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Effectiveness of *Chlorella vulgaris* inactivation during electrochemical water treatment

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ABSTRACT

In this study, an undivided electrochemical cell equipped with graphite electrodes was constructed for inactivation of algae *Chlorella vulgaris.* Suspensions containing relatively high concentration of *C. vulgaris* (10^8 cell/L) were exposed to current densities within the range 2.021–8.084 mA cm^{-2}, initial NaCl concentration 2–8 g dm^{-3} and the electrolysis was performed under batch mode. The results show that as the current density increased, the rates of inactivation increased accordingly. Complete inactivation of *C. vulgaris* cells at 8.084 mA cm^{-2} and 4 g dm^{-3} NaCl was observed after 55 min. Electrochemical treatment caused progressive destruction of the algal cell integrity and at 8.084 mA cm^{-2} lysed cells represent 69.7%. A longer time was needed for complete inactivation of algal cells at lower electrolyte NaCl concentration (2 g dm^{-3}) indicating that *C. vulgaris* appeared to be sensitive to electrogenerated active chlorine. The results implicated that electrochemical treatment can be effective as alternative treatment process for algal elimination in waters with higher mineral content.

Keywords: Electrochemical treatment; *Chlorella vulgaris*; Active chlorine; Cell viability

1. Introduction

Algal blooms have become a pressing issue in inland freshwater systems on local and global scales [1]. A set of physical, chemical and biological factors including climate changes and anthropogenic impacts are the reasons associated with substantial intensification of algal blooms occurring. As was stated in technical report elaborated by Joint Research Centre of European Commission, harmful algal blooms have a significant socio-economic impact on human health, fishery, tourism and recreation [2]. Algae overgrowth deteriorates suitability of water sources for drinking, sanitation, irrigation or industrial use due to increasing turbidity and undesirable toxin production.

In recent years, algae removal from waters and wastewaters gained importance, but their low specific gravity and small size caused difficulties in effectiveness of conventional and alternative water treatment processes such as sedimentation, coagulation, flocculation, dissolved air flotation, filtration and advanced oxidation processes [3,4]. Several processes to prevent bloom formation and for algae reduction or inactivation with and without the use of chemical/biological agents have been proposed. To enhance the removal efficiency of algae pre-oxidation step before actual treatment [5,6] and pre-chlorination are the most widely used pre-treatment processes. Chlorine also effectively degrades cyanotoxins, and algae removal efficiency depends on chlorine dosage, treatment time or pH [6–9]. However, recent concerns on chlorine rise due to its activity to react with organic matters to form carcinogenic disinfection by-products such as trichloromethanes and

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chloroacetic acids [10,11]. Interesting nature-based solution was described by Chen and Bridgeman [1]. They revealed that the UV-C radiation from lamps potentially powered by solar panels effectively reduced the concentration of *Chlorella vulgaris* with clear differences by radiation level.

Electrochemical disinfection as one of the most promising alternatives to conventionally used treatment methods has been discussed since the 1950's [12–14]. In comparison with conventional disinfection treatment, it provides advantages for both primary and residual disinfection. Moreover, the presence of chloride in the electrolyte led to the generation of highly germicidal active chlorine species (Cl_2, HOCl, ClO^-) or chlorine radicals (•Cl, $•Cl_2^-$), which enhanced the performance of cells inactivation efficiency. Another chlorine-free oxidizing species, for example, hydrogen peroxide, ozone, hydroxyl radical could also be produced during electrolysis [14–16]. In situ generation of strong oxidants reduces handling and storage hazards associated with chlorine and hypochlorite [17].

Electrochemical treatment effectiveness strongly depends on configuration of electrolytic cell, electrodes material, electrolyte solution and experimental parameters such as flow rate or current density. Electrochemical disinfection has been reported to be capable of inactivation of wide spectrum of microorganisms from viruses through bacteria and algae to larger species such as Euglena [15,18] in various types of water matrices [16,19]. However, the effectiveness of the electrochemical cells to disinfect microorganisms has been shown to vary. In recent years, numerous cell configurations and electrode materials have been investigated [20–23]. Liang et al. [24] observed significantly lower level of *Microcystis aeruginosa* disinfection when chlorine free electrolytes were used in electrochemical tube employing Ti/RuO_2 as anodes. Gao et al. [10] mention monolithic ceramic electrode (MCE, i.e., monolithic titanium suboxides) as a promising electrode material for effective electrochemical deactivation of algae. The stable deactivation of *M. aeruginosa* was associated with the oxidative species electrochemically produced on MCE. Also, other reports indicate that the use of boron-doped diamond (BDD) anodes significantly enhanced the removal of algae [25,26]. As was pointed out by Gao et al. [10], there exists a dilemma for the selection of electrode material in terms of electrical conductivity, electrochemical activity, chemical stability, economic reliability and environmental availability. It seems that for in situ application, devices with high efficiency of electrochemical treatment as well as with robust, simple and cheap design will be more suitable. Locker et al. [27] stated potential to exploit carbon-based electrodes for an ultra-cheap, disposable assembly for the production of antimicrobial hypochlorite for at-source based on simple DC applied current in a non-divided cell. Recently, Saha and Gupta [28] revealed that carbon electrodes exhibited a great potential for electrochlorination systems as an alternative to costlier platinum and DSA electrodes. Another important aspect from the perspective of practical application of electrochemical treatment for algae removal is potential to be self-powered by stand-alone photovoltaic systems to operate independently of electricity networks or subsurface urban infrastructure. Photovoltaic unit enables the use of electrochemical cells for pre-treatment or treatment of waters in distant localities such as water reservoirs, basins, tanks, dams, etc. [16,29].

Considering the above-mentioned facts, the main purpose of this study was to determine the feasibility of *C. vulgaris*, a colonial *Chlorophyceae* removal through the electrochemical water treatment process using constructed low cost, simple and undivided electrochemical cell equipped with graphite electrodes potentially powered by photovoltaic system. The viability of *C. vulgaris* cells during electrolysis and the effects of current density, electrolyte concentrations and detention time on inactivation of relatively high algae concentrations (10^8 cell/L) have been investigated. Moreover, the effects of NaCl concentration on the electrochemical active chlorine generation were also studied.

2. Materials and methods

2.1. Algal strain and culture condition

Microalgae *C. vulgaris* BEIJERINCK H1993 obtained from the Culture Collection of Algae of Charles University (Prague, Czech Republic) was adopted as model organism for electrochemical water treatment experiments. *C. vulgaris* was cultured in flasks containing 250 cm^3 of sterile diluted (1:3 v/v; pH 6.0) Hoagland growth medium (HGM, g dm^{-3}): $MgSO_4$ $7H_2O$ (0.3697), KNO_3 (0.4044), $CaCl_2$ (0.4439), NaH_2PO_4 $2H_2O$ (0.2917), Na_2HPO_4 $12H_2O$ (0.0465), $FeSO_4$ $7H_2O$ (0.0179), $NaNO_3$ (0.3399), NH_4Cl (0.2139), NH_4NO_3 (0.1601), H_3BO_3 (0.0085), Na_2MoO_4 $2H_2O$ (0.00006), $MnSO_4$ (0.005), $ZnSO_4$ $7H_2O$ (0.00066) and $CuSO_4$ $5H_2O$ (0.0008).

