samples, and significant toxicity removal was only achieved when high ozone doses were used (Silva et al. 2004). No significant changes in toxicity were noted during the photo-Fenton, solar TiO_2-heterogeneous photocatalysis, and ozonation of LLs (Marttinen et al. 2002; Prieto-Rodríguez et al. 2013; Yang et al. 2022). In this term, the implementation of EO technology for treatment of a LL complex matrix has not been fully investigated.

So far a significant number of EO-dedicated electrode materials have been tested, such as granular activated carbon, glassy carbon, polypyrrole, graphite, massive Pt, pure and doped PbO_2 (Dbira et al. 2019; Wai et al. 2020; Barrios et al. 2021; Jiang et al. 2021). Among them BDD is known as a non-active electrode with a high oxygen overpotential, which produces numerous hydroxyl radicals and other powerful oxidants effective for LL oxidation (El Ouaer et al. 2017; Dec et al. 2018; Amor et al. 2019). Furthermore, it has a wide electro-chemical window (from -1.25 to +2.3 V) as compared to a standard hydrogen electrode (Cornejo et al. 2020; Bogdanowicz and Ryl 2022). Thus, in this study, EO was evaluated using various boron-doped diamond anodes prepared on Nb substrate. Long-term studies on the effects of the boron doping level of diamond electrode and current densities (25–100 mA cm^{-2}) on the efficiency of LL treatment were conducted. The sanitary condition of LLs, as well as inactivation effectiveness of microorganisms by BDD electrodes, were investigated using conventional cultivation and microscopic analysis.

Moreover, the eco-toxicity of raw LLs and those treated by EO was tested on model species, i.e.: *Thamnocephalus platyurus*, *Daphnia magna* and *Artemia salina*.

2. Materials and methods

2.1. MSWP description and sampling of raw LLs

The LLs originated from MSWP "Eko Dolina Lezyce" located in the Pomerania region (northern Poland), from cell operated from 2003 to 2011, which was receiving mainly municipal waste mixed with food services. The samples of raw LLs were collected to polyethylene bottles in 2018 and 2019 and transported to the laboratory (at 4 ± 1 °C), where their physicochemical properties were immediately evaluated. In total, three 24-h composite samples (10 L each) as mix of discrete samples were collected.

2.2. Analytical methods

The characterization of LLs considered the measurement of the following parameters (according to the APHA 2005 standard): chemical oxygen demand (COD), total nitrogen (TN), inorganic N compounds ($N\text{-}NH_4^+$, $N\text{-}NO_3^-$, $N\text{-}NO_2^-$), total phosphorus (TP) and orthophosphate ($P\text{-}PO_4^{3-}$), which were all measured using a XION 500 spectrophotometer (Dr Lange, GmbH, Germany)(APHA 2005). Conductivity, pH and oxidation-reduction potential (ORP) were determined by a portable multi-parameter meter, the HL-HQ40d multi (HACH, Germany), and 20-day biochemical oxygen demand (BOD_{20}) by manometric respirometric BOD OxiTop® method (Fudala-Ksiazek et al. 2018b). Chloride (Cl^-) and sulphate (SO_4^{2-}) concentrations were measured by ion chromatography using: a DIONEX 3000 chromatograph (DIONEX, USA) (column: Ion Pac®AS2 [2 × 250 mm]; suppressor: ASRS-300, 2 mm; mobile phase: 4.5 mM CO_3^{2-}, 1.4 mM HCO_3^-; flow rate: 0.38 mL min^{-1}; detection: conductivity) (Szopińska et al. 2016), and total suspended solids (TSS), volatile suspended solids (VSSs) and mineral suspended solid (MSS) using the gravimetric method according to Polish Standards (PKN 2007).

2.3. Preparation of BDD electrodes

The BDD electrodes were deposited on two-inch Niobium substrates (Spinex, Poland) using a Microwave Plasma Assisted Chemical Vapour Deposition (MWPACVD) process. The optimized process parameters were 1% CH_4 of total flow equal to 300 sccm, microwave power was set to 1,300 W, the induction heating stage temperature was set to 700 °C, and process pressure was 50 Torr (Fudala-Ksiazek et al. 2018b). Three BDD electrodes characterized by different doping levels were investigated. During the BDD growth, the B_2H_6/CH_4 gas ratio was kept at 500 ppm, 10,000 ppm and 15,000 ppm, in order to obtain three BDD electrodes named 0.5k BDD, 10k BDD and 15k BDD, respectively. The time of the deposition was set to 12 hours. The molecular structure of the electrode surface was analysed using the Raman technique. The Raman spectra were recorded at room temperature using the micro-Raman system (InVia, Renishaw, UK) and a 514-nm argon ion laser was used for excitation. Spectra were recorded in the range of 600–3,500 cm^{-1}. The surface morphology was analysed using a Scanning Electron Microscope (SEM) (EVO-40, Zeiss, Germany). To determine sp^3/sp^2 ratio, the Raman spectra were deconvoluted using the Lorentzian function that allows the estimation of a real contribution of both carbon phases (OriginPro 8.0, OriginLab, Northampton, MA).

2.4. Electrochemical oxidation assay

Samples of raw LLs were diluted using deionized water (1:1 (v:v)) prior to EO. Then, 400 mL of diluted LL sample was treated by different current densities of between 25 and 100 mA cm^{-2} using a 500-mL single-chambered reactor (Fig S1, Supplementary Material). A magnetic stirrer (Electrochemical Stirrer, ES34, Wigo, Poland) with stirring speed of 300 rpm was used to maintain the homogeneity of sample. The electrochemical system for LL treatment was operated under the galvanostatic condition provided by the power supply (GW Instek GPD-23035, Taiwan). Both BDD anode and stainless-steel plates of cathode were flat and had an active area of 10.5 cm^2, and the distance between them was 1.0 cm. The assessment of the process was performed on samples collected every two hours (15 mL volume each) for the eight hours of the experiment. All samples were degassed by mixing on a multipoint stirrer (Variomag, POLY 15 KOMED, Thermofisher Scientific, USA) at 50 rpm for 15 min, then physico-chemical analyses were performed (determination of chemical oxygen demand [COD], total nitrogen [TN], inorganic N compounds [N-NH_4^+, N-NO_3^-, N-NO_2^-], 20-day biochemical oxygen demand [BOD_{20}] concentrations, pH, and ORP according to procedures described in point 2.2). Each experiment was performed in triplicate. To reduce the effect of temperature on EO process (current density above 50 mA cm^{-2} caused an increase of electrolytes' temperature) the experimental set-up was subjected to cooling, and the temperature was maintained at 25 ± 2 °C.

