Electrochemical Degradation of Cyanobacterial Toxin Microcystin-LR Using Ti/RuO₂ Electrodes in a Continuous Tubular Reactor

Wenyan Liang,^{1,2} Jiuhui Qu,^{1,*} Ke Wang,² Jinli Wang,² Huijuan Liu,¹ and Pengju Lei¹

Abstract

Occurrence of cyanobacteria and their potent toxins has imposed a risk to human health and raised concerns in drinking water treatment. In this study, the applicability of electrochemical oxidation on microcystin-LR produced by cyanobacteria was examined using Ti/RuO₂ as anodes. The experiments focused on the variation and elimination of extracellular and intracellular microcystin-LR during the cyanobacterial inactivation by electro-oxidation. The effects of applied current density, cell density, as well as electrolyte type on toxin degradation have been studied. The complete elimination of extracellular and intracellular microcystin-LR was achieved and 100% removal for total microcystin-LR was obtained at current density 5 mA/cm². The results showed that when the cell densities decreased or the applied current densities increased, the efficiency of total microcystin-LR removal could be increased. More than 98% removal for total microcystin-LR was also achieved even if the cyanobacteria were inoculated in lake water or drinking water without adding any chemicals. It can be concluded that electro-oxidation of microcystin-LR is quite an efficient method, and can be applied to remove microcystin-LR from waters during the cyanobacterial inactivation.

Key words: microcystin-LR; electro-oxidation; degradation; toxin; cyanobacteria

Introduction

UNDER CERTAIN CONDITIONS, usually a combination of high nutrient load and warm, stable conditions, cyanobacteria (blue-green algae) can grow excessively and form blooms. The occurrence of these blooms creates a significant water quality problem, as certain species of cyanobacteria are capable of producing toxins. Cyanotoxins are very diverse in their chemical structure and toxicity and often classified as dermatotoxins (lipopolysaccharides, lyngbyatoxin-a, and aplysiatoxins), neurotoxins (anatoxin-a, homoanatoxin-a, anatoxin-a(s), and saxitoxins), and hepatotoxins (microcystins, nodularin, and cylindrospermopsin) according to their toxic effects on animals (Figueiredo *et al.*, 2004; Codd *et al.*, 2005). However, the most studied hepatotoxins produced by cyanobacteria are the cyclic heptapeptides called microcystins (Vasconcelos *et al.*, 2001). The toxicity of mi-

crocystins is associated with the inhibition of protein phosphatases 1 and 2A (Gehringer, 2004; Lankoff *et al.* 2004). Microcystins are toxic to primary human hepatocytes, and they may be responsible for the liver failure cases observed after acute cyanobacterial poisoning (Batista *et al.*, 2003) and liver cancer or gene mutations in cells (Fleming *et al.*, 2002; Zhan *et al.*, 2004). The first documented human deaths from cyanobacterial hepatotoxins occurred in a dialysis clinic in Caruaru city, Brazil, during 1996 following intravenous exposure. Fifty-two of 131 patients had died due to the consumption of water contaminated with microcystins (Azevedo *et al.*, 2002).

Far more than 70 structural variants of microcystins have been identified from natural samples (Grützmacher *et al.*, 2002). They share a general structure: cyclo (D-Ala¹-X²-D-MeAsp³-Y⁴-Adda-Arg⁵-D-Glu⁶-Mdha⁷-) where, X and Y are variable amino acids, D-MeAsp is D-erythro-β-methylas-

¹State Key Laboratory of Environmental Aquatic Chemistry, Research Center for Eco-Environmental Science, Chinese Academy of Science, Beijing 100085, People's Republic of China.

²The Key Laboratory for Silviculture and Conservation of Ministry of Education, College of Natural Resources & Environment, Beijing Forestry University, Beijing 100083, People's Republic of China.

*Corresponding author: Research Center for Eco-Environmental Science, Chinese Academy of Science, Beijing 100085, P.R. China. Phone: +86-10-62849151; Fax: +86-10-62923558; E-mail: jhqu@mail.rcees.ac.cn

partic acid, Adda is (2S, 3S, 8S, 9S)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid, and Mdha is *N*-methyldehydroalanine. Although structural variations can arise in all seven amino acids, the most frequently encountered microcystin variants are characterized according to the methylation pattern, and the two variable L-amino acids located at positions X and Y of the structure (McElhiney and Lawton, 2005). Combinations of the two variable L-amino acids, X and Y, are used in the nomenclature of the toxins. The XY variable amino acids for MC-LR, MC-RR, and MC-YR are leucine (L), arginine (R), and tyrosine (Y). Based on biochemical and histological studies, MC-LR was found to be the most potent toxin followed by MC-YR and MC-RR (Gupta *et al.*, 2003). Because of its acute and chronic toxicity, the World Health Organization (WHO) published a provisional guideline value for MC-LR of 1 $\mu\text{g/L}$ in drinking water (WHO, 1998). The development of methods to effectively reduce the toxin concentrations in waters has thus become an important focus of current research efforts.