Flasks were placed on laboratory shaker (Multi-Shaker PSU 20) at 100 RPM for optimal aeration and to avoid sticking of algal cells on flask walls and incubated in a laboratory growth chamber (Binder KBWF720) at 25°C ± 1°C. The light intensity 11,450 lx was provided in a 16 h/8 h light/dark cycle (white fluorescent cool light, Philips TLD18W/865). Measurements of algal growth parameters (cell number, cell size) were realized by digital cellometer (Nexcelom Cellometer™ Auto T4) every 48/72 h. The logarithmic phase of growth was attained after 24 d of cultivation. Before electrochemical experiments, content of cultivation flasks with algae culture in the log phase of growth were mixed, stirred, biomass harvested and size distribution of algae cells were measured.

2.2. Experimental apparatus

Electrochemical experiments were carried out in an undivided electrochemical cell under conditions of batch system (Fig. 1). The electrochemical reactor consists of 5 dm^3 glass tank (22.5 × 15 × 15 cm) with effective volume of 2 dm^3 equipped with two graphite electrodes (electrode consists from three graphite blocks 80 × 23 × 10 mm with density 1.8 g cm^{-3}; specific electrical resistance 1.3 mΩ cm and medium grain size 6 μm. Elero Inc., Slovak Republic) connected by a monopolar connection. Electrodes were positioned vertically and parallel to each other with an inter-electrode distance of 1.5 cm. The surface area of the anode and the cathode immersed in solution was 15.7 cm^2.

The electric power was supplied with a regulated DC power supply Elektrolyser (VEB MLW Labortechnik Ilmenau, Germany) and managed by two separate digital multimeters (METEX M-3850D and AXIO MET AX-18B).

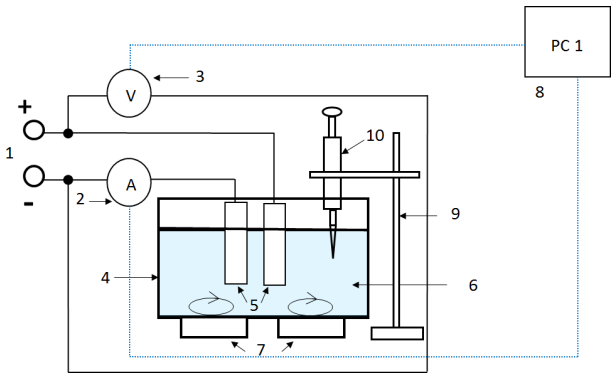

Fig. 1. Experimental apparatus used in experiments. (1) DC power supply; (2) ampere meter; (3) volt meter; (4) electrochemical reactor; (5) carbon electrodes; (6) electrolyte; (7) magnetic stirrers; (8) PC; (9) laboratory rack; (10) sampling micropipette.

The algal suspension was continuously stirred at 100 RPM by two magnetic stirrers during electrolysis to maintain a uniform concentration of the electrolyte solution and algae distribution within the electrochemical system.

2.3. Experimental procedure

For batch electrochemical experiments, 2 dm^3 of artificially polluted water were prepared. A batch culture of *C. vulgaris* was concentrated by centrifugation (1,500 RPM; 3 min) and mixed with electrolyte (final volume 2 dm^3) to give an initial cell density of 7×10^5 cell/mL. Before electrolysis initial viability and algal cell density were determined. All electrochemical experiments were performed at galvanostatic conditions at 22°C and NaCl was used as electrolyte in concentration range from 2 to 8 g dm^{-3} to test the effect of NaCl on treatment process. The applied current densities ranged from 2.021 and 8.084 mA cm^{-2}. During electrochemical process, aliquot samples were collected for analysis at given time intervals. Electrolysis was conducted continuously until more than 99% mortality (6 log removal) was observed in collected samples.

To evaluate the electrochemical process UV/Vis spectrum (190–800 nm) was measured in time intervals using UV/Vis spectrophotometer (Varian Cary 50).

2.3.1. Cell counting, dye-staining and autofluorescence

A direct microscopic method for *C. vulgaris* cell counting (cell/mL) in samples was performed using Bürker counting chamber and optical microscope at magnification 100× (Olympus CX41 with a camera Olympus E-450, Japan). Algal cell counting was also conducted using cellometer (Nexcelom Cellometer™ Auto T4). Each sample was analyzed three times with differences within 5% in all cases.

To test the viability of *C. vulgaris* cells for estimating the survival ratio, two modified methods according to Pouneva [30] and Imase et al. [31] were used. Control and cells from electrochemical experiments were stained with methylene blue (MB). MB solution (0.2 cm^3, 20 mg cm^{-3}) was added into 1 cm^3 of algal samples and incubated for 10 min in the dark at room temperature and subsequently observed by optical microscope. Cells stained blue were considered to be dead.

The viability of algal cells was also investigated after observing the autofluorescence of chlorophyll. In the autofluorescence testing, cells emitting red fluorescence were considered to be live. For both qualitative and quantitative observations epifluorescence microscope (Olympus CX41, Japan) with excitation LED light source (PRO-LM-LED-FLUO B; 460 nm) and filter set (CX-DMB-2 cube; excitation filter 420 to 480 nm; dichroic mirror DM500; 515 nm emission filter) was used. Survival rates were calculated according to the following Eq. (1):

$$\text{Survival rate } \% = \frac{N_t}{N_0} \times 100 \tag{1}$$

where N_t number of viable cells in time t, N_0 number of viable cells in t_0. In the present work, the term inactivated algal cell represents both dead and lysed cell. Lysed cells were calculated according to Eq. (2):

$$\text{Lysed cell (cell/ml)} = N_{T_0} - N_{T_t} \tag{2}$$

where N_{T_0} total number of cells in time t_0, N_{T_t} total number of cells in time t.

2.3.2. Active chlorine determination

Electrochemically generated active chlorine was determined by modified o-tolidine-based photometric method [32]. The colourless o-tolidine (3,3′-dimethyl-4,4′-diamino-diphenyl) with active chlorine forms a yellow product. For specific reaction of o-tolidine with chlorine, pH < 1.3 has to be maintained. Into 25-cm^3 volumetric flask, 1.25 cm^3 0.135% o-tolidine solution was added and filled with electrochemically treated water containing active chlorine. Solution was mixed and incubated in the dark for 5 min due to photosensitivity of yellow product. Subsequently, absorbance at 435 nm was measured using UV–Vis spectrophotometer. Concentration of active chlorine in treated water was determined from calibration curve.

In order to prepare calibration curve, calibration solutions as mixture of two standard solutions $CuSO_4$ $5H_2O$ (1.5 g $CuSO_4$ was diluted in distilled water containing 1 cm^3 concentrated H_2SO_4 and filled up to 100 cm^3) and $K_2Cr_2O_7$ (25 mg $K_2Cr_2O_7$ was diluted in distilled water containing 1 cm^3 concentrated H_2SO_4 and filled up to 100 cm^3) were prepared. Solutions corresponding to active chlorine concentration of 0.01, 0.05, 0.07, 0.1, 0.2 and 0.5 mg dm^{-3} were prepared by mixing of 0, 0.4, 1.2, 1.8, 1.9 and 2 cm^{-3} $CuSO_4$ $5H_2O$ and 0.8, 5.5, 7.5, 10, 20 and 45 cm^{-3} of $K_2Cr_2O_7$ and filled with distilled water to 100 cm^{-3}. The absorbance of calibration solutions was measured spectrophotometrically at 435 nm and data were used for calibration curve (absorbance of $CuSO_4$ $5H_2O$ and $K_2Cr_2O_7$ mixtures vs. corresponding concentration of active chlorine).