The energy consumption [W, kWh] was calculated by multiplying the applied current [A], electrolysis time [8 h] and the average cell voltage (E_{cell}) [V]. Next, this value (W, kWh) was recalculated and expressed in kWh m^{-3}, or specific energy consumption was calculated in units of COD mass [kWh kg^{-1} COD] and per unit N-NH_4^+ mass [kWh kg^{-1} N-NH_4^+]. The specific energy consumption (EC) for the electrochemical cell operation was estimated from Eq. 1 per unit COD mass, or by Eq. 2 per unit N-NH_4^+ mass (Flox et al. 2007)

$$\textbf{Eq. (1)}\ EC_{COD} = (1000 \times E_{cell} \times I \times \Delta t) / (Vs\ [\Delta COD]) \quad (1)$$

$$\textbf{Eq. (2)}\ EC_{N\text{-}NH4+} = (1000 \times E_{cell} \times I \times \Delta t) / (Vs\ [\Delta N\text{-}NH_4^+]) \quad (2)$$

where: 1000 is a conversion factor (in mg/g), E_{cell} is the average cell voltage (in V), I is the applied current (in A), Δt is the electrolysis time (in h), V_s is the solution volume (in L), and (ΔCOD) is the experimental COD concentration decay (in mg L^{-1}).

2.5. Microbiological and toxicological assays

Conventional plate count was used to evaluate the sanitary condition of raw LLs. Disinfection capability of EO was also tested by calculating the inactivation rate (R) as the ratio between bacterial occurrence in raw (N_0) and electrochemically treated (N_t) LLs at the respective time of EO experiment (samples were taken after t = 2 h, 4 h, 6 h, 8 h) according to the formula below:

$$\textbf{Eq. (3)}\ R = 1 - N_t \times N_0^{-1} \quad (3)$$

The presence of faecal indicators (*E. coli* and *Enterococcus* spp.) were tested using membrane filtration according to EN ISO 9308-1:2014/A1:2017 and EN ISO 7899-2:2000, respectively (ISO 2000; PKN 2014). The number of mesophilic and psychrophilic bacteria was evaluated using agar plates incubated in 37 °C and 22 °C, respectively, for seven days. All results were expressed as colony-forming units (CFU) per mL.

Total prokaryotic cell number (TCN), average cell volume (ACV) and prokaryote biomass (PB) in raw and treated LLs were determined by DAPI staining and direct counting using standard epifluorescence microscopy technique (Porter and Feig 1980). Samples were stained in 1 μg mL^{-1} final DAPI concentration for 10 minutes in darkness, filtered through 0.2-μm polycarbonate Whatman filters (Merck, Germany) and then rinsed twice with: bacterium-free distilled 1 mL of water and 1 mL of particle-free 80% ethanol. Filters were examined under UV light (BO-103W high-pressure mercury burner, 330–380-nm excitation filter, 420-nm barrier filter and 400-nm dichroic mirror) with an epifluorescence microscope (Nikon Eclipse 80i) under 1000 × magnification. Bacteria were counted in two repeats of 10 separate fields. The image analysis system of Świątecki (Świątecki 1997) was applied. Bacterial biomass was estimated using the conversion factors of Norland (Norland 1993).

The toxicity of raw and treated LLs was determined using acute toxicity microbiotests: Thamnotoxkit F (24-hour test based on Thamnocephalus platyurus crustaceans), Rapidtoxkit F (a 30–60-minute test for inhibition of food intake based on Thamnocephalus platyurus crustaceans.), Daphtoxkit F. magna (24–48-hour test based on Daphnia magna crustaceans) and Artoxkit M. (4–48-hour test based on Artemia franciscana crustaceans). The tests were performed according to the procedures described by the manufacturer MicroBioTests Inc. (Nazareth, Belgium).

The following concentrations of LLs were used: 0.4, 0.8, 1.6, 3.1, 6.2, 12.5, 25, 50, and 100%. Tests were performed in triplicate. The test organisms were prepared for the experiment by incubating their cryptobiotic forms in Standard Freshwater (SF) (for *T. platyurus*), in Standard Medium (SM) (for *D. magna*) and in Standard Marine Water (SMW) (for *A. franciscana*). The assay plates containing larvae were incubated at 20 °C in the dark. Mortality percentage was calculated after 24 hours. Additionally, *T. platyurus* and *D. magna* organisms were monitored at different times of exposure (1, 5, 20 min and 1.5, 3, 12 h). The individuals were presumed dead if they did not show any movement for 15 seconds of observation. The test was valid when the mortality of individuals in the control sample did not exceed 10%.

In this work, microbiotest for rapid detection of water contamination was performed using *Thamnocephalus platyurus* (MicroBioTests Inc., Nazareth, Belgium). This test measures a sub-lethal toxic stress after 30 minutes or 1 hour exposure of the crustaceans to water samples suspected to contain toxicants.

3. Results and discussion

3.1. Characteristics of the Raw LL

The LLs were collected from the cell (operated in years 2003-2011), which was receiving mainly municipal waste mixed with food services. The observed chemical profile of tested LLs matrix (Table 1), together with the stable methane production (according to personal communication), confirmed the metagenomic phase of the landfill cell (Fudala-Ksiazek et al. 2018b; Gomes et al. 2019; Tejera et al. 2019). The raw LLs were characterized by dark brown colour, high COD (3608 ± 123 mg O_2 L^{-1}) and TN (2148 ± 108 mg TN L^{-1}) but relatively low BOD_{20} value (403 ± 54 mg O_2 L^{-1}). Such characteristic, together with LLs low biodegradability (BOD_{20}/COD ratio <0.15), high ammonia concertation (N-NH_4^+/TN ratio from 0.95 to 0.98) and low TSS (< 90 mg L^{-1}) are typical for LLs generated by mature sites (Ying et al. 2013; Peng et al. 2020). In addition to the age of the landfill cell, its exploitation also had an impact on LL quality, here especially the fact that (1) the deposition of biodegradable substances had gradually been limited

(Wilk et al. 2019) and that (2) the concentrate generated during on-site, reverse-osmosis treatment of LLs was recycling back the landfill cell. The latter one can explain high concentrations of chloride (2690 ± 70 mg Cl^- L^{-1}), sulphate (1353 ± 70 mg SO_4^{2-} L^{-1}) and electrolytic conductivity of raw LLs ranging from 21.2 to 26.8 mS cm^{-1}.