Conventional flocculation–filtration–chlorination procedures only resulted in a relatively small decrease in the toxin concentrations, and these processes clearly did not detoxify water containing hepatotoxic cyanobacteria (Himberg *et al.*, 1989). If conventional treatment processes were combined with activated carbon, they generally removed more than 80% of microcystins from raw water, but a residual concentration of 0.1–0.5 $\mu\text{g/L}$ equivalents of MC-LR was observed considering both granular activated carbon and powered activated carbon treatment facilities (Lambert *et al.*, 1996). Under normal conditions, microcystins could be degraded by organisms in practical biological treatment facilities sometimes as a sole carbon source (Saitou *et al.*, 2003; Ishii *et al.*, 2004). Primary degradation of MC-LR occurred rapidly in the reservoir water with a first half-life of 3–4 days (Cousins *et al.*, 1996). But the complete degradation of MC-LR cost 1 week to several weeks by biological treatment (Holst *et al.*, 2003; Sugiura *et al.*, 2003).

Compared to the biological treatment, microcystin degradation by chemicals can be realized in a very short time. Ozone has been shown to be effective for the destruction of some classes of toxins under special conditions. One hundred percent destruction of MC-LR was related to a residual ozone concentration present after 5 min (Rositano *et al.*, 2001). The toxins were easily decomposed by chlorination with sodium hypochlorite, and no noxious products were detected from the chlorination process of MC-LR (Tsuji *et al.*, 1997). It showed that the MC-LR could be decomposed by Fenton oxidation with very low initial concentrations of H_2O_2 and Fe^{2+} (Fe^{3+}) in the reaction mixture. Its characteristic UV absorption at 238 nm dropped below their detection limit after 30 min (Gajdek *et al.*, 2001). When the Fenton process was complemented with radiation (ultraviolet, visible, or both), the resulting process, known as photo-Fenton, exhibited enhanced reaction efficiency for the degradation of MC-LR (Bandala *et al.*, 2004).

In recent years, electrochemical oxidation has become a new alternative for water treatment because it is a promising and versatile method that has the potential to replace or complete already existing processes. Electrochemical techniques use the electron, a “clean reagent,” as the main reagent. These processes can operate at ambient temperature without the need of temperature control (Fernandes *et al.*,

2004). The use of electrochemical methods has been proven to be effective to significantly reduce the amount of pollutants present in highly colored industrial wastewater, particularly when the wastewater contains biorefractory organic pollutants or microtoxic substances (Lima Leite *et al.*, 2002; Barrera-Díaz *et al.*, 2003). Moreover, electrolytic methods have shown promise as a method of disinfection, whereas low levels of free chlorine, sodium hypochlorite, or hypochlorous acid may be produced *in situ* in NaCl-containing solutions (Matsunaga *et al.*, 1994 Venczel *et al.*, 1997; Bergmann *et al.*, 2002). Although the mechanism of electrochemical disinfection has been largely attributed to the action of electrogenerated active chlorine, Jeong *et al.* (2006) found that the $\text{OH}\cdot$ was the major lethal species responsible for the *Escherichia coli* inactivation in the chloride-free electrochemical disinfection process employing boron-doped diamond as anode.

In previous works, the effect of electro-oxidation treatment on the cyanobacterial inactivation was examined and it was found that the electro-oxidation processes could decrease the cell numbers efficiently and made the cyanobacteria lose the potential to survive and grow. Scanning electron microscopy investigation of algae revealed surface damage and apparent leakage of intracellular contents after electrochemical cycling process. The chlorophyll-*a* released from the cells was almost degraded by electrochemical oxidation (Liang *et al.*, 2005). The present study on MC-LR degradation by electro-oxidation also addresses the use of dimensionally stable anodes (DAS) Ti/RuO₂ under different experimental conditions. Unlike most studies focused on the elimination of MC-LR extracts from cyanobacteria, this paper focuses on the variation and elimination of extracellular and intracellular (cell-bound) MC-LR during the inactivation by electro-oxidation. The influence of experimental conditions such as applied current density, cell density, as well as electrolyte type on the MC-LR degradation has been also studied.

