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ELECTROCOAGULATION/FLOCCULATION OF CYANOBACTERIA FROM SURFACE WATERS

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Highlights

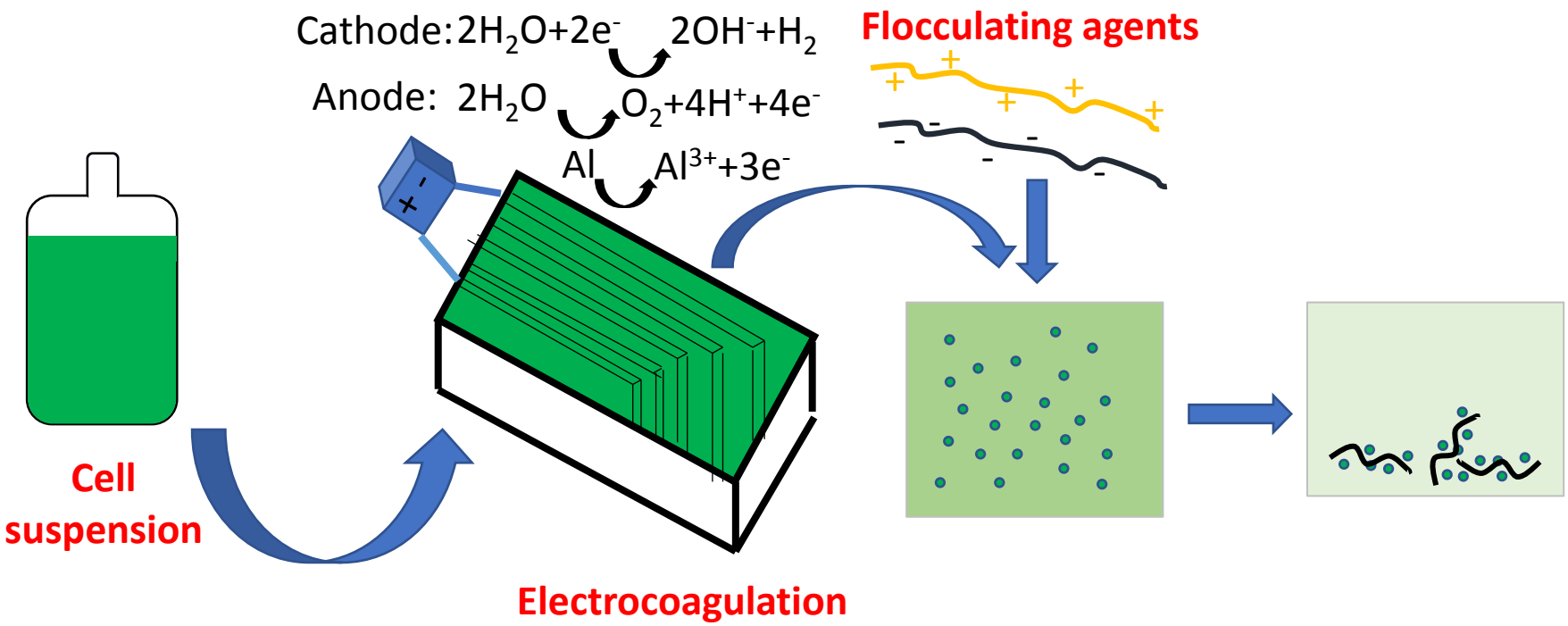
Electrocoagulation of cyanobacteria under low charge loading was optimal

Cell inactivation did not occur through complete lysis

Low doses of anionic flocculants reduce turbidity very efficiently

Extracellular organic matter interferes during flocculation

Combination of electrocoagulation and flocculation is economically feasible


Cathode: $2H_2O+2e^-$ $2OH^-+H_2$
Anode: $2H_2O$ $O_2+4H^++4e^-$
Al $Al^{3+}+3e^-$
Flocculating agents
Cell suspension
Electrocoagulation