2.3.3. Electrolysis analysis

The specific energy consumption was calculated from data recorded during electrochemical treatment according to Eq. (3) [22,33]:

$$E_{sp} = \frac{I \times U \times T}{V} 1000 \quad (3)$$

where E_{sp} is the specific energy consumption (kWh m^{-3}), I is the current intensity (A), U is the electrical potential (V), T is the treatment time (h) and V is the volume of treated water (m^3).

3. Results and discussion

In preliminary experiments, the electrochemical elimination of green alga *C. vulgaris* from freshwater at conditions 4.042 mA cm^{-2}, NaCl 4 g dm^{-3} and 22°C were examined. The absorption UV–Vis spectra of electrochemically treated water containing algal cells are shown on Fig. 2(a). The absorbance at 680 nm corresponds to the maximum absorbance of chlorophyll-a in algal cells. As was reported by Poelman et al. [34] chlorophyll-a represents approximately 1%–2% of the dry weight of planktonic algae. The UV–Vis spectra (Fig. 2(a)) indicated that due to the damage of *C. vulgaris* cells, the chlorophyll-a released from the cells was degraded by electrochemical oxidation and significant decolourization of treated water (Fig. 2(b)) during electrolysis was observed. Peak at 680 nm completely disappeared after 100 min. After 20 min of electrolysis, significant absorption peak appeared at 290 nm which could be attributed to the formation of hypochlorite ions (ClO^-) during electrochemical disinfection. Similarly, Mascia et al. [25] observed maximum at 292 nm in UV spectrum attributed to ClO^- absorption.

To test the viability of *C. vulgaris* cells during electrochemical disinfection, staining with MB and observation of chlorophyll autofluorescence were used (Fig. 3). MB is hydrophilic dye able to penetrate into the cell only if lipophilic membrane had been seriously damaged and therefore blue stained cells were evaluated to be dead. The degree of cell membrane damage affects permeability and also the staining time. As seen by comparison with an intact cell, MB stained cell clearly after 10 min (Fig. 3(a)) indicating higher permeability of cell membranes as a result of electrochemical treatment. Imase et al. [31] in experiments with *Chlorella* cells exposed to oxidative stresses revealed that at least 20 min is required for clear staining with MB. Autofluorescence of chlorophyll pigments is very sensitive and easily decomposed by oxidation [30,35] and only live algae cells emitted red fluorescence. From Figs. 3(b) and (c) it is evident that live *C. vulgaris* cell emitted red chlorophyll autofluorescence after blue light excitation. Dead cell emitted none or very low green signal intensity and could be observed only under light microscope. Results confirmed degradation of chlorophyll revealed by spectral analysis (Fig. 2(a)). Moreover, the disappearance of chlorophyll autofluorescence could be an appropriate index of the survival rates of *Chlorella* cells during electrochemical disinfection and although both methods provided similar viability count, only autofluorescence was used in further experiments.

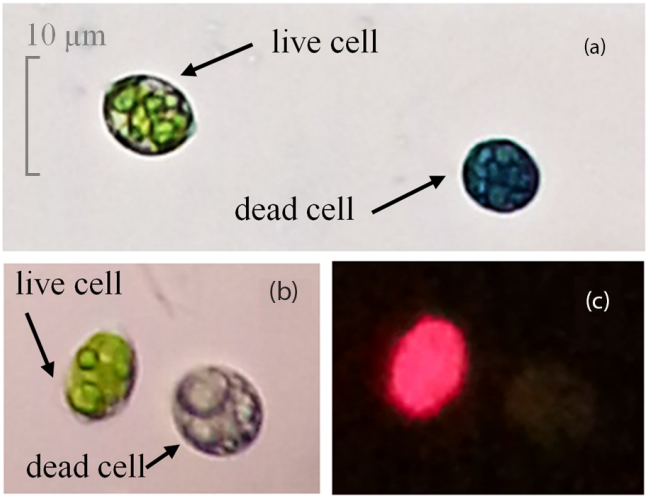

Fig. 3. Microscopic images of *Chlorella vulgaris* (a) after 10 min staining with MB; (b) before and (c) after emission of chlorophyll autofluorescence.

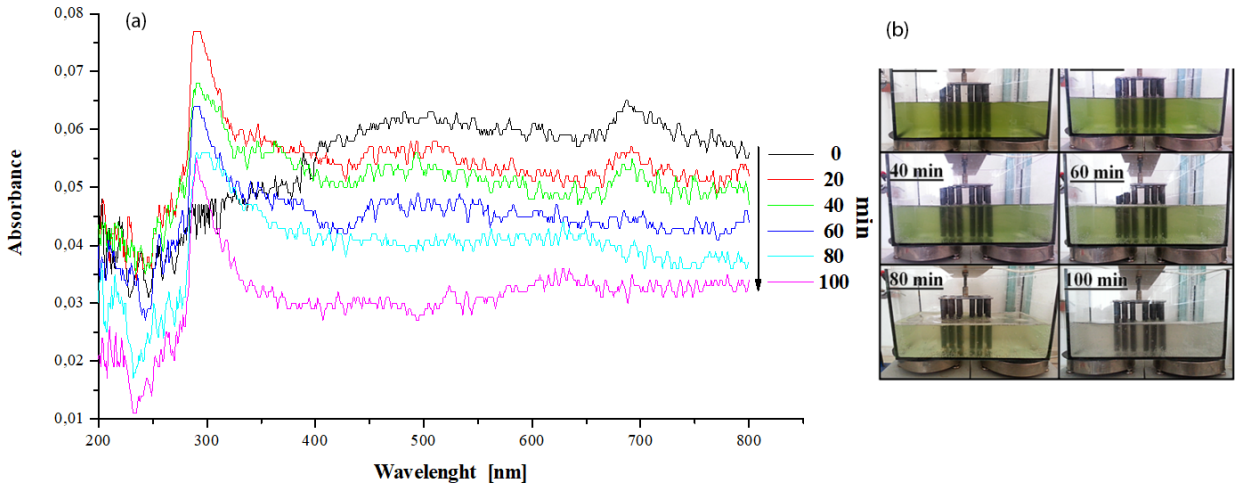

Fig. 2. UV–Vis spectra of water containing *Chlorella vulgaris* during electrochemical disinfection (a), glass tank at various treatment time (b). Experimental conditions: current density 4.042 mA cm^{-2}; electrolyte 4 g dm^{-3} NaCl; initial cell density 7 × 10^5 cell/mL; initial pH 5,8; T = 22°C.