Materials and Methods

Cyanobacteria culture

The cyanobacterium used in this research was *Microcystis aeruginosa* FACHB 496, a kind of cyanobacteria commonly found in freshwater. *M. aeruginosa* was cultured in the late log growth phase which corresponded to 14 days after inoculation in batch mode in 500-mL Erlenmeyer flasks containing 200 mL of the sterilized growth media MA. The flasks were housed in an incubator at 30°C under illumination (28 $\mu\text{mol/m}^2 \cdot \text{s}$) on a 14-h light/10-h dark cycle. Growth was monitored daily by cell counting. Stock cultures were prepared weekly by aseptic transfer of a known number of cyanobacteria from a culture in the log growth phase to a freshly sterilized flask with growth media.

Apparatus

As shown in Fig. 1, the tubular electrochemical apparatus consisted mainly of a power supply, rod anode and tubular cathode, peristaltic pump, and reservoir. In this study, a common water pipe constructed from iron (i.d. = 17 mm, length = 260 mm) with an insulated material around it was used as cathode and reactor, and the anode was a commercial rod (D = 5 mm) of titanium coated with RuO₂. The an-

FIG. 1. Schematic view of the electrochemical tubular apparatus: (1) DC power source; (2) tubular reactor; (3) cathode; (4) insulated layer; (5) anode; (6) ampere meter; (7) flow meter; (8) peristaltic pump; (9) reservoir; (10) stirring apparatus.

ode was placed at the center of the reactor. The distance between cathode and anode was 6 mm. A constant voltage/current controlled DC power source was used as the power supply. A peristaltic pump was used to pump the samples feed from the reservoir into the tubular reactor.

MC-LR extraction

To extract the intracellular microcystin, cells were harvested by centrifugation at $7,500 \times g$ for 5 min (Beckman J2-HS Centrifuge), and the pellet was lyophilized. The supernatants were filtered through a microfiber filter (GF/C) and then applied directly to the conditioned octadecyl silyl (ODS) silica gel cartridge (0.5 g, Varian Bond Elut C18) to get the extracellular microcystin. The dried cells were resuspended in 20 mL 80% (v/v) methanol and stirred for 4-h at room temperature to extract the toxins from cells. The procedure was repeated and the combined extracts were centrifuged at $7,500 \times g$ for 15 min. The supernatants were evaporated at 60°C and then were resuspended in 50 mL MilliQ H₂O (18 M Ω /cm at 25°C). Then they were applied to conditioned C18 cartridges to get the intracellular microcystin. The C18 cartridges had been previously conditioned with 10 mL HPLC-grade methanol and 20 mL MilliQ H₂O. The cartridges were rinsed with 30 mL 5% (v/v) methanol and then eluted with 10 mL 80% (v/v) methanol to give the desired fractions. The eluate was evaporated at 60°C and taken up in 0.5 mL water. If necessary, the concentrated samples were filtered through a $0.2\text{-}\mu\text{m}$ filter before HPLC analysis.

MC-LR analysis

The microcystin concentrations were determined by HPLC (Hewlett Packard 1050) with Agilent C18 4.6×250 mm analytical column at monitoring wavelength 238 nm. MC-LR was identified by its characteristic UV spectra using a diode array detector. Analytical MC-LR standards were from Sigma (St. Louis, MO). Stock solutions were prepared in methanol and diluted as required with MilliQ H₂O for use as working solutions. Mobile phase A was MilliQ water containing 0.05% (v/v) trifluoroacetic acid (TFA) and mobile phase B was acetonitrile. The flow rate was 1 mL/min. In-

cluding preconcentration, a detection limit of $0.2\text{ }\mu\text{g/L}$ for individual MC-LR was achievable.

Electro-oxidation of samples

The samples containing live *M. aeruginosa* were poured into the reservoir and fed to the column to fill it completely and then recycled with the application of the specified current density. The supporting electrolytes used in working solutions were the culture media, the lake water from Guanting reservoir, or the tap water. All samples were prepared from certain electrolyte with an added amount of *M. aeruginosa* concentrated by centrifugation. To study the effect of direct current on MC-LR degradation, the *M. aeruginosa* suspensions were exposed to current densities ranging from 1–10 mA/cm². At appropriate time intervals, samples were taken from the reservoir for analysis to determine the change of extracellular and intracellular MC-LR during electro-oxidation. The initial samples without any treatment taken from

FIG. 2. Variation of MC-LR concentrations and removal efficiency of total MC-LR with time during the electro-oxidation processes at 5 mA/cm^2 . The results shown are mean data from triplicate experiments, and error bars indicate the standard deviations.

the reservoir were used as the control samples. All the experiments were performed at room temperature and in triplicate.