Table 1. Characteristic of raw landfill leachates [mean[1] ± SD]

	Parameter	Value
Organic and inorganic matter characteristics [mg L^{-1}]	COD	3608 ± 123
	BOD_{20}	403 ± 54
	BOD_{20}/COD	0.12 ± 0.01
	TSS	71 ± 10
	MSS	38 ± 12
	VSS	33 ± 10
Nitrogen forms [mg L^{-1}]	$N\text{-}NH_4^+$	2069 ± 93
	$N\text{-}NO_3^-$	9.4 ± 7.1
	$N\text{-}NO_2^-$	0.40 ± 0.10
	TN	2148 ± 108
	$N\text{-}NH_4^+$/TN	0.97 ± 0.02
Phosphate forms [mg L^{-1}]	$P\text{-}PO_4^{3-}$	10.7 ± 1.2
	TP	15.3 ± 1.0
	$P\text{-}PO_4^{3-}$/TP	0.70 ± 0.10
Other ions [mg L^{-1}]	Cl^-	2690 ± 70
	SO_4^{2-}	1353 ± 70
	S^{2-}	8.70 ± 0.80
Physicochemical parameters	pH	7.80 ± 0.10
	redox [mV]	-414.0 ± 7.6
	Conductivity [mS cm^{-1}]	24.0 ± 2.8

[1)] number of samples n = 3

3.2. Results of the electrochemical oxidation of LL

According to the Comninellis group model (Panizza et al. 2008, 2010; Urtiaga et al. 2012), oxidation of organic matter is mainly caused by hydroxyl radicals (•OH) through direct and/or indirect oxidation. Although, in complex matrix as LLs, a BDD anode is suspected to generate strong oxidants from salts such as: sulfates, carbonates and chlorides (Serrano 2014; Patra et al. 2020; Du et al. 2021). Taking into consideration the high concertation of chloride ions (2620–2760 mg L^{-1}) in the tested LLs, active chlorine (HOCl, OCl^-, Cl_2) may also play a significant role during EO-treatment, applied in this study. Ding et al. (2017) and Lacasa et al. (2012) have described that active chlorine has a significant impact on $N\text{-}NH_4^+$ removal from wastewater (Lacasa et al. 2012; Ding et al. 2017). Detailed EO mechanisms of ammonium species in the presence of Cl^- ions is presented in Fig. S2 (see in Supplementary

Material). Furthermore, recent studies (Ukundimana et al. 2018; Ding et al. 2020) have shown that if wastewater has an excess of active chlorine needed for oxidation of ammonium compounds, an improvement in the oxidation of organic compounds is also achieved at high current densities. Nevertheless, above a threshold NaCl concentration, the indirect oxidation process near the anode and in the bulk solution can hinder the oxygen evolution reaction on the BDD surface by the interaction between hydroxyl radicals (physisorbed) and Cl^- to form active chlorine species (de Moura et al. 2015). Strong oxidants such as sulfate radicals ($SO_4^{\bullet-}$) can be electrogenerated using a BDD anode, by reaction of HSO_4^- and undissociated H_2SO_4 present in LLs with ($^{\bullet}OH$) (Serrano et al. 2002). Available scientific publications report that the removal of organic pollutants can be 10–15-fold higher when sulfate ions are present in electrolyte compared to $^{\bullet}OH$-based anodic oxidation via indirect oxidation (Agustina et al. 2019; Cheng et al. 2020). Nevertheless, it should be emphasized that sulfate radicals do not have such a significant effect on the removal of ammonia as chloride ions. Moreover, the role of sulfate radicals in mineralization of organic matter and ammonia oxidation in LLs matrix still remains little known (Lan et al. 2017; Wang et al. 2021). Hence, salinity of LLs and the related multistep oxidation pathway process need to be further elucidated in terms of organic matter mineralization and nitrogen removal (Chen et al. 2019).

In this study, the COD removal using three BDD electrodes with different boron doping levels is shown in Fig. 1a as a function of normalized concentration (C_t/C_0) and EO-treatment time.

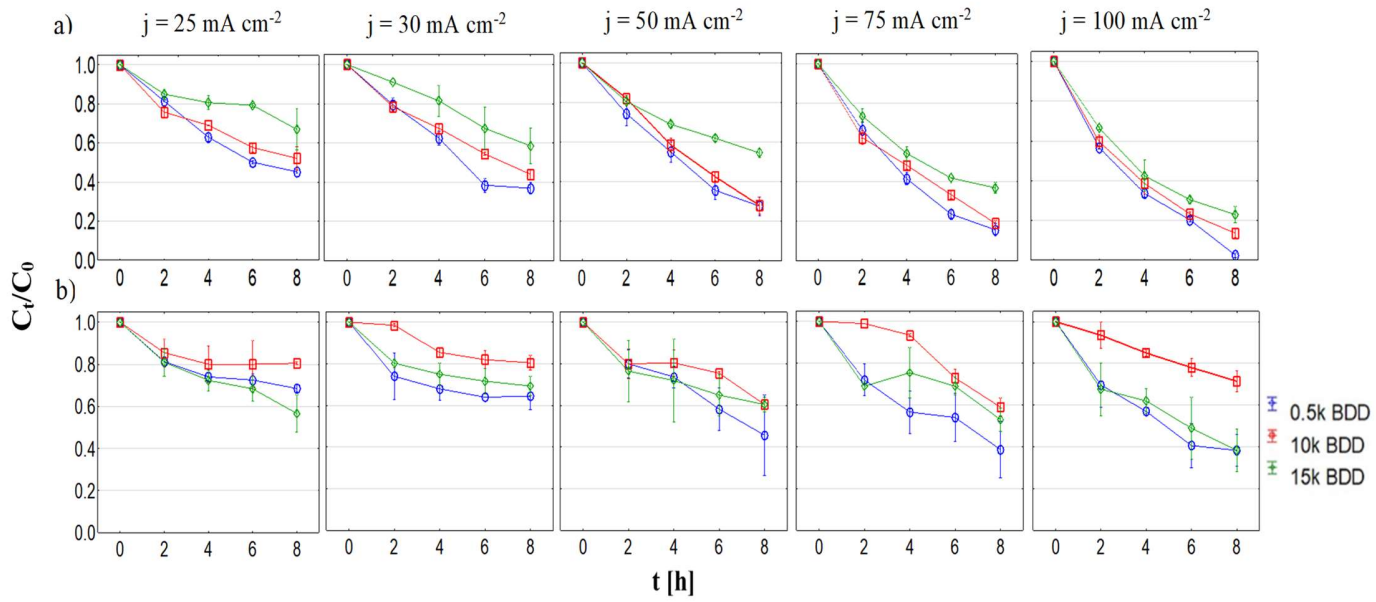

Fig. 1 (a) COD and (b) $N\text{-}NH_4^+$ changes in LLs with different BDD anodes and current densities (25–100 mA cm^{-2}) during 8 h electrolysis time

It can be ascertained that the rate of COD reduction increased with increasing current densities. In general, all experiments showed that the most effective electrode in COD and BOD_{20} removal was 0.5k BDD (Fig. 1a and Supplementary Material, Table S1). After eight hours of treatment with an applied current density of 100 mA cm^{-2}, the C_t/C_0 for COD and BOD_{20} was equal to 0.03 and 0.1, respectively. In comparison, under the same conditions, C_t/C_0 for COD was 0.23 using 15k BDD and 0.14 using 10k BDD. Moreover, after 8 h C_t/C_0 for BOD_{20} was 0.03 and 0.37

for 15k and 10k, respectively. Efficient organic matter removal can therefore be achieved by using BDD electrodes with a higher sp^3/sp^2 ratio (the lower-doped), which increases production of hydroxyl radical (Espinoza et al. 2018). The largest amount of COD was removed during the first four hours of the process (Fig. 1a and Supplementary Material, Table S2). With applied current density of 100 mA cm^{-2} an increasing biodegradability index (BOD_{20}/COD ratio) was observed in the EO-treated wastewater (e.g. from 0.11 in non-treated LLs to 0.26 using 0.5k BDD, and from 0.11 LLs to 0.29 using 10k BDD). However, using the 15k BDD electrode, an inverse relationship was noted (from 0.11 to 0.06). Changes in the biodegradability index were not high, though, which may have been influenced by the formation of non-biodegradable intermediates during the EO-treatment process.