3.1. Effect of current density on cell density and integrity

According to our previous results [36], the current density significantly influenced the electrochemical oxidation efficiency of dyes in wastewaters. Therefore, the effect of current density on *C. vulgaris* cells density and integrity was studied. Electrochemical disinfection experiments were performed at different current densities within the range of 2.021–8.084 mA cm^{-2}, taking into account the energy consumption (i.e., economic efficiency of water treatment process). Constant electrolyte volume (2 dm^3) and concentration of NaCl (4 g dm^{-3}) were used. Moreover, appropriate agitation (100 RPM) was selected to ensure good mixing of the oxidants forming by electrolysis considering the results of Alfafara et al. [37] and Xu et al. [38] who pointed out that agitation exhibits higher algal removal efficiency at lower current densities (<5 mA cm^{-2}).

The effects of various current densities on survival of *C. vulgaris* are shown in Fig. 4. When 2.021 mA cm^{-2} was applied, cells viability progressively decreased, about 5.36 and 1.19 × 10^5 cell/mL of viable cells were observed in 50 and 150 min, respectively. Complete inactivation of algal cells was obtained after 225 min. The results show that as the current density increased, the rates of inactivation increased accordingly. Interestingly, at 8.084 mA cm^{-2}, dramatic increase of dead (2.11 × 10^5 cell/mL) and lysed (1.97 × 10^5 cell/mL) algal cells was observed after 27 min. Complete inactivation of *C. vulgaris* cells at 8.084 and 4.042 mA cm^{-2} was recorded after 55 and 95 min, respectively (Fig. 4). After 55 min of exposure, the pH value of electrolytes decreased at all current densities used from 5.8 to 5.5. It was also found that electric charge required for complete inactivation of algal cells increased with decreasing of applied initial current density (data not shown). Our results are also consistent with previous findings of electrochemical inactivation of cyanobacteria *Cylindrospermopsis raciborskii* [23] and photoautotrophic gram-negative bacteria *M. aeruginosa* [39]. Xu et al. [38] observed similar effect of increasing current density on inactivation of *M. aeruginosa*. After 30 min of electrochemical treatment with combination of graphite-stainless electrodes, 95.3% inhibition ratio was recorded at current density 16 mA cm^{-2}. Lin et al. [40] concluded that electrolysis by current densities higher than 10 mA cm^{-2} led to the complete inhibition of *M. aeruginosa* cells at 5 × 10^5 cell/mL initial cell density.

Previous studies [25,41,42] showed that even the presence of very low concentration of Cl^- ions allows the oxidations of chlorides, which lead to dominant constitution of active chlorine species. During the electrolysis, adsorption and dissolution of chlorine generated from the oxidation of chloride ions

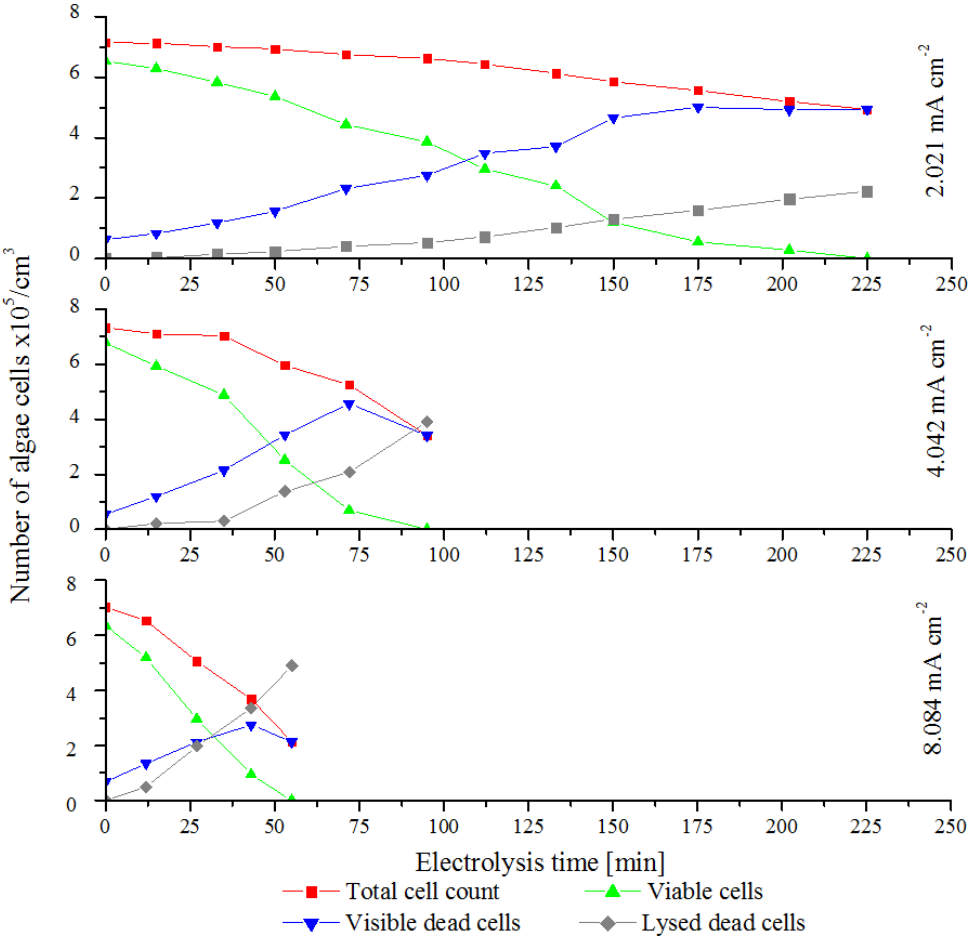

Fig. 4. Rates of inactivation of *Chlorella vulgaris* cells during electrolysis performed at various current densities (2.021–8.084 mA cm^{-2}; electrolyte: 4 g dm^{-3} NaCl; initial cell density 7 × 10^5 cell/mL; initial pH 5.8, T = 22°C).

occur at the anode surface while oxygen evolves from water discharge [43,44] (Eqs. (4) and (5)):

$$2Cl^- \rightarrow Cl_2 + 2e^- E^0 = -1.36V \quad (4)$$

$$2H_2O(l) \rightarrow O_2(g) + 4H^+ + 4e^- E^0 = -1.23V \quad (5)$$

Subsequently, dissolved chlorine reacts with water and hypochlorous acid and hypochlorite ions are produced (Eqs. (6) and (7)):

$$Cl_2 + H_2O \rightarrow HClO + Cl^- + H^+ E^0 = -0.93V \quad (6)$$

$$HClO \rightarrow ClO^- + H^+ \quad (7)$$

Electrochemical generation of various active chlorine forms strongly depends on electrolyte pH value. According to chlorine speciation [45], chlorine dominates at pH below 2.5 while hypochlorite ions dominate above pH 8.0. In our experiments, pH during electrolysis decreased to 5.5 indicating mainly the presence of HClO and hypochlorite ions forms. In cathodic reaction zone reduction of the active chlorine and the hydrogen evolution is favoured (Eqs. (8) and (9)):

$$ClO^- + 2e^- + H_2O \rightarrow Cl^- + 2OH^- \quad (8)$$

$$H_2O + 2e^- \rightarrow H^+(g) + 2OH^- E^0 = -0.83V \quad (9)$$

Since NaCl (4 g dm^{-3}) was used as supporting electrolyte during electrochemical disinfection, electrogenerated active chlorine (total concentration of hypochlorite ions, hypochlorous acids and dissolved chlorine) was also analyzed. The effect of current intensity and electrolysis time on the active chlorine formation in experiments without algae cells in solution is shown in Fig. 5. There is only negligible difference in the maximum values of produced active chlorine species at different current densities. However, time needed to reach the maximum level of active chlorine differed significantly. At current densities 8.084 and 4.042 mA cm^{-2} maximum level of active chlorine (0.46 mg dm^{-3}) has been achieved after 40 and 100 min, respectively. Active chlorine generation at 2.021 mA cm^{-2} was markedly slower and maximum concentration 0.43 mg dm^{-3} was recorded after 180 min. When 7 × 10^5 cell/mL of algae was present in electrolyte during first 55 min of electrolysis at all current densities no active chlorine was detected (not shown). As was pointed out by Gao et al. [3], the decrease of active chlorine concentration in the presence of algae cells could be attributed to the fact that the active chlorine would react with the algae cells immediately after generation.