Results and Discussion

Removal efficiency for MC-LR

Continuous tubular reactor experiments were carried out to determine the electro-oxidation effects on MC-LR degradation. 1.5-L *M. aeruginosa* suspension (5×10^9 cells/L) was electrochemically treated at current density 5 mA/cm², using culture media as the supporting electrolyte to reduce any influence from the electrolyte on cell activity. At appropriate intervals, 200 mL samples were taken for analysis to determine the intracellular, extracellular, and total (intra- plus extracellular) MC-LR variations.

As can be seen in Fig. 2, the extracellular MC-LR in the supernatant water produced by the *M. aeruginosa* increased with detention time and amounted from 0 to 11.2 µg/L at 5.4 min. It indicated that the electrochemical treatment on the *M. aeruginosa* caused deterioration on the cell integrity and components of cells and resulted in the leakage of MC-LR from cells. After 5.4 min the extracellular MC-LR concentrations decreased with time and were undetectable after 52 min. The degressive trend showed that MC-LR released from the cells had been completely removed by the electro-oxidation. The intracellular MC-LR decreased with duration time and their characteristic UV absorption at 238 nm dropped below the detection limit after 130 min. As shown in Fig. 2, the initial concentration of the total toxin was 38.2 µg/L, and almost all of toxins were in the cell-bound form. The total concentrations of MC-LR studied exceeded those occurring naturally in lakes and drinking water in order to analyze the toxin. It can be seen that total MC-LR removal efficiencies were about 50 and 95% at 15 and 52 min, respectively. Above 130 min, all of the MC-LR was eventually

FIG. 3. Variation of MC-LR concentrations during the static culture stage when electro-oxidation processes at 5 mA/cm² was stopped. The results shown are mean data from triplicate experiments, and error bars indicate the standard deviations.

FIG. 4. Effect of applied current on the MC-LR degradation and the removal efficiency of total MC-LR. The initial (0) means the MC-LR concentration before the electro-oxidation processes.

consumed in the suspensions. Including preconcentration, a detection limit of 0.2 µg/L for individual MC-LR was achievable, so that the degradation of MC-LR by electro-oxidation was complete and 100% MC-LR removal was achieved.

Under the conditions of the present studies, most authors put forward that two mechanisms can be responsible for the removal of organic matters by electro-oxidation: direct anodic oxidation and indirect oxidation in the bulk solution. In the former process, the organics are destroyed at the electrode's surface. Whereas during indirect electrochemical oxidation, the organics are destroyed by the appropriate anodically formed oxidants such as Cl₂, hypochlorite, peroxide, ozone, Fenton's reagent, or peroxodisulphate (Grimm *et al.*, 1998; Jüttner *et al.*, 2000; Szpyrkowicz *et al.*, 2001). In the presence of chloride, elimination of organic pollutants was improved because of the mediation of electrogenerated active chlorine at the anode (Panizza and Cerisola, 2003). The chlorine treatment of cyanobacteria has been proved to be effective in destroying hepatotoxins such as microcystins and nodularin as long as a free chlorine residual of at least 0.5 mg/L was present after 30 min (Newcombe and Nicholson, 2004). Moreover, the hypochlorous acid produced by the reaction of chlorine with water is a strong oxidant which can help to oxidize MC-LR (Sheng *et al.*, 1998; Ögütveren *et al.*, 1999). But in waters with a relatively high demand, removal is slower than those with pure toxins in high purity water because of the competitive reactions with the natural organic materials or the matters released from the cyanobacterial cells. The low chlorine doses utilized are probably consumed by the naturally occurring organic materials and are therefore insufficient to destroy the microcystins present (Newcombe and Nicholson, 2004).

Another experiment was carried out to determine the effect of chlorine, hypochlorous acid, or other oxidative matters produced through anode reaction on MC-LR elimination when the electro-oxidation process stopped. *M. aeruginosa* suspension (1.5-L) with the cell density of 5×10^9 cells/L was treated by electro-oxidation at a detention time of 6 min and then put into the illumination incubator. At appropriate time intervals, 200 mL samples were taken from