In turn, nitrogen compounds were not removed as effectively as was COD. The reasons for this could be insufficient Cl^- concentration in the tested samples and excessive organic matter content. According to the literature, $N\text{-}NH_4^+$ is commonly oxidized via a chlorine-mediated pathway (Cl_2/HOCl) and production of chlorinated compounds increased by using BDD electrodes with a lower sp^3/sp^2 ratio (Medeiros De Araújo et al. 2014; Espinoza et al. 2018). BDD anodes promote the generation of hydroxyl radicals, and the high content of chloride ions induces the simultaneous formation of free chlorine, which is responsible for the indirect oxidation of ammonium (Supplementary Material, Fig. S2). However, chlorine evolution may be enhanced at lower COD concentrations (Fernandes et al. 2014). A higher active chlorine concentration may be found at lower COD concentration because of the limited reaction with organic compounds. Since the production of chlorinated compounds, possibly follows the reaction pathway (Laheäär et al. 2015)

Eq. (4) $Cl^- \rightarrow Cl\bullet$, after that

Eq. (5) $Cl\bullet \rightarrow Cl_2$ or

Eq. (6) $Cl^- + {}^{\bullet}OH \rightarrow ClO^-$,

the presence of organic matter hinders the production those stable oxidants, by reacting directly with the hydroxyl radical, thus limiting also the further production of oxochlorinated compounds:

Eq. (7) $Cl^- + {}^{\bullet}OH \rightarrow ClO^- + H^+ + e^-$

Eq. (8) $ClO^- + {}^{\bullet}OH \rightarrow ClO_2^- + H^+ + e^-$

Eq. (9) $ClO_2^- + {}^{\bullet}OH \rightarrow ClO_3^- + H^+ + e^-$

Eq. (10) $ClO_3^- + {}^{\bullet}OH \rightarrow ClO_4^- + H^+ + e^-$

Hence, higher COD concentration could result in limited ammonium oxidation rates. The effective removal of high ammonium concentrations by EO can be achieved using very high chlorine concentrations (*ca* 5,000 to 20,000 mg L^{-1}). However, this may lead to the formation of hazardous organic compounds as final products, e.g. trihalomethane. In research conducted by Perez et al. (2012) it was found that during 2 h of LLs electrolysis 64, 82 and 98% of $N\text{-}NH_4^+$ was removed from samples containing 5,000, 10,000 and 20,000 mg L^{-1} of Cl^-, respectively (Pérez et al. 2012). This phenomenon was also confirmed by Ding et al. (2017) and Lacasa et al. (2012).

In our study, increasing specific current densities achieve higher $N\text{-}NH_4^+$ and TN removal rates (Fig. 1b; Supplementary Material, Fig. S3c). After eight hours of treatment with an applied current density of 100 mA cm^{-2}, the

313 C_t/C_0 for TN was 0.61, 0.69 and 0.51 using 0.5k BDD, 10k BDD and 15k BDD, respectively. In turn, C_t/C_0 after 8 h
314 for $N\text{-}NH_4^+$ (j = 100 mA cm^{-2}) was 0.38 (0.5k BDD), 0.59 (10k BDD) and 0.38 (15k BDD). $N\text{-}NH_4^+$/TN for raw LLs
315 was in the range 0.88–0.99, and after 8 h of treatment (j = 100 mA cm^{-2}) it was in the range 0.62–0.82. This difference
316 shows that after EO-treatment ammonia ions are either transformed to nitrate or nitrite forms, and only partly to
317 gaseous nitrogen. For the current density range of 25–75 mA cm^{-2} both TN and $N\text{-}NH_4^+$ were most effectively removed
318 in the first four hours of the process. For j = 100 mA cm^{-2} TN and $N\text{-}NH_4^+$, removal rates are more stable over the
319 whole electrolysis time (8 h) (Fig. 1b; Supplementary Material, Fig. S3c). A similar dependence was observed by
320 Ghazouani et al. (2017) and Zhou et al. (2016) using BDD silicon-based electrodes (with a set-up of three electrodes
321 with 70 cm^2 surface area each) for municipal wastewater treatment. During the process, ammonium nitrogen was
322 removed mainly in the first hour of EO, after which its concentration stabilized.

323 Nevertheless, as already mentioned in previous studies (Fudala-Ksiazek et al. 2018b) it was expected that,
324 due to the high concentration of ammonia nitrogen in the tested non-treated LLs, nitrate is generated during EO and,
325 in fact, the total nitrogen will not be removed at a significant level. Nitrate concentrations show a notable increase for
326 all tested current densities during the application of 0.5k BDD (Supplementary Material, Fig. S3a). With the
327 application of 0.5k BBD, due to the lower boron doping level, direct oxidation of ammonium is predominant reaction
328 (in comparison to other tested BDD electrodes) with the reaction expressed in Eq. 11 (Wilk et al. 2021).

329 **Eq. (11)** $NH_{3(ad)} + 3H_2O \rightarrow NO_3^- + 8e + 9H^+$

330 No increase in nitrate is observed during the EO process with the application of 10k BDD, and in the case of 15k BDD
331 the increase in this species varied irregularly according to the current applied (Supplementary Material, Fig. S3a).
332 Hence, it is observed that the current density affects the process of nitrate formation, while the boron doping affects
333 the efficiency, resulting in the better performance of this reaction (Eq. 11) at the lower doping level. At the same time,
334 an increase in nitrite ions (from 0.22 mg L^{-1} up to 28.3 mg L^{-1} for 15k BBD, j = 100 mA cm^{-2}) was detected during
335 eight hours of process (Supplementary Material, Fig. S3b). The nitrite increase during electrolysis is directly related
336 to the increase in nitrates and the reaction given in Eq. 12 (Ghazouani et al., 2017). This reaction is reversible; hence,
337 it is hard to observe any tendency in nitrite concentration during the EO process.