ABSTRACT

Cyanobacterial blooming episodes in surface waters used for drinking purposes are increasing due to eutrophication of water ecosystems. Water treatments should be optimised to remove phytoplankton cells as well as their associated toxins. The performance of a multicell electrocoagulation reactor operating under a continuous flow and coupled with flocculation for cyanobacteria removal was examined. Electrocoagulation of a suspension of the unicellular cyanobacteria *Microcystis aeruginosa* and its related toxin microcystin-LR, as a model of toxic cyanobacterium and cyanotoxin, respectively, showed that cell removal occurred through charge neutralisation with the Al hydroxides generated in the system, whereas the toxin did not undergo electrolytical processes. Cell inactivation-was a function of the charge loading and the cell population. Inactivation of a 10^5 cells/ mL suspension was 34% for a charge loading of 50 C/L whereas only 3% was achieved by increasing cyanobacteria concentration by one-order of magnitude. Flocculation of the electrocoagulated suspensions with anionic polyelectrolytes revealed a bridging mechanism, whereas an electrostatic patch aggregation was involved with cationic flocculants. Electrocoagulation/flocculation of a cyanobacterial blooming surface water (about 10^6 cells/mL) showed quite good concordance of cell inactivation values (9.7-10.7%) with those detected previously with *Microcystis aeruginosa* (5.4%). However, the performance of the flocculants was slightly worse as determined from the residual turbidity values (17% vs. 7% in pure cultures of *M. aeruginosa*) due to the different compositions of the extracellular organic matter.

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Key words: electrocoagulation, flocculation, cyanobacteria, microcystins

1. INTRODUCTION

Cyanobacteria (blue-green algae) are found in many water ecosystems. High light irradiation and the presence of a high concentration of nutrients, especially relevant nitrogen and phosphorus, are responsible for cyanobacterial blooms with subsequent deterioration of water quality by scum formation, hypoxia, toxin formation, bad taste and odors (Lopez et al., 2008). Cyanobacterial blooming episodes have been increasing as a result of the eutrophication of freshwater systems, many of which are used for drinking purposes (Roegner et al., 2014; Huisman et al., 2018). The maintenance of installations used in water treatment plants requires the control and removal of cyanobacterial blooms for safer operation conditions, to avoid the clogging of devices. In addition, removal of secondary metabolites (cyanotoxins) is needed because of their high toxicity. Cyanotoxins are diverse in chemical structure and toxicity. For example, microcystins are cyclic peptides with more than 80 variants but only four (LR, RR, LA and YR) are of special concern due to their high hepatoxicity and as potent tumour promoters (Westrick et al., 2010). The WHO has established a guideline of 1µg/ L in drinking waters for the LR-variant.

On one hand water purification requires the removal of intact algae to avoid the release of endotoxins by cell lysis; on the other hand, it requires the removal of extracellular toxins. Therefore, the sequence of several water treatment units should be optimised. Studies of the performance of different utilities used in drinking water treatment plants (DWTP) for cyanobacteria removal are scarce. Clarification processes are very efficient for cell removal (Drikas et al., 2001; Teixeira et al., 2017), but the efficiency of these processes is species dependent (Henderson et al., 2008). Zamyadi et al. (2012) obtained values larger than 99% for removal by conventional coagulation-flocculation (C/F) processes of *Microcystis*, *Anabaena* and *Pseudoanabaena*; however,

Aphanizomenon removal with sand-anthracite filters was relatively poor (54-74%) and lysis of cyanobacterial cells was observed. Further chlorination did not reduce the total number of cells below the WHO Alert Level 1. Monitoring studies in seven DWTPs in Egypt showed poor performance in the removal of *Oscillatory limnetica* using conventional treatments (Mohamed, 2016). In several cases, microcystin concentration was detected above the WHO guideline due to cell lysis during the C/F process. Shang et al. (2018) also reported that the efficiency of the C/F processes in cyanobacteria removal in DWTP~~s~~ depended on the dominant seasonal species.

In recent years, electrocoagulation (EC) has received significant attention as an alternative method to conventional C/F processes for remediation of waters (El-Ashtoukhy et al., 2017; Gilhotra et al., 2018). The advantages of EC over conventional C/F can be summarised as: (i) coagulants are electrogenerated in situ so that the presence of competitive/interfering anions added as coagulant salts is avoided; (ii) adjustment of pH is not necessary except for extreme cases; (iii) more effective and rapid organic matter separation; (iv) flotation processes through the attachment of pollutants/particles to microbubbles of oxygen and hydrogen generated in the system increased the separation efficiency; (v) a smaller amount of coagulant is required with the subsequent lower sludge production; and (vi) the operating costs are lower (Mollah et al., 2004; Garcia-Segura et al., 2017).