TABLE 1. EFFECT OF CELL DENSITY ON MC-LR REMOVAL

Cell density (cells/L)	MC-LR before treatment (ug/L)			MC-LR after treatment (ug/L)			Removal for total MC-LR (%)
	Extracellular	Intracellular	Total	Extracellular	Intracellular	Total	
8.2×10^8	0 ± 0	8.4 ± 0.4	8.4 ± 0.4	0 ± 0	0.1 ± 0.2	0.1 ± 0.2	98 ± 2
2.4×10^9	0 ± 0	21.3 ± 0.1	21.3 ± 0.1	2.1 ± 0.1	2.5 ± 1.7	4.6 ± 1.8	78 ± 9
5.3×10^9	0 ± 0	40.0 ± 1.3	40.0 ± 1.3	8.2 ± 0.6	12.0 ± 4.3	20.2 ± 4.9	49 ± 11

The data are means $\pm$ standard deviations.

the treated suspensions to detect the variations of the extracellular and intracellular MC-LR during the static culture stage. As shown in Fig. 3, 10.0 $\mu\text{g/L}$ MC-LR, about 25% of the total MC-LR, was released into the supernatant when electro-oxidation process stopped. However, 96% MC-LR entered into the suspensions due to the cell damages a day after the ceasing of electro-oxidation process. The increased extracellular MC-LR concentrations indicated that the chlorine, hypochlorous acid, or other oxidative matters produced through anode reactions were not sufficient to destroy the MC-LR gradually released into the suspensions.

Effect of applied current density

Applied current density is a parameter of great importance for the electro-oxidation of organic compounds (Lima Leite *et al.*, 2002). The effect of current upon the degradative behavior of MC-LR was studied by electrolyzing 200-mL cyanobacterial suspension in the range 1–10 mA/cm^2 at a detention time of 18 min. The results were reported in Fig. 4. Culture media were used as electrolytes and the initial total MC-LR concentration before treatment was 38.3 $\mu\text{g/L}$ with undetectable extracellular MC-LR. For the studied current densities, it can be seen that the efficiency of MC-LR removal was dependent on the current density, that is, removal efficiency was directly proportional to the applied current density as might have been expected in other electrochemical processes (Ögütveren *et al.*, 1999). When the current density increased, a more rapid decay of the total MC-LR was observed. After electro-oxidation treatment at 1 mA/cm^2 , most of the MC-LR was still in the cells due to the slight injury of the cells. However, when the current densities increased to 2.5 mA/cm^2 and 5 mA/cm^2 , the percents of the extracellular MC-LR to the total MC-LR increased and a certain amount of extracellular MC-LR was degraded by the electro-oxidation. Because of the high current density at 10 mA/cm^2 , all of the released MC-LR was degraded and resulted in undetectable extracellular MC-LR concentration. The rate of electro-oxidation on MC-LR was faster than that of MC-LR released from cells at 10 mA/cm^2 .

As shown in Fig. 4, an increase in the current density caused a higher effect on the total MC-LR removal which reached 86% at 10 mA/cm^2 . The faster degradation efficiency with increasing current density in anodic oxidation can be related to a quicker reaction of pollutants with the concomitant higher concentration of adsorbed hydroxyl radicals $\text{OH}\cdot$ produced on the anodes (Boye *et al.*, 2003). The electrochemical oxidation of aqueous solutions which contain organic matters, by the use of traditional anodes (Ti/Pt, Ti/PbO₂, Ti/IrO₂ . . .) proceeds in two steps: the first step

is the formation of $\text{OH}\cdot$ on the anodes; after this, the absorbed $\text{OH}\cdot$ oxidize the organic matters. The dimensionally stable anodes (DAS) represent a class of electrodes where a metallic titanium substrate is covered by metallic oxides (IrO₂, RuO₂, PbO₂, SnO₂, etc.). They have good effect due to the coating materials showing electrocatalytic activity toward $\text{OH}\cdot$ production (Panizza *et al.*, 2001), and that $\text{OH}\cdot$ is a nonselective, very powerful oxidant that can react with organics yielding dehydrogenated or hydroxylated derivatives. Therefore, the results that the efficiency of MC-LR removal was proportional to the applied current density showed that the radical $\text{OH}\cdot$ may play an important role on the destruction of MC-LR and the anode material may be very important in MC-LR elimination.

Effect of cell density

Experiments were conducted to investigate the effect of cell density on MC-LR removal by the electrochemical treatment. The results were displayed in Table 1. As seen in this table, when the cell density decreased from 5.3×10^9 cells/L to 8.2×10^8 cells/L, corresponding to the total MC-LR of 40.0 to 8.4 $\mu\text{g/L}$, the removal efficiency of the samples treated by electro-oxidation at 5 mA/cm^2 at a detention time of 18 min increased from 49 to 98%, a significant improvement by about 49%. Such an increase in the total MC-LR removal was apparently attributable to a decrease in the oxidation load at a lower initial MC-LR concentration of the present electrochemical treatment. The results showed that the lower the initial MC-LR concentration the faster it could be removed

FIG. 5. Effect of electrolyte type on the MC-LR degradation and the removal efficiency of total MC-LR.

from the suspension by anodic oxidation. For the cell density of 8.2×10^8 cells/L, the extracellular MC-LR was undetectable after treatment.