338 **Eq. (12)** $NO_3^- + H_2O + 2e^- \rightarrow NO_2^- + 2OH^-$

339 The pH of treated LLs basically increased over the process duration (Supplementary Material, Fig. S4),
340 reaching the maximum value after eight hours of EO. An increase was observed from 7.81 up to 9.58 (0.5k BDD, j =
341 100 mA cm^{-2}). Together with the pH increase, the current efficiency for hydroxyl radical formation may decrease and
342 at the same time the oxygen evolution reaction may occur more intensively (Zhang et al. 2013). Hence, having more
343 alkaline conditions, oxygen is generated at a greater rate, which consequently slows down the rate of $^{\bullet}OH$ generation
344 (McBeath et al. 2019). Moreover, competitive reactions of $^{\bullet}OH$ with the organic matter (Fig. 1a) may also influence
345 NH_4^+ degradation rate, which was also confirmed by our previous studies (Wilk et al. 2021). Then, a decrease in
346 pollutant degradation rate is observed. As already mentioned, this phenomenon is observed after approximately four
347 hours of process (see Fig. 1).

Another important aspect of EO control is energy consumption analysis. Detailed evaluation of energy consumption during eight hours of processes is presented in Fig. 2. An increase in applied specific current results in a higher EC_{COD} and $EC_{N\text{-}NH4+}$ value (Fig. 2a, c). The highest COD removal rate, which was 97.1%, required energy equal to 205 kWh kg^{-1} COD and 386 kWh m^{-3} (0.5k BDD, j = 100 mA cm^{-2}). The highest N-NH_4^+ removal rate, equal to 62% was achieved by 0.5k BDD and 15k BDD after 8 h of process with an applied current density of 100 mA cm^{-2}. Under these conditions $EC_{N\text{-}NH4+}$ was 575 and 530 kWh kg^{-1} N-NH_4^+, for 0.5k BDD and 15k BDD, respectively. We see the difference between maximum achieved removal for COD – 97.1% and N-NH_4^+– 62%. This also influence specific energy consumption. That is why higher EC values are we observed for N-NH_4^+, than for COD. A result obtained by Ghazouani et al. (2017) with the application of BDD electrode (j = 37.7 mA cm^{-2}) shows that for 91% of COD removal from municipal wastewater 230 kWh kg^{-1} COD is required. However, the initial concentration of the studied wastewater was 920 mg L^{-1}. At the same time, the authors pointed out that an increase in COD content involved a significant decrease in EC_{COD}, which was also highlighted by Fernandes et al. (2013). Hence, this tendency promotes the BDD EO technique for the treatment of high COD-load LLs and also condensed by-products generated during the application of reverse osmosis.

Moreover, as was also mentioned by Ghazouani et al. (2017), the EO process is regarded as relatively expensive in comparison to the conventional treatment and, from an economical point of view, should be considered more as a combined process or as an auxiliary unit. Despite its relatively high energy consumption, the big advantage of applying EO in LLs (and other industrial wastewater) treatment is that it generates no by-products, such as highly contaminated condensate, which always occurs with reverse osmosis and any other filtration method (Pressman et al. 2012; Chen et al. 2021). EO-treatment of LLs can be also regarded as a competitive method for activated carbon (Jin et al. 2013; Bourgin et al. 2018) and coagulation (Zhao et al. 2013) technologies.

On the other hand, interesting results presented by Fernandes et al. (2013) show that energy consumption decreases in experiments, which were performed in a semi-pilot plant operating in batch mode with recirculation. With the initial COD of 8,900 ± 800 mg L^{-1}, 69% COD removal was achieved during following experimental condition: j = 200 mA cm^{-2}, flow = 360 L h^{-1}, reactor volume 5 L, where specific energy consumption was estimated at 91.1 kWh kg^{-1} COD, which is far less than in the case of batch experiments without LL recirculation.

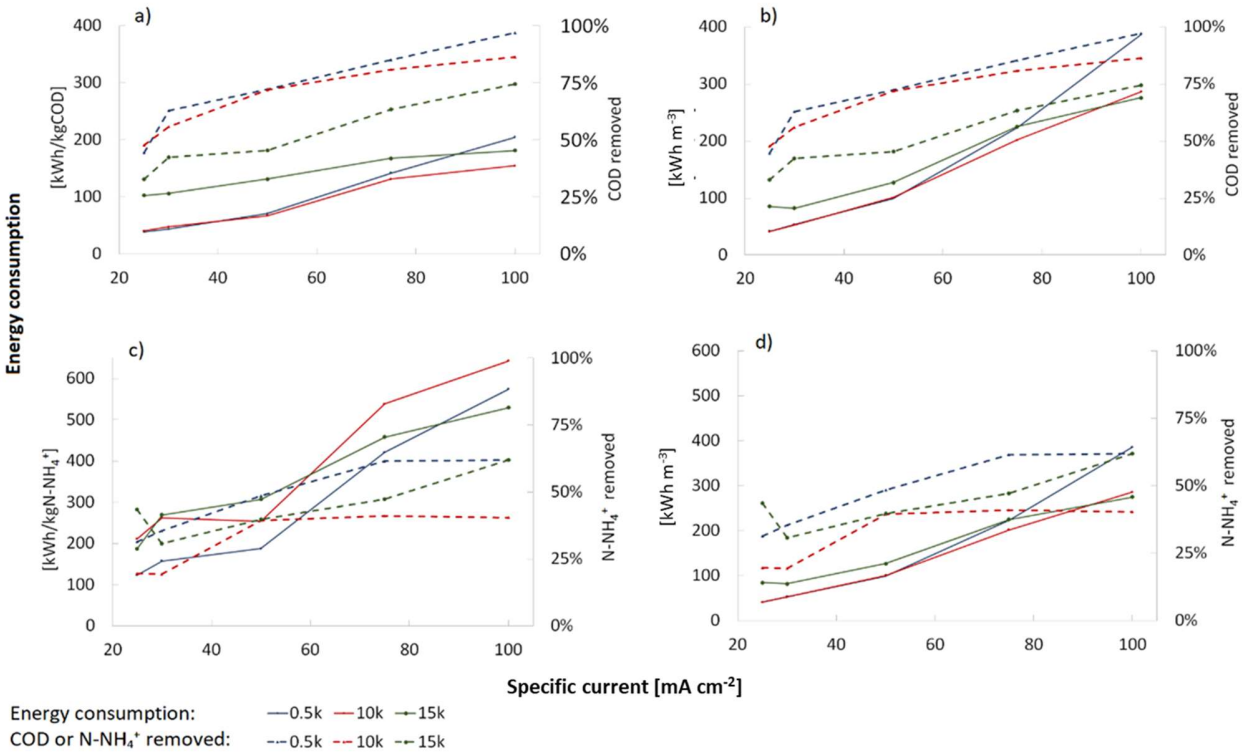

Fig. 2 (a and b) Specific energy consumption after 8 h of EO process expressed in [kWh kg^{-1} COD], [kWh kg^{-1} N-NH_4^+] and [kWh m^{-3}], at the different current densities plotted against COD removed [%] and (c and d) N-NH_4^+ removed [%]

3.3. Evaluation of the raw and treated LLs microbiological quality

Based on the presented results (Fig.1), the most effective electrode in terms of COD, BOD_{20} and N-NH_4^+ removal was 0.5k BDD. 10k BDD also removed COD and BOD_{20} highly effectively, but in the case of N-NH_4^+, the removal results were less satisfactory. Taking into account all the results obtained, 0.5k BDD and 10k BDD electrodes were selected for toxicological and microbiological assay.