Application of EC to algae removal is mainly aimed at harvesting biofuel production (Fayad et al., 2017; Shi et al., 2017). Most studies have focused on the influence of operating parameters such as current intensity, initial algae cell density, material of the electrodes used, pH, etc. Aluminium and iron are the most commonly used electrodes, but in the particular case of microalgae, the removal efficiency of Al electrodes was higher than those of Fe, with the added advantage of reduced energy

consumption because of its larger dissociation and reduced electrode passivation by oxide formation (Gao et al. 2010; Souza et al., 2016). The effect of current intensity is related to the ohmic resistance of the suspension, and therefore, the greater the inter-electrode distance, the greater the applied potential should be for identical metal dissolution of the sacrificial anode. This effect was more pronounced with Fe electrodes (Valero et al., 2015). Another factor influencing the efficiency of the EC process is the composition of the biomass; the presence of chloride ions can generate chlorine and enhance cell inactivation (Hakizimana et al., 2016). Cell abatement during the EC process is positively correlated with temperature, pH and conductivity (Boudjema et al., 2014). In the vast majority of studies, the system used consisted of a two-electrode EC cell operating in a galvanostatic mode and tested in batch. In order to operate at a workable rate of metal dissolution under realistic conditions, the use of electrodes with large surface areas are required. This may be achieved by using several pairs of electrodes connected in monopolar mode (Mollah et al., 2004). In the current study, the EC/sedimentation of an algae suspension using a multi-electrode cell was studied. During the first stage, the removal of the unicellular *Microcystis aeruginosa* and microcystin-LR, as a model of toxic cyanobacterium and cyanotoxin, respectively, was tested. Then, flocculation of the coagulates was assayed using polyelectrolytes of different ionicities and molecular weights. Finally, the conditions previously determined with *Microcystis* sp.were used to examine cyanobacteria removal from bloomed surface water.

2. MATERIALS AND METHODS

2.1.Materials.

Dream Taq PCR Master Mix, DNA loading and 100 bp DNA Ladder gTPbio were supplied by Thermo Fisher Scientific (MA, USA), ethidium bromide by Sigma-Aldrich (MO, USA) and low EEO D1 agarose by BIOMOL (Hamburg, Germany). The polyelectrolytes Chemifloc AN 270 (AN270), Chemifloc AN 870 (AN870), Chemifloc PA47 (PA47) and Chemifloc AA (CAA) were kindly supplied by Chemipol S.A. (Barcelona, Spain). The polyelectrolytes AN270, AN870 and CAA are anionic flocculants with ionicity % mole of 20, 10 and 8. Their molecular weights are 10×10^3 kDa for AN270, 11×10^3 kDa for AN870 and 8×10^3 kDa for CAA. A cationic starch (CS) with ionicity 22% mole was purchased from Penford Co. (CO, USA). Cyanobacteria bloom surface water was collected from a water reservoir located in Carrión de los Céspedes (Sevilla, Spain).

2.2. Cyanobacterial culture.

Microcystis aeruginosa PCC7806 (hereafter referred to as *Microcystis* sp.) was obtained from the Pasteur Culture Collection and grown in BG11 medium (Rippka et al., 1979) supplemented with 17.5 mmol/L $NaNO_3$ and 10 mmol/L $NaHCO_3$, under a bubbling mixture of air/CO_2 (1% v:v) at 25ºC with a light intensity of 75 µE m^2 /s^{-1} under continuous light.

2.3. Electrochemical set-up.

A cylindrical tank (11 cm radius and 33 cm height) with 10 L of cyanobacteria suspension was connected to a peristaltic pump followed by a flowmeter that permitted a set-up flow between 5 and 35 L/h, before the EC unit began operating under

continuous flow to reduce the time for electrocoagulation, and at the same time, increase the amount of purified water relative to batch EC systems.-The surface of every electrode used was 162 cm^2. The electrocoagulation unit was formed by five lead 3 mm thick Al electrodes in parallel connected in monopolar mode to a generator providing a current intensity (j) up to 37 mA/cm^2. The inter-electrode distance was 0.5 cm. The EC unit worked in a bottom-top mode and operated in a potentiostatic mode between 5 and 20 V, the intensity being recorded. The volume of the EC cell was 0.604 L (8.5 cm base x 12.5 cm width x 5.5 cm height). The hydraulic retention time (HRT) in the cell, i.e., the EC time, was set up to 1.0 and 2.2 min. A cubic receiver tank was fixed at the output with a capacity of 3.4 L (15 cm base). After conducting an EC experiment, the electrodes were washed with a 2% HCl solution, and then rinsed with water until no chloride was detected as a precipitate by addition of $AgNO_3$.

The dosage rate of coagulant in the reactor is dependent on the EC time and the current density which determines the consumption of the material of the anode. These two parameters are related through the charge loading, which is defined as the charges transferred in electrochemical reactions for a given amount of water treated.

The charge loading was calculated by:

$$Q = \frac{I\, t_{EC}}{V} \qquad \text{Eq. [1]}$$

where Q is the charge loading (C/L), I is the applied current (A), V is the volume of the EC cell, and t_{EC} is the EC time or HRT (min).