Our results suggest that electrochemical disinfection caused progressive destruction of the algal cell integrity (Fig. 6). At 8.084 mA cm^{-2} lysed cells represent 4.9 × 10^5 cell/mL of total cell count in initial sample. As expected, the lowest proportion of lysed cells (2.22 × 10^5 cell/mL) was observed at 2.021 mA cm^{-2}. With increasing current density and formation of active chlorine, the proportion of lysed cells significantly increased during electrolysis (Fig. 6). Moreover, morphological changes of *C. vulgaris* cells after electrochemical treatment were observed. Prior to electrolysis, cells have a distinct cell envelope while electrochemical treatment induced significant disruption of cell envelope (not shown). The smaller diameter and crumpled shape of algal cells indicate that during electrolysis, the cells could be dehydrated with only water leaving the cell, or cell lysis may be occurring and releasing intracellular contents. Similarly, Daghrir et al. [46] observed that current intensity (0.6 A) applied for 100 min of electrolysis enlarged the porosity and the permeability of the cell membrane of *C. vulgaris* and promotes the release of intracellular compounds into the medium. Gao et al. [3] and Monasterio et al. [4] revealed that exposition of algae to an external electric field led to the induction of permanent or transient pores in the membrane. If electrochemically generated oxidants are present, these pores may allow the oxidants free access to the interior of the cell, which increases the efficiency of inactivation process

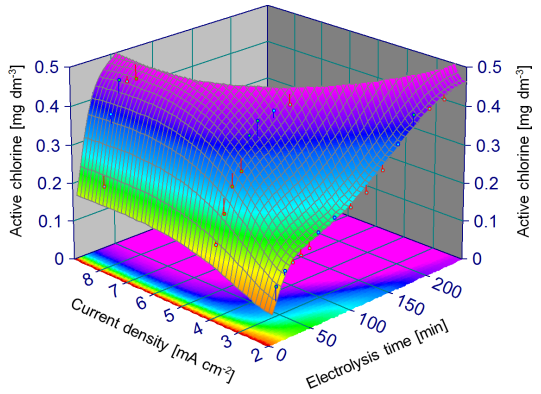

Fig. 5. Effect of current density and electrolysis time on active chlorine formation (mg dm^{-3}) during electrolysis (electrolyte: 4 g dm^{-3} NaCl, initial pH = 5.8, T = 22°C) without the presence of algal cells. Surface plot predicted using nonlinear regression in TableCurve3D.

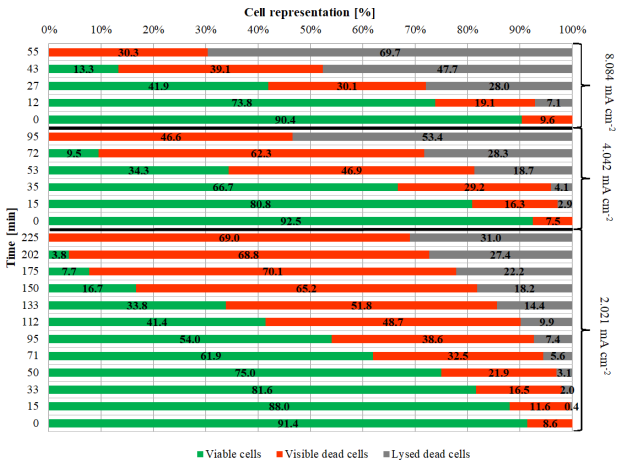

Fig. 6. Proportion of live, dead and lysed *Chlorella vulgaris* cells during electrolysis at various current densities.

(active chlorine can damage the nucleus, protoplasm and proteins of the cell membrane). It is indicated that the results of other researchers (e.g., Mascia et al. [47]), we suppose that the main mechanism of algal cells inactivation is the reaction with bulk oxidants (see discussion below).

3.2. Effect of electrolyte concentration

The supporting electrolytes used in most electrochemical disinfection processes are chlorides, sulfates, nitrates and phosphate salts [48,49]. In this study, various concentrations of NaCl as a support electrolyte for increase of conductivity of treated water and generation of active chlorine were used. Moreover, it was found that the presence of chloride ions eliminates the negative effects of other anions (SO_4^{2-}, HCO_3^-) and the precipitation of Ca^{2+} and Mg^{2+} which could lead to the formation of an insulating layer on electrode surface and decrease in the current efficiency [50].

Increasing in NaCl concentration caused to increase the active chlorine ions (Fig. 7) and the cell conductivity, due to increasing the mass transport of chloride ions to the anode surface. Although the initial concentration of NaCl significantly influenced the maximum concentration of electrogenerated active chlorine, the time needed to reach maximum at all NaCl concentrations was approximately 100 min. Maximum concentration of active chlorine of 0.65 mg dm^{-3} was obtained at 8 g dm^{-3} NaCl electrolyte concentration. The lower maximum concentrations of active chlorine (0.33 and 0.46 mg dm^{-3}) were determined during electrolysis with 2 and 4 g dm^{-3} NaCl. Similarly to previous experiments, when 7×10^5 cell/mL of algae was present in electrolyte during first 55 min of electrolyses, no active chlorine was detected.

The effects of various concentration of NaCl on survival of *C. vulgaris* are shown in Fig. 8. Experiments without current density exposure revealed only negligible influence

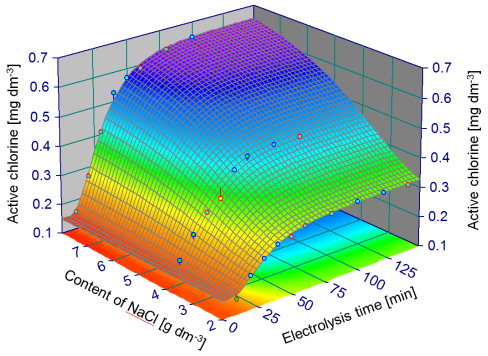

Fig. 7. Effect of NaCl concentration (g dm^{-3}) and electrolysis time on active chlorine formation (mg dm^{-3}) during electrolysis (current density: 4.042 mA cm^{-2}, initial pH = 5.6–5.8, T = 22°C) without the presence of algal cells. Surface plot predicted using nonlinear regression in TableCurve3D.