As suspected, faecal indicators were not detected in tested, raw LLs (which in this study were collected from mature prism) because both enterococci and *E. coli* are considered to be good markers of rather recent faecal contamination. Besides faecal indicators also presence of mesophilic and psychrophilic bacteria were analysed in raw LLs, and were calculated at levels of 0.4×10^3 CFU mL^{-1} and 3.4×10^3 CFU mL^{-1}, respectively. Obtained values were much lower as compared with raw municipal wastewater, where they reach up to 10^7–10^8 CFU mL^{-1} (Michałkiewicz et al. 2018). In the case of DAPI staining, small prokaryote cells were detected in raw LLs (Fig. 3b), in total numbered 8.01×10^5 cells mL^{-1}, with the highest amount of rods, constituting 80%.

The disinfection capabilities were tested for two electrodes: 0.5k BDD and 10k BDD, which were the most effective at COD and N-NH_4^+ removal. During the EO 8-h tests, samples were collected every two hours for

bacteriological assay. According to the results, the inactivation of bacterial cells was readily achieved for 10 k BDD electrode, after 2 h (the C_t/C_0 was 0.002 for psychrophilic bacteria and 0.076 for mesophilic) (Fig. 3a).

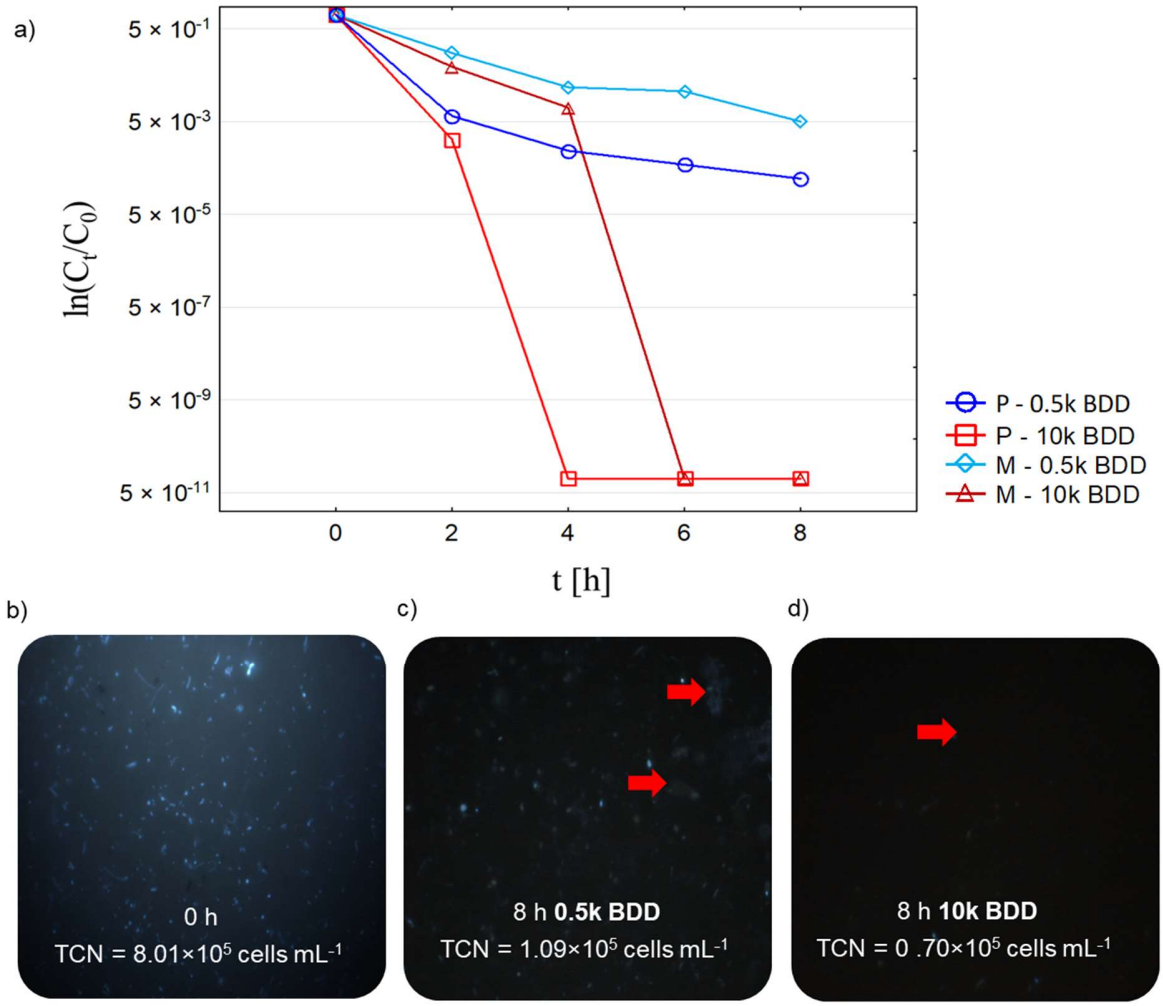

Fig. 3 Change of bacterial concentration during the 0.5k BDD and 10k BDD experiments, presented as (a) $\ln(C_t/C_0)$ of colony forming units of psychrophilic (P) and mesophilic (M) bacteria as well as (b) microscopic observation of DAPI stained prokaryotic cell in LLs prior to experiment, and (c) after 8h of treatment by 0.5k BDD electrode and (d) 10k BDD electrode; presence of some "bacterial ghosts" representing inactivated cells, which have lost cytoplasmic content (including DNA) via perforated membranes are marked with red arrow