The cell density in natural water bodies is often less than that in the experiment. Only during cyanobacteria blooms could the cell density reach 10^8 – 10^9 cells/L. So the normal MC-LR concentration can be eliminated in a short time. But if the blooms are brought out, it must take a long time to eliminate the MC-LR completely during the inactivation of *M. aeruginosa*.

Effect of electrolyte type

Another important factor for the electrochemical processes, which consist of heterogeneous ion transfer reactions, is the electrolyte type of the suspension to be treated. The supporting electrolytes used in most studies on the electro-oxidation of organic matters are chloride, sulfate, and nitrate salts (Motheo *et al.* 2000; Torres *et al.*, 2003; Fernandes *et al.*, 2004). The photocatalytic degradation of microcystins showed that the use of untreated natural lake water as the water matrix resulted in a slower decomposition of microcystins than distilled water (Shephard *et al.*, 2002). When the cyanobacteria lose activity and die, they will lyse, resulting in the cell-bound MC-LR entering into the water (Figueiredo *et al.*, 2004). In order to reduce the influence of electrolyte on the cell activity, the culture media was used as electrolyte in the former studies. So the following experiment was carried out to determine the effect of electrolyte type on the MC-LR electro-oxidation.

Besides the culture media, the natural filtered lake water from Guanting reservoir and the tap water were used as electrolytes in the MC-LR degradation at current density 5 mA/cm² with a detention time of 18 min. *M. aeruginosa* were inoculated in different electrolytes after concentration by centrifugation and the suspensions were electrolyzed immediately. Results were depicted in Fig. 5. All of the extracellular MC-LR before and after treatment was undetectable due to the lower cell density (8 – 10×10^8 cells/L). As shown in Fig. 5, there were no significant differences between these three kinds of electrolytes on the MC-LR removal. When lake water and culture media were used, the total removal efficiency for MC-LR reached more than 98%. When tap water was used as an electrolyte, the removal efficiency dropped a little to 96%. So it can be concluded that the MC-LR in the natural water or tap water can be degraded by the electro-oxidation without adding any chemicals. The active matters including the hydroxyl radicals generated by water discharge on the electrode and direct anodic oxidation may play certain role in destroying the MC-LR.

Conclusion

The results described in this work give strong evidence about the possibilities of the electrochemical oxidation process in MC-LR decomposition. The process proved it feasible to be conducted in a simple undivided cell reactor equipped with Ti/RuO₂ anode. A complete elimination of MC-LR has achieved after electro-oxidation at 5 mA/cm². Changes of current density and initial cell density have a significant influence on the efficiency of the MC-LR elimination processes. The study showed that the efficiency of treatment increased when the cell density decreased and the applied current increased. Furthermore, MC-LR removal has also

achieved even when the *M. aeruginosa* cells were inoculated in the natural lake water or drinking water without adding any chemicals. Thus, it can be said that electro-oxidation of MC-LR is quite an efficient method and can be applied to the removal of MC-LR from waters.

Acknowledgments

This work was supported by grants from National Natural Science Foundation of China (Grant No. 50678024), National Science Fund for Distinguished Young Scholars (Grant No. 50225824).

Author Disclosure Statement

The authors state that no competing financial interests exist.