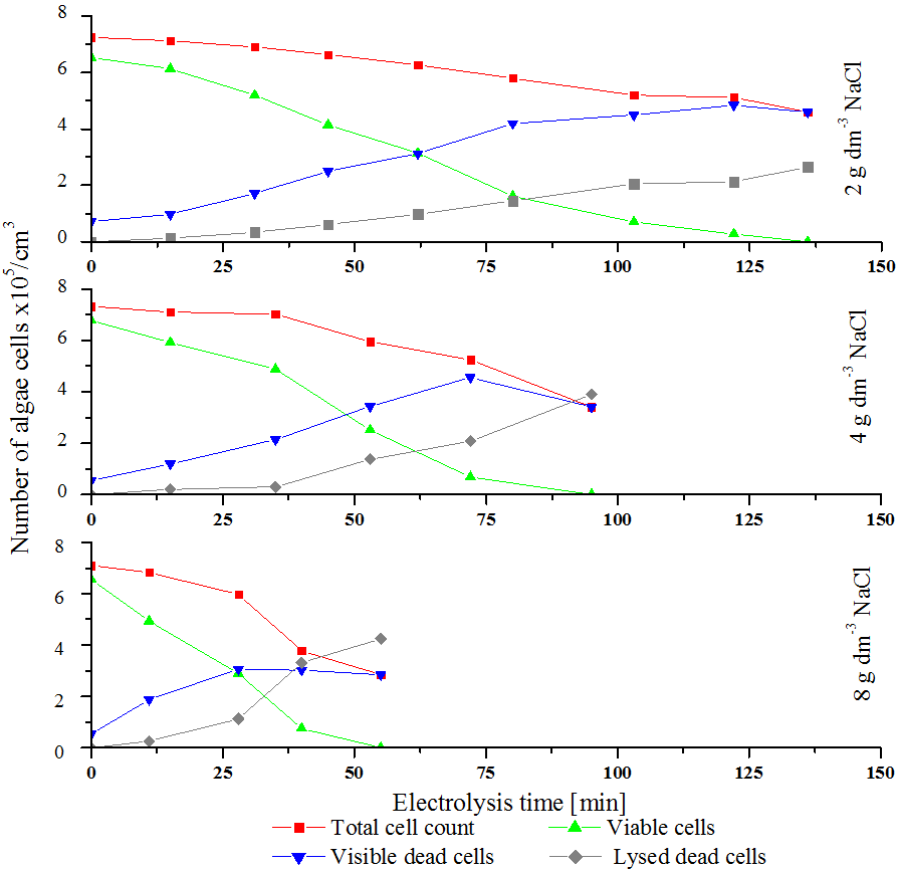

Fig. 8. Rate of inactivation of *Chlorella vulgaris* cells during electrolysis performed at various concentration of NaCl (current density 4.042 mA cm^{-2}; initial cell density 7 ′ 10^5 cell/mL; initial pH 5.6–5.8, T = 22°C).

of NaCl on *C. vulgaris* viability (not shown). With increasing initial NaCl concentration at current density 4.042 mA cm^{-2}, disinfection rate significantly increased and time needed to complete inactivation of algal cells decreased to 55 min. A longer time was needed (95 and 136 min) for complete inactivation of algal cells at lower electrolyte NaCl concentrations (2 and 4 g dm^{-3}). The pH value of electrolyte decreased from 5.8 to 5.4 (8 g dm^{-3} NaCl), 5.5 (4 g dm^{-3} NaCl) and 5.6 (2 g dm^{-3} NaCl) at the end of electrolysis.

The stability of cell membranes was monitored by a determination of lysed cell counts. At 4.042 mA cm^{-2} and 2 g dm^{-3} NaCl up to 2.65 × 10^5 lysed cell/mL (36.6%) was observed at the end of the experiment. As expected, significantly higher numbers of lysed cells (3.91 and 4.25 × 10^5 lysed cell/mL, respectively, 53.4% and 59.9%) were observed at 4 and 8 g dm^{-3} NaCl (Fig. 8). As was discussed above, changes in permeability of cell membrane are one of the main reasons of this effect. Higher current densities caused significant destabilization of cell membrane and increase their permeability. Therefore, significantly lower concentrations of generated active chlorine are needed for effective algal cells inactivation.

The main problem in cell damage evaluation is that parallel mechanisms exist and that the analysis of disinfecting species is extremely difficult due to the high reaction rates [20]. Table 1 shows the effects of various disinfectants on living cells. As was pointed out by Monasterio et al. [4], concentration of active chlorine in treated water will be strongly reduced by the presence of algae. Higher algal inactivation efficiency of electrochemically generated active chlorine was observed in comparison with injection of electrochemically generated chlorine presented by Sun et al. [51]. They used significant higher chlorine dosage (5 mg dm^{-3}) injected into *C. vulgaris* suspension (1 × 10^5 cell/cm^3), however full inactivation of *C. vulgaris* was recorded after 24 h for one trial injection and for multi-trial injection of 1.5 mg dm^{-3} chlorine after 10 h. It is obvious that also reactive oxygen species (ROS) are generated in undivided electrochemical cell equipped with graphite electrodes and therefore, their role in algal cell inactivation cannot be neglected, although contradictory findings can be found in literature. Locker et al. [27] confirmed that the antimicrobial efficacy of electrochemically generated hypochlorite using carbon-based electrodes is not enhanced by cogenerated reactive oxygen species. To identify the role of ROS in algal cell inactivation, further detailed research is needed.

To compare electrochemical disinfection with other processes, specific energy consumption (Fig. 9) was calculated. It could be observed that as current density varied from 2.021 to 8.042 mA cm^{-2}, energy consumption increased correspondingly. The energy required for a complete inactivation of *C. vulgaris* (initial algae concentration 7 × 10^5 cell/cm^3) was found to be 1.25, 1.55 and 2.82 kWh m^{-3}. Similarly, as NaCl concentration increased (from 2 to 8 g dm^{-3}; current density 4.042 mA cm^{-2}), the energy required for complete inactivation of *C. vulgaris* was found to be 2.76, 1.55 and 0.99 kWh m^{-3}. Obtained values are in a good agreement with those observed by Mascia et al. [25]. For complete inactivation of *Chlorella* cells values from 2.5 to 3.0 kWh m^{-3} were calculated (electrolysis with BDD anodes, current density 25–100 A m^{-2}). Interestingly, for inactivation of *M. aeruginosa* cells, significantly higher values (up to 150 kWh m^{-3}) were obtained indicating higher resistance of *M. aeruginosa* to electrochemical treatment (electrolysis with BDD anodes, current density 10–60 A m^{-2}) [47]. When electrocoagulation was used for *M. aeruginosa* removal, the energy consumption increased dramatically from 0.2 to 2.28 kWh m^{-3} as the current density varied from 0.5 to 5.0 mA cm^{-2} [3]. As expected it is important to optimize the current input in electrochemical disinfection processes to avoid extra-higher energy consumption.