It is suggested that inactivation of bacterial cells by both electrodes in the studied LLs was mainly the synergic effect of hydroxyl radicals (•OH) and active chlorine (HOCl, OCl^-, Cl_2). In contrast to hydroxyl radicals, whose instability causes them to react mostly non-selectively with cellular components (causing damage to the outer membrane), active chlorine compounds can penetrate the bacterial cell and cause decarboxylation of amino acids, and can react with nucleic acids and key enzymes (Long et al. 2015). According to scientific studies (Jeong et al. 2009; Li and Ni 2012; Martínez-Huitle and Brillas 2021), bacterial inactivation occurs faster in electrolytes with a lower

concentration of organic substances and ammonia. In our study, the greatest decrease in bacterial concentration was observed during the first 4 h, which corresponds to the greatest COD and N-NH_4^+ removal in 1st phase of the process. At high concentrations of COD and N-NH_4^+ in LLs, the rate of inactivation of bacterial cells reached the maximum. In this study, however, taking into consideration the presence of sulphate ions (1353 ± 275 mg L^{-1}) in the tested LLs, also other electroactive ions and their oxidation products needs to be considered. Long et al. (2015) reported that electrogenerated oxidants of sulphate radicals ($SO_4^{\bullet-}$) and peroxydisulphate (Shin et al. 2019) attacked the cell membrane proteins, causing damage to the K^+ ion transport systems, inhibiting cell division and ATP synthesis, while reactions with the intracellular enzymes was mostly negligible. The changes in bacterial community structure during the EO assay were indirectly confirmed by imaging DAPI staining and the presence of so called "bacterial ghosts" (Fig. 3c-d) – inactivated cells that have lost cytoplasmic content (including DNA) via perforated membranes (Taddese et al. 2018). Cultivation method also confirmed that dissemination of LLs microorganism is mitigated, since a high inactivation rate of mesophilic and psychrophilic bacteria was achieved (Fig. 3). According to the obtained data, the electrosynthesis of oxidants BDD system may have effectively inactivated bacterial cells, and thus the toxicity of EO-treated LLs needs to be tested against other species.

3.4. Toxicity assessment of electrochemical oxidation process using in vivo microassays

The current work examined the toxic effects of raw and EO-treated LLs on aquatic crustaceans *Thamnocephalus platyurus*, *Dapnia magna* and *Artemia franciscana*. The bioassays were performed for raw LLs and LLs treated with 0.5k BDD and 10k BDD electrodes. On the basis of the experimental data it was found that, among the tested LL samples, raw LLs were characterized by the strongest toxicity of all tested species (Fig. 4) probably as a synergistic toxic effect of different compounds present in the complex LL matrix (Wang et al. 2016). Toxicity of LLs after EO-treatment decreased but remained high, especially on *T. platyurus* and *D. magna* assays (Fig. 4).

Thamnotoxkit F and Rapidtoxkit F

The most sensitive organism among those tested proved to be *T. platyurus*, which is prone to environmental contamination and frequently used in bioassays (Kalka 2012; Aydin et al. 2015). In this study, raw LLs indicated strong toxicity towards *T. platyurus* (Fig. 4a, Fig. 5a). In the case of treated LL samples, lower toxicity was observed (Fig. 4, Fig. 5b-c).

The samples treated with 10k BDD anode were less toxic towards the aforementioned organisms than were those treated with 0.5k BDD. A significant decrease in toxic effect was observed in samples at concentrations 0.4% and 0.8% – by 46% and 70%, respectively (Fig. 4). Detailed observations showed that in samples at concentration above 6.2%, *T. platyurus* individuals were dead after a 1-minute exposure. With decreasing sample concentrations (0.4–3.1%) toxic effects from 1.5 to 24 hours were observed (Supplementary Material, Fig. S5). Researchers Mavakala et al. (2016) and Melnyk et al. (2014) indicate that the main causes of *T. platyurus* mortality in LLs are extremely high concentrations of salts and COD (Melnyk et al. 2014; Mavakala et al. 2016). However, in such a complex LL matrix, it is difficult to indicate the direct cause of toxicity.

Additionally, a microbiotest for rapid detection of contamination was carried out using *Thamnocephalus platyurus*. Exposed to harmful substances, these organisms react by slowing their filtration rate. In this experiment,

the raw LL samples showed higher toxicity compared to leachates after EO (Fig. 5). Raw LL samples caused particle uptake inhibition at a concentration of 12.5%. In the case of treated samples the inhibition effect was only noted at the highest concentration (100%). During the test, inhibition was not observed to change with time of exposure. The results of the "rapid assay" correspond to those of the 24-hour test – i.e. the strongest toxicity was observed for highly concentrated samples.

Daphtoxkit F. magna

LLs were also toxic to the *Daphnia magna* species. The highest mortality of *D. magna* exposed to raw and treated LLs was observed in samples with concentrations ranging from 6.2 to 100% (Fig. 4b). Treated LLs at concentrations below 3.1% were less toxic than raw samples. Additional observations of the organisms showed that in the samples with concentrations above 6.2% crustaceans died with a 3-hour exposure. At sample concentrations of 25%, 50% and 100%, all crustaceans died after 1–5 minutes.

According to Kalcikova et al. (2011), the toxic effects of LLs on daphnids are mainly attributed to high levels of ammonium compounds and COD. There are also publications in which no relationship between the mortality of daphnia and high levels of the aforementioned contaminants was found (Isidori et al. 2003; Kalcikova et al. 2011). To exactly determine the main factors responsible for the acute toxicity towards the selected organism, it would be necessary to include many chemical compounds potentially present in the LLs in a long-term monitoring programme. This would also allow the conditions and concentrations that cause death of the daphnia species to be determined with more confidence.

Artoxkit M.

The survival of *Artemia franciscana* in raw and treated LLs was also studied, and as for the *Daphnia magna* species and *T. platyurus*, it depended on the concentration of the samples. During 24-hour exposure to raw LLs, the strongest toxic effect was observed for the two most concentrated samples (50% and 100%) (Fig. 4c). In the samples treated by 10k BDD, crustacean mortality decreased by *ca* 40%. The most diluted samples of LLs slightly affected *A. franciscana* (Fig. 4c).

The observed response of aquatic crustaceans to LLs is in general species-dependent. In this work, the sensitivity of two species of crustaceans *T. platyurus* and *D. magna* was observed, while lower mortality was noted for *Artemia franciscana*, which lives naturally in inland saltwater lakes and tolerates a wide range of salinities (35–110%) (Vanhaecke et al. 1984). This makes *A. franciscana* a good indicator of LL toxicity, as compared to other compounds such as chlorine ions.

It can be concluded that LL toxicity is a very complex issue, and there remains insufficient knowledge about LL compounds and their toxicity. Thus, effective tools for estimating leachate strength and toxic potency are needed. This is of special concern if LL treatment is implemented, as treatment benefits connected with the effective removal of nutrients could be nullified by the generation of toxic compounds. In this study, EO-treated LLs were less toxic than raw samples, especially in the case of diamond electrodes with higher levels of boron doping.