The amount of dissolved Al was theoretically determined by the use of Faraday's law:

$$w = \frac{j\, t\, M}{n\, F} \qquad \text{Eq. [2]}$$

where *w* is the quantity of electrode material dissolved (mg of Al / cm^2), *j* the current density (A/ cm^2), *t* is the time in *s*, *M* is the relative molar mass of the electrode (26.982 g/ mol), *n* the number of electrons in oxidation/reduction reaction (3) and *F*- is Faraday's constant (96485 C/ mol).

2.4. Cell enumeration.

Microcystis aeruginosa grown in axenic cultures was quantified using a flow cytometer based on its autofluorescence. In surface water samples, the enumeration associated with three size ranges for unicellular (3-8μm), filamentous (9-17 μm) cyanobacteria and larger (18-30 μm) microorganisms were estimated using a particle counter (Beckmann Coulter Z2, USA).

2.5. DNA extraction and PCR analysis.

A 50 mL aliquot of water was harvested by centrifugation (10000g for 5 min at 4ºC), and the DNA of the cell pellets was extracted by a standard phenol-chloroform procedure (Sambrook and Russell, 2001). PCR analysis of the extracted DNA was performed using the primer pairs in Table S1 under the conditions described in detail in Gkelis and Zaoutsos (2014). Thermal cycling was carried out using an Eppendorf MasterCycler Pro. PCR products were separated with a 2% (w/v) agarose gel in 1X TAE buffer stained with ethidium bromide and photographed under UV transillumination.

PCR products were cloned into *E. coli* using a TOPO-TA cloning kit (Invitrogen, USA). Random clones were sequenced. A homologue search using BLAST (NCBI) allowed identification of the closest homologues.

2.6. Flow cytometer analysis

A FC500 flow cytometer (Beckman Coulter, USA) equipped with an air cooled 20 mW argon laser and an additional red diode laser emitting at fixed wavelengths of 488 and 630 nm, respectively, was used for measurement. Fluorescent filters and detectors were all standard with green fluorescence collected in channel FL1 (525 nm) and orange fluorescence in channel FL3 (620 nm). Probe fluorescence, chlorophyll a fluorescence, forward scatter-FSC (cell size) and side scatter-SCC (cell granularity) data were collected and analysed using CXP software. FSC and FL1 were used to quantify and gate the cyanobacterial cells. The histogram plot was divided into two regions: counting of particles emitting fluorescence were assigned to live cells~~,~~ and particles with no emission of fluorescence to dead cells. FSC was previously fitted to determine cyanobacteria only.

Flow cytometer analysis with surface waters were performed after sonication for 2 min under 360 W, for disruption of colonies and filamentous cells. Under these conditions, a good agreement between the total cell count using a cell counter and those determined by flow cytometer analysis was obtained.

2.7. Microcystin analysis.

Microcystin-LR was extracted from water samples (50 mL) using OASIS HLB solid-phase extraction cartridges as described in Meriluoto and Spoof (2005). Microcystin recoveries were greater than 95%. Analytical determination was performed using a Shimadzu HPLC equipped with a PDA detector. The reverse phase column used was a 15 cm Kromasil 100 C18 and the flow rate was 1 mL/min. The isocratic mobile phase was acetonitrile with water acidified to pH 3.0 by trifluoroacetic acid at a 38:62

196 (v:v) ratio. The wavelength was fixed at 270 nm, retention time was 5 min, and the
197 detection limit was 0.015 µg/L.
198
199 2.8. Flocculation experiments.
200 One hundred mL of water samples (cyanobacterial cultures, electrocoagulated
201 suspensions) were placed into 250 mL beakers. In the case of electrocoagulated
202 suspensions, two charge loadings were used, 10 and 50 C/L,corresponding to low and
203 intermediate voltages. A magnetic stirrer was inserted into the beakers and the different
204 volumes of flocculants were added at 700 rpm. Stirring continued at the same speed for
205 3 min and then decreased to 200 rpm for 15 min. After settling times of 15 min, reading
206 of supernatant turbidity was performed. Residual turbidity was calculated as the fraction
207 remaining in the supernatant from the initial value (expressed as percent).
208
209 2.9 Analytical determinations.
210 Analyses were performed according to the ISO tools for conductivity
211 (7888:1985), concentrations of nitrate (ISO 7890-1-11986), chemical oxygen demand
212 (ISO 15705) and dissolved oxygen (ISO 17289). Measurement of ORP was conducted
213 using a Hach Lange sensor MTC101. pH was measured using a GLP21 Crison pH
214 meter. An ICP-OES (Varian model 720-ES) was used for the determination of the
215 concentrations of P, K, Na, Ca, Mg, S, and Al in waters. For the Al analysis, the
216 solutions were treated previously with a 4 N HNO_3 solution for dissolution of
217 precipitates. A Shimadzu TOC-VSCH analyser was used for organic carbon analysis
218 after filtration of water samples through 0.22 µm membrane filters (Prat Dumas,
219 France). UV_{254} was measured using a UV-Visible Shimadzu model 1201
220 spectrophotometer. A Shimadzu Turbiquant model DR3000 was used for turbidity

measurements. Zeta potential and particle dimension measurements were performed using a Zetasizer Nanosystem and a Mastersizer 2000 system (Malvern Instruments, GB), respectively.