Table 1
Effects of various disinfectants on living cells according to Bergmann [52] and Ghernaout et al. [20]

Disinfectants	Mechanism
Chlorine	DNA replication enzymes inhibition
ClO_2	Changes in cell walls due to oxidation of amino groups
	Disruption of proteo-synthesis
	Disruption of glucose oxidase
O_3	Destruction of cell membranes
	Reaction with glutathione
	Damages of chromosomal DNA
Hydroxyl radicals	Strand breaking in DNA radicals
	Reaction with nucleic acids
	Cell deformation lead to their rupture

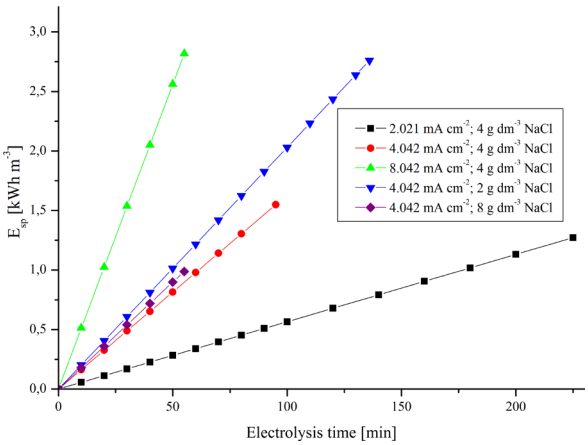

Fig. 9. Energy consumption E_{sp} during electrochemical disinfection of water with *Chlorella vulgaris* at various current density and NaCl content.

4. Conclusions

An undivided electrochemical cell equipped with graphite electrodes was constructed and used for inactivation of relatively high concentration of *C. vulgaris* (10^8 cell/dm^3) in water. Suspensions of *C. vulgaris* were exposed to current densities within the range 2.021–8.084 mA cm^{-2} and initial NaCl concentration 2–8 g dm^{-3}. The viability of algal cells obtained after observing the autofluorescence of chlorophyll clearly decreased with increase in current density and electrolysis time. Complete inactivation of *C. vulgaris* cells at 8.084 mA cm^{-2} and 4 g dm^{-3} NaCl was observed after 55 min. Electrochemical disinfection caused progressive destruction

of the algal cell integrity and at 8.084 mA cm^{-2} lysed cells represent 69.7%. A longer time was needed for complete inactivation of algal cells at lower electrolyte NaCl concentration (2 g dm^{-3}) indicating that *C. vulgaris* appeared to be sensitive to electrogenerated active chlorine. Maximum concentration of active chlorine 0.65 mg dm^{-3} was obtained at 8 g dm^{-3} NaCl electrolyte concentration. Based on our results and the results of other researchers, we suppose that the main mechanism of algal cells inactivation is the reaction with bulk oxidants. The results implicated that electrochemical treatment can be effective as alternative treatment process for algal elimination in waters with higher mineral content. Based on the energy consumption calculations, possible treatment system self-powered by photovoltaic panels could potentially be constructed on the basis of our simple electrochemical cell and implemented in in situ algal elimination.

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References

[1] E.S. Chen, T.B. Bridgeman, The reduction of *Chlorella vulgaris* concentrations through UV-C radiation treatments: a nature-based solution (NBS), Environ. Res., 156 (2017) 183–189.
[2] I. Sanseverino, D.S. Conduto Atónio, L. Pozzoli, S. Dobricic, T. Lettieri, Algal Bloom and Its Economic Impact, Publications Office of the European Union, Italy, 2016.
[3] S. Gao, J. Yang, J. Tian, F. Ma, G. Tu, M. Du, Electro-coagulation-flotation process for algae removal, J. Hazard. Mater., 177 (2010) 336–343.
[4] S. Monasterio, F. Dessì, M. Mascia, A. Vacca, S. Palmas, Electrochemical removal of *Microcystis aeruginosa* in a fixed bed reactor, Chem. Eng. Trans., 41 (2014) 163–168.
[5] J.J. Chen, H.H. Yeh, I.C. Tseng, Effect of ozone and permanganate on algae coagulation removal – pilot and bench scale tests, Chemosphere, 74 (2009) 840–846.
[6] M. Ma, R. Liu, H. Liu, J. Qu, Chlorination of *Microcystis aeruginosa* suspension: cell lysis, toxin release and degradation, J. Hazard. Mater., 217–218 (2012) 279–285.
[7] B. Mukherdee, P.N. Pandey, S.N. Singh, Mathematical modelling and system analysis of inorganic carbon in the aquatic environment, Ecol. Model., 152 (2002) 129–143.
[8] J.L. Acero, E. Rodriguez, J. Meriluoto, Kinetics of reactions between chlorine and the cyanobacterial toxins microcystins, Water Res., 39 (2005) 1628–1638.
[9] L. Ho, G. Onstad, U. von Gunten, S. Rinck-Pfeiffer, K. Craig, G. Newcombe, Differences in the chlorine reactivity of four microcystin analogues, Water Res., 40 (2006) 1200–1209.
[10] Y. Gao, J. Zhang, X. Bai, S. You, Monolithic ceramic electrode for electrochemical deactivation of *Microcystis aeruginosa*, Electrochim. Acta, 259 (2018) 410–418.
[11] Y.L. Zhang, B.P. Han, B. Yan, Q.M. Zhou, Y. Liang, Genotoxicity of disinfection by-products (DBPs) upon chlorination of nine different freshwater algal species at variable reaction time, Aqua, 63 (2014) 12–22.
[12] A. Reis, Die anodische Oxidation als Inaktivator pathogener Substanzen und Prozesse, Klin. Wschr., 29 (1951) 484–485.
[13] H. Bergmann, T. Iourtchouk, K. Schops, K. Bouzek, New UV irradiation and direct electrolysis-promising methods for water disinfection, Chem. Eng. J., 85 (2002) 111–117.
[14] M.I. Kerwick, S.M. Reddyb, A.H.L. Chamberlain, D.M. Holt, Electrochemical disinfection, an environmentally acceptable method of drinking water disinfection?, Electrochim. Acta, 50 (2005) 5270–5277.
[15] H.F. Diao, X.Y. Li, J.D. Gu, H.C. Shi, Z.M. Xie, Electron microscopic investigation of the bactericidal action of electrochemical disinfection in comparison with chlorination, ozonation and Fenton reaction, Process. Biochem., 39 (2004) 1421–1426.
[16] X. Huang, Y. Qu, C.A. Cid, C. Finke, M.R. Hoffmann, K. Lim, S.C. Jiang, Electrochemical disinfection of toilet wastewater using wastewater electrolysis cell, Water Res., 92 (2016) 164–172.
[17] A. Kraft, M. Stadelmann, M. Blaschke, D. Kreysig, B. Sandt, F. Schröder, J. Rennau, Electrochemical water disinfection Part I: Hypochlorite production from very dilute chloride solutions, J. Appl. Electrochem., 29 (1999) 859–866.
[18] D. Ghernaout, A. Badis, A. Kellil, B. Ghernaout, Application of electrocoagulation in *Escherichia coli* culture and two surface waters, Desalination, 219 (2008) 118–125.
[19] A. Cano, P. Cañizares, C. Barrera-Díaz, C. Sáez, M.A. Rodrigo, Use of conductive-diamond electrochemical-oxidation for the disinfection of several actual treated wastewaters, Chem. Eng. J., 211–212 (2012) 463–469.
[20] D. Ghernaout, M.W. Naceur, A. Souabed, On the dependence of chlorine by-products generated species formation of the electrode material and applied charge during electrochemical water treatment, Desalination, 270 (2011) 9–22.
[21] A. Vacca, M. Mascia, S. Palmas, A. Da Pozzo, Electrochemical Treatment of Water Containing Chlorides under Non Ideal Flow Conditions with BDD Anodes, J. Appl. Electrochem., 41 (2011) 1087–1097.
[22] M. Zhou, H. Särkkä, M. Sillanpää, A comparative experimental study on methyl orange degradation by electrochemical oxidation on BDD and MMO electrodes, Sep. Purif. Technol., 78 (2011) 290–297.
[23] B. Bakheet, M.A. Islam, J. Beardall, X. Zhang, D. McCarthy, Electrochemical inactivation of *Cylindrospermopsis raciborskii* and removal of the cyanotoxin cylindrospermopsin, J. Hazard. Mater., 344 (2018) 241–248.
[24] W. Liang, K. Wang, L. Chen, L. Ruan, L. Sui, Variation of algal viability during electrochemical disinfection using Ti/RuO_2 electrodes, Water Sci. Technol., 64 (2011) 162–170.
[25] M. Mascia, A. Vacca, S. Palmas, Electrochemical treatment as a pre-oxidative step for algae removal using *Chlorella vulgaris* as a model organism and BDD anodes, Chem. Eng. J., 219 (2013) 512–519.
[26] X. Wang, P. Xiang, Y. Zhang, Y. Wan, H. Lian, The inhibition of *Microcystis aeruginosa* by electrochemical oxidation using boron-doped diamond electrode, Environ. Sci. Pollut. Res., 25 (2018) 20631–20639.
[27] J. Locker, P. Fitzgerald, D. Sharp, Antibacterial validation of electrogenerated hypochlorite using carbon-based electrodes, Lett. Appl. Microbiol., 59 (2014) 636–641.
[28] J. Saha, S.K. Gupta, An endeavor towards competitive electro-chlorination by comparing the performance of easily affordable carbon electrodes with platinum, Chem. Eng. Commun., 204 (2017) 1357–1368.
[29] S. Hostin, P. Benedekovič, A. Michalíková, Chlorine production for water disinfection by the means of photovoltaic panels, Nova Biotechnol. Chim. -Supplement, 9 (2009) 1–6.
[30] I. Pouneva, Evaluation of algal culture viability and physiological state by fluorescent microscopic methods, Bulg. J. Plant. Physiol., 23 (1997) 67–76.
[31] M. Imase, Y. Ohko, M. Takeuchi, S. Hanada, Estimating the viability of *Chlorella* exposed to oxidative stresses based around photocatalysis, Int. Biodeterior. Biodegrad., 78 (2013) 1–6.
[32] M.L.L. Nollet, L.S.P. De Gelder, Handbook of Water Analysis, CRC Press, New York, 2007.
[33] H. Särkkä, M. Vepsäläonen, M. Pulliainen, M. Sillanpää, Electrochemical inactivation of paper mill bacteria with mixed metal oxide electrode, J. Hazard. Mater., 156 (2008) 208–213.
[34] E. Poelman, M. De Pauw, B. Jeurissen, Potential of electrolytic flocculation for recovery of micro-algae, Resour. Conserv. Recycl., 19 (1997) 1–10.
[35] C. Rioboo, J.E. O'Connor, R. Prado, C. Herrero, A. Cid, Cell proliferation alterations in *Chlorella* cells under stress conditions, Aquat. Toxicol., 94 (2009) 229–237.