References

- Azevedo, S.M.F.O., Carmichael, W.W., Jochimsen, E.M., Rinehart, K.L., Lau, S., Shaw, G.R., and Eaglesham, G.K. (2002). Human intoxication by microcystins during renal dialysis treatment in Caruaru-Brazil. *Toxicology* 181–182, 441.
- Bandala, E.R., Martínez, D., Martínez, E., and Dionysiou, D.D. (2004). Degradation of microcystin-LR toxin by Fenton and Photo-Fenton processes. *Toxicon* 43, 829.
- Barrera-Díaz, C., Ureña-Núñez, F., Campos, E., Palomar-Paradavé, M., and Romero-Romo, M. (2003). A combined electrochemical-irradiation treatment of highly colored and polluted industrial wastewater. *Radiat. Phys. Chem.* 67, 657.
- Batista, T., Sousa, G., Suput, J.S., Rahmani, R., and Šuput, D. (2003). Microcystin-LR causes the collapse of actin filaments in primary human hepatocytes. *Aquat. Toxicol.* 65, 85.
- Bergmann, H., Iourtchouk, T., Schöps, K., and Bouzek, K. (2002). New UV irradiation and direct electrolysis—Promising methods for water disinfection. *Chem. Eng. J.* 85, 111.
- Boye, B., Dieng, M.M., and Brillas, E. (2003). Anodic oxidation, electro-Fenton and photoelectro-Fenton treatments of 2,4,5-trichlorophenoxyacetic acid. *J. Electroanal. Chem.* 557, 135.
- Codd, G.A., Morrison, L.F., and Metcalf, J.S. (2005). Cyanobacterial toxins: Risk management for health protection. *Toxicol. Appl. Pharm.* 203, 264.
- Cousins, I.T., Bealing, D.J., James, H.A., and Sutton, A. (1996). Biodegradation of microcystin-LR by indigenous mixed bacterial populations. *Water Res.* 30, 481.
- Fernandes, A., Morão, A., Magrinho, M., Lopes, A., and Gonçalves, I. (2004). Electrochemical degradation of C. I. Acid Orange 7. *Dyes Pigm.* 61, 287.
- Figueiredo, D.R., Azeiteiro, U.M., Esteves, S.M., Gonçalves, F.J.M., and Pereira, M.J. (2004). Microcystin-producing blooms—A serious global public health issue. *Ecotox. Environ. Safe.* 59, 151.
- Fleming, L.E., Rivero, C., Burns, J., Williams, C., Bean, J.A.K., Shea, A., and Stinn, J. (2002). Blue green algal (cyanobacterial) toxins, surface drinking water, and liver cancer in Florida. *Harmful Algae* 1, 157.
- Gajdek, P., Lechowski, Z., Bochnia, T., and Kępczyński, M. (2001). Decomposition of microcystin-LR by fenton oxidation. *Toxicon* 39, 1575.
- Gehring, M.M. (2004). Microcystin-LR and okadaic acid-induced cellular effects: A dualistic response. *FEBS Lett.* 557, 1.
- Grimm, J., Bessarabov, D., and Sanderson, R. (1998). Review of electro-assisted methods for water purification. *Desalination* 115, 285.