3. RESULTS AND DISCUSSION

3.1. EC of *Microcystis* sp. cultures

A study of cell survival of *Microcystis aeruginosa* as a function of the charge loading was performed (Fig. 1). For a 10^5 cells/mL concentration, a charge loading of 10 C/L yielded survival values of 81.4±1.8 and 85.7±2.7% for 1.0 and 2.2 min HRT, respectively, which are not significantly different. However, the current intensity used for a HRT of 2.2 min was about two-fold lower. An additional advantage of using higher HRTs is the extension in Q values by operating under identical current intensities. Q reached maximum values of approximately 50 C/L with 34.8±0.5% of inactivated cells for HRT of 2.2 min, whereas lower values were obtained for an HRT of 1 min (18C/L with 22.0±1.8% of inactivation).

An analysis of cell survival based on Q values showed that this was strongly related to the initial concentration of the cyanobacteria. The survival decreased concomitantly with increased Q values and was steepest for the lowest cell concentration used. When the total concentration was 10^5 cells/mL, the survival decreased from 85.7±0.5% for a Q value of 5 C/L to 65.7±0.1% for a Q of 50 C/L. On the contrary, the latter charge loading only reduced ~~a~~live cells up to 92.8±0.2% while the concentration of *Microcystis* sp. increased by one order of magnitude. Again, no difference was noticed on cell survival based on Q values obtained with different HRTs and current intensities. As an example, a Q value of 28 C/L yielded 93.9 ± 0.3 and 95.5 ± 0.7% survival for HRT of 1 and 2.2 min, respectively.

246 Microcystin-LR was monitored during the EC process (Fig.2). The guideline value
247 of 1.0 µg/L set by the WHO for drinking waters was already reached by both initial
248 *Microcystis* sp. suspensions, which was especially relevant for the highest cell
249 concentration used, exceeding 2.5-fold the value above the regulatory limit. In both
250 suspensions, the use of Q values larger than 20 C/L increased the microcystin-LR
251 concentration drastically, from 2.06±0.02 to 6.74±1.14 µg/L for a 10^5 cells/mL
252 suspension and from 3.30±0.20 to 4.78±0.18 µg/L for 10^6 cells/mL . This increase was
253 more pronounced with the10^5 cells/mL suspension of cyanobacteria which paralleled
254 the inactivation percentages (Fig. 1) that increased from 20% for 20 C/L to 34% for 50
255 C/L, whereas only a 3% inactivation was achieved for a 10^6 cells/mL suspension.
256 The microcystin- LR concentrations in the electrocoagulated suspensions were
257 relatively low if inactivation occurred with full rupture of the cells, because intracellular
258 microcystins can amount to 98% of the total (Chow et al., 1997). Cell inactivation has
259 been assigned to irreversible permeabilisation of the cellular membrane with leakage of
260 cytoplasmic components by formation of transient pores when exposed to an external
261 electric field (Ghernaout and Ghernaout, 2010). The formation of these transient
262 aqueous pathways in the lipid bilayer should exceed a threshold-value, below which no
263 decreased viability is observed (Canatella et al., 2001). In principle, the electric field
264 across the cellular membrane should exceed 1 V to form water-filled pores in the
265 membrane's lipid bilayer (Nuccitelli et al., 2006).
266 This transmembrane potential is dependent on experimental conditions as the pulse
267 or duration of the electric field (Abderrahmane et al., 2008). For a 10^5 cells/ml
268 *Microcystis* sp. suspension, Q values had low inactivation values due to the fact that the
269 threshold value for electroporation was barely reached. The larger inactivation amounts
270 observed at higher Q values are mainly associated with increased EC times that avoid

271 the restoration of the membrane, thus largely increasing ion transfer and provoking
272 irreversible cellular damage. Other factors affecting the magnitude of the cell
273 overpotential are the ionic concentration in the bulk, types of electrolyte and cell
274 concentration (Jiménez Izquierdo et al., 2010). The lowest inactivation observed for
275 similar Q values, with increasing cell concentration from 10^5 to 10^6 cells/mL, was due
276 to increased local perturbations with denser cell suspensions of the electric field caused
277 by neighbouring cells (Canatella et al., 2001).