[36] M. Valica, S. Hostin, Electrochemical treatment of water contaminated with methyl orange, Nova Biotechnol. Chim., 15 (2016) 55–64.
[37] C.G. Alfafara, K. Nakano, N. Nomura, T. Igarashi, M. Matsumura, Operating and scale-up factors for the electrolytic removal of algae from eutrophied lakewater, J. Chem. Technol. Biotechnol., 77 (2002) 871–876.
[38] Y. Xu, Y. Yang, M. Ou, Y. Wang, J. Jia, Study of *Microcystis aeruginosa* inhibition by electrochemical method, Biochem. Eng. J., 36 (2007) 215–220.
[39] W. Liang, J. Qu, L. Chen, H. Liu, P. Lei, Inactivation of *Microcystis aeruginosa* by continuous electrochemical cycling process in tube using Ti/RuO_2 electrodes, Environ. Sci. Technol., 39 (2005) 4633–4639.
[40] L. Lin, C. Feng, Q. Li, M. Wu, L. Zhao, Effects of electrolysis by low-amperage electric current on the chlorophyll fluorescence characteristics of *Microcystis aeruginosa*, Environ. Sci. Pollut. Res., 22 (2015) 14932–14939.
[41] H. Bergmann, J. Rollin, Product and by-product formation in laboratory studies on disinfection electrolysis of water using boron-doped diamond anodes, Catal. Today, 124 (2007) 198–203.
[42] A.M. Polcaro, A. Vacca, M. Mascia, S. Palmas, J. Rodiguez Ruiz, Electrochemical treatment of waters with BDD anodes: kinetics of the reactions involving chlorides, J. Appl. Electrochem., 39 (2009) 2083–2092.
[43] C. Comninellis, A. Nerini, Anodic oxidation of phenol in the presence of NaCl for wastewater treatment, J. Appl. Electrochem., 25 (1995) 23–28.
[44] G. Chen, Electrochemical technologies in wastewater treatment, Sep. Purif. Technol., 38 (2004) 11–41.
[45] L. Wang, M. Bassiri, R. Najafi, K. Najafi, J. Yang, B. Khosrovi, W. Hwong, E. Barati, B. Belisle, C. Celeri, M. Robson, Hypochlorous acid as a potential wound care agent: Part I. Stabilized hypochlorous acid: a component of the inorganic armamentarium of innate immunity, J. Burns Wounds, 6 (2007) e5.
[46] R. Daghrir, L. Igounet, S.K. Brar, P. Drogui, Novel electrochemical method for the recovery of lipids from microalgae for biodiesel production, J. Taiwan Inst. Chem. Eng., 45 (2014) 153–162.
[47] M. Mascia, S. Monasterio, A. Vacca, S. Palmas, Electrochemical treatment of water containing *Microcystis aeruginosa* in a fixed bed reactor with three-dimensional conductive diamond anodes, J. Hazard. Mater., 319 (2016) 111–120.
[48] H.D. Al-Hamaiedeh, Effect of electrolyte components on electrochemical generation and disinfection efficiency of active chlorine, Desal. Wat. Treat., 12 (2009) 369–374.
[49] A.C. Ndjomgoue-Yossa, C.P. Nanseu-Njiki, I.M. Kengne, E. Ngameni, Effect of electrode material and supporting electrolyte on the treatment of water containing *Escherichia coli* by electrocoagulation, Int. J. Environ. Sci. Technol., 12 (2015) 2103–2110.
[50] J. Basiri Parsa, M. Golmirz, M. Abbasi, Degradation of azo dye C.I. Acid Red 18 in aqueous solution by ozone-electrolysis process, J. Ind. Eng. Chem., 20 (2014) 689–694.
[51] J. Sun, J. Wang, X. Pan, H. Yuan, A New Treatment Strategy for Inactivating Algae in Ballast Water Based on Multi-Trial Injections of Chlorine, Int. J. Mol. Sci., 16 (2015) 13158–13171.
[52] M.E.H. Bergmann, In: C. Comninellis, G. Chen, Electrochemistry for the Environment, Springer, New York, 2010, pp. 163–204.