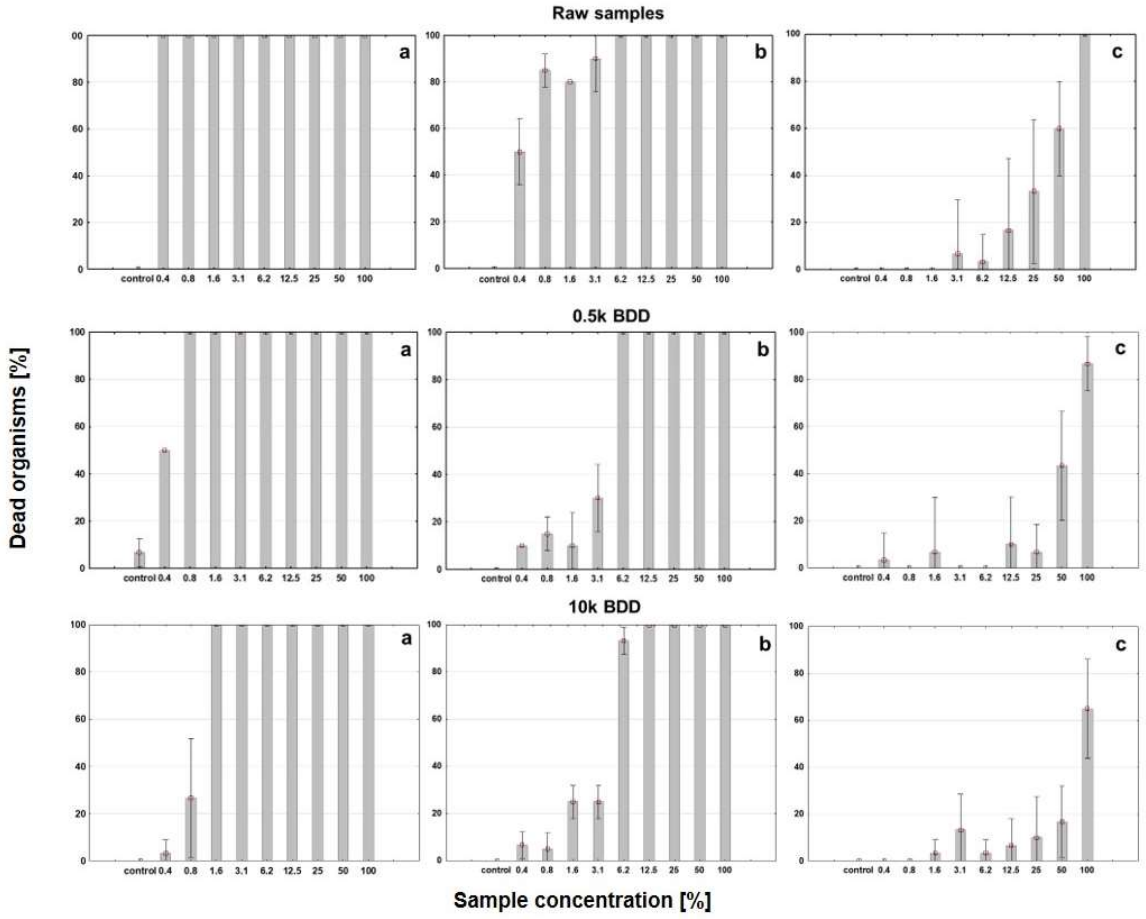

Fig. 4 (a) Thamnocephalus platyurus, (b) Daphnia magna, (c) Artemia franciscana and their mortality percentage to raw and treated LL samples with 0.5k BDD and 10 k BDD Nb electrodes, 24-hour tests

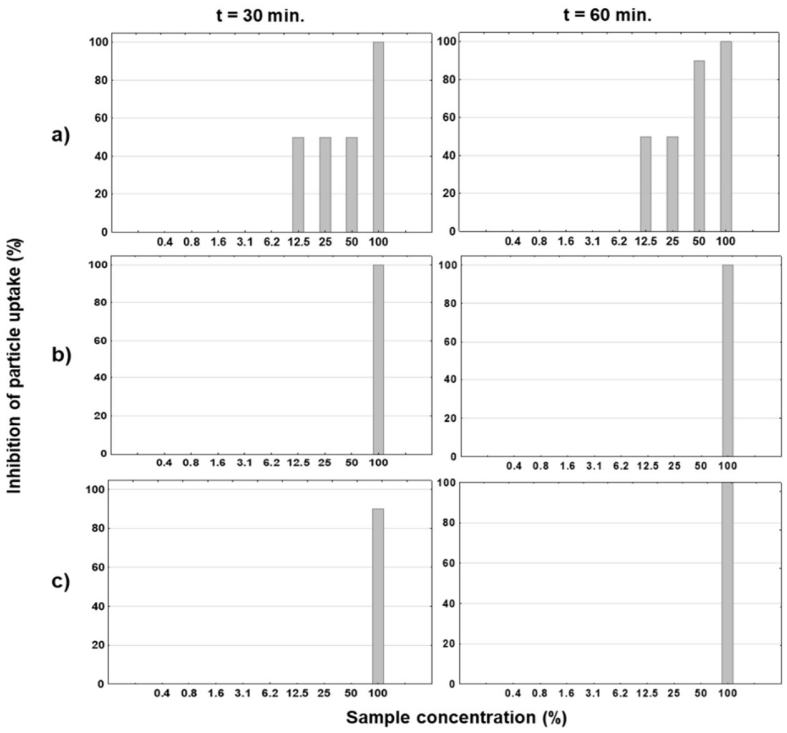

Fig. 5 (a) Effect of raw LL samples, (b) treated LLs with 0.5k BDD electrode and (c) treated LLs with 10k BDD electrode on T. platyurus inhibition of particle uptake, Rapidtoxkit test

4. Conclusions

This study indicates the potential of EO by means of BDD electrodes technique as a promising method for remediating sanitary LLs. The effectiveness of removal of the selected macropollutants from LLs was tested by the EO method using three BDD electrodes with boron doping concentrations of 500, 10,000 and 15,000 ppm of B and applied current densities 25–100 mA cm^{-2}. In general, the highest current density used (j = 100 mA cm^{-2}) resulted in the best pollutant-removal efficiency. Among the tested electrodes, the 0.5k BDD showed the best organic and N-NH_4^+ compound removal efficiency (97% COD, 90% BOD_{20}, 62% N-NH_4^+). Moreover, results demonstrated that most of the N-NH_4^+ was converted mainly to nitrate or nitrite forms: thus, the simultaneous removal of COD and N-NH_4^+ via BDD oxidation requires further investigation towards the total nitrogen removal. The EO method is also effective to mitigate the dissemination of LLs microorganism, reaching over 80% of mesophilic and up to 99% of psychrophilic bacterial cell inactivation within 2 h. It is suspected that in a such complex matrix as LLs, the EO process is based on combined effect of electrogenerated oxidants such as: hydroxyl radicals (•OH), active chlorine (HOCl, OCl^-, Cl_2) and sulphate radicals. Interestingly, the toxicity assays, indicated that EO-treated LLs were less toxic than raw ones, which confirmed that LL toxicity was more connected with the high concentration of salts and other pollutants, than with the presence of EO by-products. Thus, it is believed that by choosing appropriate electrolysis parameters the toxicity effect can be diminished, without compromising nutrient removal and disinfection capability.

5. Declarations

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable.

Availability of data and materials: All data generated or analyzed during this study are included in this published article.

Competing interests: The authors declare no competing interests.

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