278 Oxidative stress could also be responsible for inactivation of *Microcystis* sp. cells.
279 Wei et al. (2011) pointed out that in the vicinity of the electrodes, a pH gradient may be
280 formed and if the system is not buffered, the production of toxic hydrogen peroxide at
281 the cathode is feasible. As oxygenic photosynthetic organisms, cyanobacteria are
282 regularly challenged by oxidative stress caused by reactive oxygen species, such as
283 superoxide anion radicals, hydrogen peroxide and hydroxyl radicals produced in the
284 light as a consequence of photosynthetic electron transport and oxygen evolution.
285 Therefore, cyanobacteria as well as plants and algae are equipped with potent
286 antioxidant systems (Latifi et al., 2009). Strong resistance to externally added hydrogen
287 peroxide was found in the cyanobacterial strains *Synechocystis* sp. PCC6803 and
288 *Anabaena* sp. PCC7120 (Pascual et al., 2010). Thus, cultures of *Synechocystis* and
289 *Anabaena* in their mid-exponential phase were able to sustain growth in the presence of
290 7.5 mmol/L and 2.5 mmol/L hydrogen peroxide, respectively. Indeed, hydrogen
291 peroxide added to such cultures at a concentration of 0.5 mmol/L was completely
292 decomposed after 5 min in *Synechocystis* and after 12 min in *Anabaena* (Pascual et al.,
293 2010). The content and composition of antioxidant enzymes among cyanobacteria vary
294 between species. Peroxiredoxins are thiol-dependent peroxidases and gene encoding
295 peroxiredoxins (e.g. 2-Cys Prx), found in all cyanobacterial genomes examined. Some

296 species, such as *Synechocystis* sp. PCC6803, also contain a bifunctional catalase-
297 peroxidase (KatG), whereas others, such as *Anabaena* sp. PCC7120 and *Microcystis* sp.,
298 do not (see Supplementary Table 2 in Pascual et al., 2010). Therefore, it would be
299 reasonable to assume that *Microcystis sp*. has a tolerance towards hydrogen peroxide
300 similar to that of *Anabaena*, for example up to 2.5 mmol/L concentration. This is an
301 order of magnitude above the concentration that could be generated next to the cathode
302 in this system at the lowest charge loading, considering the stoichiometry reaction
303 between the dissolved Al and the potential H_2O_2 generated (0.5 mmol/L). Moreover, as
304 long as the EC proceeded, pH became more basic from initial values near neutrality that
305 paralleled lower redox potentials (Table 1). These two features avoided/decreased
306 generation of hydrogen peroxide at the cathode interface, contrasting with the trends in
307 cell inactivation (Fig. 1).
308 As shown in Table 1, the use of higher Q values in the EC electrocoagulation
309 process yielded a more reductive solution (ORP values decreased); therefore, other
310 redox reactions can take place. An inspection of Table 1 indicates that nitrate reduction
311 on the cathode occurred, accompanied by release of hydroxyls increasing the final pH
312 values.
313 Several mechanisms can be operated during the electrocoagulation of the
314 cyanobacteria and formation of settled flocs: charge neutralisation, sweep flocculation,
315 extracellular organic matter acting as an aid coagulant, hydroxide flocs filling gaps
316 between bristles, etc. (Henderson et al., 2008; Ghernaout et al., 2010). In Figure 3, the
317 residual turbidity and zeta potential clearly showed a parallel relationship with increased
318 Q values. The zeta potential of the cell suspensions were negative, -10.7±1.0 and -
319 13.9±0.9 mV for 10^5 and 10^6 cells/mL concentrations, respectively. This is due to
320 dissociation of functional groups with particular relevance to carboxylic acids located at

the cell surface and in extracellular organic matter present in the suspension (Pivokonsky et al., 2016). For the 10^5 cells/mL suspension, the residual turbidity was reduced to 81%, and subsequently, 22% by increasing Q values from 5 to 10 C/L~~,~~ with a concomitant reduction of zeta potential to -6.4 ± 0.7 and -2.2 ± 0.3 mV. At larger Q values electroneutrality was achieved and the remaining turbidity reached very low values because the vast majority of the cells were already settled in the flocs. This pattern indicated that charge neutralisation was responsible for elimination of cells during EC. Better evidence was noted using a denser cell population, which broadened the range of observations before electroneutrality was reached. This was due to the fact that larger amounts of Al, arising from the dissolution of the anode, would be necessary for coagulation of the cells, for example, larger Q values in the case of charge neutralisation. Again, an identical pattern was obtained, which agrees with the observation that removal by charge neutralisation can be obtained if cells are spherical and free from protruding appendages (Pieterse and Cloot, 1997) as with *Microcystis*. Beyond electroneutrality, ~~Z~~eta potential values were not reached and can be rationalised on the basis of the Al chemistry. At low pH the cation Al^{3+} dominates, whereas the hydroxide $Al(OH)_4^-$ is prevalent at pH values higher than 9. At intermediate pH, such as those recorded in the EC of the cyanobacteria (Table 1), the insoluble hydroxide $Al(OH)_3$ is dominant in equilibrium with very small amounts of soluble Al species (Lindsay, 1979).