- Grützmacher, G., Böttcher, G., Chorus, I., and Bartel, H. (2002). Removal of microcystins by slow sand filtration. *Toxicon* 17, 386.
- Gupta, N., Pant, S.C., Vijayaraghavan, R., and Rao, P.V.L. (2003). Comparative toxicity evaluation of cyanobacterial cyclic peptide toxin microcystin variants (LR, RR, YR) in mice. *Toxicology* 188, 285.
- Himberg, K., Keijola, M., Hiisvirta, L., Pyysalo, H., and Sivonen, K. (1989). The effect of water treatment processes on the removal of hepatotoxins from *Microcystis* and *Oscillatoria cyanobacteria*: A laboratory study. *Water Res.* 23, 979.
- Holst, T.S., Jorgensen, N.O.G., Jorgensen, C., and Johansen, A. (2003). Degradation of microcystin in sediments at oxic and anoxic, denitrifies conditions. *Water Res.* 37, 4748.
- Ishii, H., Nishijima, M., and Abe, T. (2004). Characterization of degradation process of cyanobacterial hepatotoxins by a Gram-negative aerobic bacterium. *Water Res.* 38, 2667.
- Jeong, J., Kim, J., and Yoon, A. (2006). The role of reactive oxygen species in the electrochemical inactivation of microorganisms. *Environ. Sci. Technol.* 40, 6117.
- Jüttner, K., Galla, U., and Schmieder, H. (2000). Electrochemical approaches to environmental problems in the process industry. *Electrochim. Acta* 45, 2575.
- Lambert, T.W., Holmes, C.F.B., and Hruidey, S.E. (1996). Adsorption of microcystin-LR by activated carbon and removal in full scale water treatment. *Water Res.* 30, 1411.
- Lankoff, A., Carmichael, W.W., Grasman, K.A., and Yuan, M. (2004). The uptake kinetics and immunotoxic effects of microcystin-LR in human and chicken peripheral blood lymphocytes in vitro. *Toxicology* 204, 23.
- Liang, W., Qu, J., Chen, L., Liu, H., and Lei, P. (2005). Inactivation of *Microcystis aeruginosa* by continuous electrochemical cycling process in tube using Ti/RuO₂ electrodes. *Environ. Sci. Technol.* 39, 4633.
- Lima Leite, R.H., Cognet, P., Wilhelm, A.M., and Delmas, H. (2002). Anodic oxidation of 2,4-dihydroxybenzoic acid for wastewater treatment: study of ultrasound activation. *Chem. Eng. Sci.* 57, 767.
- Matsunaga, T., Nakasono, S., Kitajima, Y., and Horiguchi, K. (1994). Electrochemical disinfection of bacteria in drinking water using activated carbon fibers. *Biotechnol. Bioeng.* 43, 429.
- McElhiney, J., Lawton, and L.A. (2005). Detection of the cyanobacterial hepatotoxins microcystins. *Toxicol. Appl. Pharm.* 203, 219.
- Motheo, A.J., and Pinheiro, L. (2000). Electrochemical degradation of humic acid. *Sci. Total. Environ.* 256, 67.
- Newcombe, G., and Nicholson, B. (2004). Water treatment options for dissolved cyanotoxins. *J. Water SRT-Aqua* 53, 227.
- Ögütveren, Ü.B., Törü, E., and Koparal, S. (1999). Removal of cyanide by anodic oxidation for wastewater treatment. *Water Res.* 33, 1851.
- Panizza, M., and Cerisola, G. (2003). Electrochemical oxidation of 2-naphthol with in situ electrogenerated active chlorine. *Electrochim. Acta.* 48, 1515.
- Panizza, M., Michaud, P.A., Cerisola, G., and Comninellis, C.H. (2001). Electrochemical treatment of wastewaters containing organic pollutants on boron-doped diamond electrodes: Prediction of specific energy consumption and required electrode area. *Electrochem. Commun.* 3, 336.
- Rositano, J., Newcombe, G., Nicholson, B., and Sztajnbock, P. (2001). Ozonation of NOM and algal toxins in four treated waters. *Water Res.* 35, 23.
- Saitou, T., Sugiura, N., Itayama, T., Inamori, Y., and Masatoshi, M. (2003). Degradation characteristics of microcystins by isolated bacteria from Lake Kasumigaura. *J. Water SRT-Aqua* 52, 13.
- Sheng, H.L., Shyu, C.T., and Sun, M.C. (1998). Saline wastewater treatment by electrochemical method. *Water Res.* 32, 1059.
- Shephard, G.S., Stockenström, S., Villiers, D., Engelbrecht, W.J., and Wessels, G.F.S. (2002). Degradation of microcystin toxins in a falling film photocatalytic reactor with immobilized titanium dioxide catalyst. *Water Res.* 36, 140.
- Sugiura, T.S., Itayama, T., Inamori, Y., and Matsumura, M. (2003). Biodegradation of microcystis and microcystins by indigenous nanoflagellates on biofilm in a practical treatment facility. *Environ. Technol.* 24, 143.
- Szyrkowicz, L., Juzzolino, C., Daniele, S., and Faveri, M.D. (2001). Electrochemical destruction of thiourea dioxide in an undivided parallel plate electrodes batch reactor. *Catal. Today* 66, 519.
- Torres, R.A., Torres, W., Peringer, P., and Pulgarin, C. (2003). Electrochemical degradation of *p*-substituted phenols of industrial interest on Pt electrodes. Attempt of a structure—reactivity relationship assessment. *Chemosphere* 50, 97.
- Tsuji, K., Watanuki, T., Kondo, F., Watanabe, M.F., Nakazawa, H., Suzuki, M., Uchida, H., and Harada, K. (1997). Stability of microcystins from cyanobacteria-IV. Effect of chlorination on decomposition. *Toxicon* 35, 1033.
- Vasconcelos, V.M., Oliveira, S., and Teles, F.O. (2001). Impact of a toxic and a non-toxic strain of *Microcystis aeruginosa* on the crayfish *Procambarus clarkii*. *Toxicon* 39, 1461.
- Venczel, L.V., Arrowood, M., Hurd, M., and Sobsey, M.D. (1997). Inactivation of *Cryptosporidium parvum* oocysts and *Clostridium perfringens* spores by a mixed-oxidant disinfectant and by free chlorine. *Appl. Environ. Microb.* 63, 1598.
- WHO (1998). Guidelines for Drinking Water Quality, 2nd ed., Addendum to vol. 2, Health Criteria and Other Supporting Information, World Health Organization, Geneva, Switzerland, 1998.
- Zhan, L., Sakamoto, H., Sakuraba, M., Wu, D.S., Zhang, L.S., Suzuki, T., Hayashi, M., and Honma, M. (2004). Genotoxicity of microcystin-LR in human lymphoblastoid TK6 cells. *Mutat. Res.* 557, 1.