The experimental dissolved Al from the sacrificial anode was lower than that theoretically estimated by Faraday's law (Fig. 4). Oxygen and hydrogen gases produced along the course of the EC act as gas- insulating spheres that increase the electrical resistance and reduce the effective intensity for Al dissolution under a low turbulence regime (Mollah et al., 2004). Another source of this discrepancy may be certain

passivation of the electrode surface by patches of calcium and magnesium precipitates with carbonate ions present in the solution. The water used contained 312 mg/L of HCO_3^-, 22. mg/L of Mg^{2+} and 86.1 mg/L of Ca^{2+}. On the other hand, experimental amounts of dissolved Al were greater for higher cell concentrations used at the same Q values, which can be explained by lower solution resistance when using a solution with greater conductivity (590 vs 530 µS/cm; Table 1). Therefore, a higher effective potential and subsequent intensity were applied on the electrode for its dissolution.

3.2. **Flocculation of electrocoagulated suspensions of *Microcystis aeruginosa*.**

Application of charge loading of 10 and 50 C/L to a blooming *Microcystis aeruginosa* concentration of 10^6 cells/mL yielded very different suspensions with negative zeta potential and moderate turbidity for a charge loading of 10C/L, whereas suspension near neutrality and high turbidity was observed with 50 C/L (Table 2). Two of the flocculant concentrations used were those recommended in the use of commercial flocculants for dewatering (10 and 100 mg/L); a lower one (1 mg/L) was also used.

For anionic flocculants, AN870 was the polyelectrolyte with better flocculation properties. With the electrocoagulated suspension at 10 C/L, the residual turbidity showed the lowest values relative to those of AN270 and CAA at the same dose. Especially remarkable was the high residual turbidity with AN270 at the lowest dose. On the contrary, the residual turbidity was already negligible for the 50 C/L suspension, and the use of these flocculants only slightly improved the flocculation process. The mechanism with polyelectrolytes of low charge densities (≤20 mol%) and high molar masses, as with AN270 and AN870, was reported to occur via bridging between particles (Bolto and Gregory, 2007). Cells were coagulated by charge neutralisation; then, the overall surface of the particles (poly(Al)hydroxide-cell) was expected to be

neutral and capable of interaction with the non-charged portion of the polymer by Van-der-Waals forces. The fact that higher removal (lower residual turbidity) was observed with the polyelectrolyte of lower charge density (AN870), which is expected to develop a more uncoiled conformation in solution revealing higher surface for interaction with particles, seemed to corroborate it. The same mechanism was observed in the flocculation of cationic polyacrylamides with negatively charged clay particles (Petzold et al., 2004). Further evidence was obtained from the analysis of the particle size distribution presented as volume fraction versus particle diameter (Figs. 5 and 6). An increase in the size of the flocs with concentration revealed a bridging mechanism where the inter-particle association via the hydrophobic segments of the polyelectrolyte resulted in bigger flocs. This bridging mechanism was also predominant with the anionic CAA as noted in its bimodal distribution; the only exception being the highest dose for a charge loading of 50 C/L. A charge neutralisation mechanism would have produced a unimodal distribution with a narrow and smaller range of particle diameters (Ghimici and Nichifor, 2012).

The cationic polyelectrolytes PA47 and CS showed a similar pattern. With a 10 C/L charge loading (Fig. 5), both polymers presented a multimodal distribution of flocculated particles independent of the dose used; however, another mechanism was operating related to the fact that the positive charge of the polyelectrolytes interacted with the negative charge of the cell membrane. The addition of CS decreased the zeta potential and practically reached electroneutrality with a 10 mg/L dose paralleled by a slight decrease in the residual turbidity (Table 2). By increasing the dose 10-fold, the zeta potential was reversed and the turbidity increased two-fold, indicating the presence of an electrostatic patch aggregation mechanism. The binding of the polymer with the cell did not neutralise the overall charge of the cell membrane, but only locally reversed

396 the charge resulting in patches of opposite charge on the cell. Electrostatic interactions
397 between opposite charged patches, located in different polymer-cell particles, induced
398 their flocculation. The addition of an excess of positive charge through increasing CS
399 concentration will increase the electrostatic repulsion with the subsequent restabilisation
400 of particles, as evidenced by the increased turbidity.
401 At the lowest polymer concentration, a peak was always present in a very narrow
402 size range (1.9-6.6 μm) and pointed to a charge neutralisation mechanism of free cells
403 in the suspension with the aluminium species, in accordance with the size of
404 *Microcystis aeruginosa* ranging between 3 and 7 μm (Henderson et al., 2008). At a 50
405 C/L charge loading (Fig. 6) the absence of this peak is explained by sweep flocculation ,
406 due to the larger of interaction of the (poly(Al)hydroxide-cell) particles with polymer
407 chains driving massive aggregation and increasing on the size of the flocs.
408 In general, using a concentration larger than 10 mg/L did not increase the efficiency
409 of the flocculation process. The anionic flocculants performed better than the cationic
410 ones; therefore, the polyelectrolytes AN270 and CAA were selected as probes for
411 flocculation of electrocoagulated suspensions of bloomed surface waters.
412

413 **3.3. Application of EC/flocculation to surface waters**

414 The water selected came from a lake with repetitive episodes of cyanobacteria blooming
415 (physico-chemical properties in Table S2). Cyanobacteria specific primers targeting the
416 16S rRNA gene revealed the presence of cyanobacteria species, the closest homologues
417 being *Microcystis elabens*, *Cyanobium gracile*, *Lengtong bryaceae* and *Synechococcus*
418 *rubescens*. PCR-based detection of a gene involved in the synthesis of microcystins
419 (*mcyB*) showed the presence of strains bearing this gene (Fig. S1). In general, nontoxic
420 strains do not contain *mcy* genes, but in some cases these strains may have fragments or

mutant forms of these genes that can be amplified with *mcy* primers, although they are not able to produce toxins (Pham et al., 2015). Further corroboration of the presence of toxic cyanobacteria strains was obtained by detection and quantification of microcystin-LR (4.3 ± 0.7 µg/L). Microcystin can be produced by unicellular and filamentous (pluricellular) cyanobacteria. The analysis of cell size using a cell counter indicated that the amount of unicellular and filamentous bacteria amounted to 90.9±1.8 and 9.2±0.9%, respectively. The total amount of cyanobacteria was determined to be $4.1x10^6$ cells/mL.

Application of EC at charge loadings of 10 and 50 C/L only increased the amount of inactivated cells by 5% (up to approximately 10%) (Table 3). These results are in good concordance with those of inactivation of a 10^6 cells/mL suspension of *Microcystis aeruginosa*, which showed percentages of 3.8±0.3% for 10 C/L and 7.2±0.2% for 50 C/L (Fig. 1). After the electrocoagulated suspensions were treated with flocculants, the amount of cyanobacteria in the supernatant was drastically reduced by two and three orders of magnitude. In addition, the cells in the supernatant were completely inactivated.

The flocculants performed worse than those with only *Microcystis* sp. cultures, as revealed in the higher residual turbidity in the supernatant. This was especially the case for the lower charge loading, with values of about 17% for both flocculants (Table 4) versus values of about 7% in *Microcystis* sp. flocculation tests (Table 2). Based on the analysis of the zeta potential, no statistical difference existed at the lower charge loading and thus, similar flocculation should be expected. At larger charge loading with surface water, the slightly positive value of the zeta potential was presumed to decrease the residual turbidity. This was made even higher by increased electrostatic interactions of aluminium particles with negatively charged surfactant molecules; however, the opposite trend was observed.

Reasoning behind this trend is the effect of the composition of the extracellular organic matter (EOM) formed by photosynthesis and secondary metabolism by-products, which differed between the species. The composition of EOM in *Microcystis aeruginosa* amounts to 60% of hydrophilic compounds of which about 10% are charged (Li et al., 2012). These hydrophilic compounds comprise a wide range of chemicals: carbohydrates, hydroxyacids, peptides, low MW alkyl alcohols, aminoacids, aminosugars, etc. (Pivokonsky et al., 2016). The low hydrophobicity is reflected in low values of SUV_{254} as an estimation of the content of aromatic structures and conjugated double bonds, which was 1.5 L/(m mg) for *Microcystis* sp. cultures. A small fraction of the aluminium released during EC might combine with some components of the EOM, such as negatively charged polysaccharides, which have been described for multiple species associated with units of uronic acids (Li et al., 2001). In that case, the charge neutralised fraction of EOM can behave as a better flocculant than AN270 and CAA, via interactions with the neutral hydroxoaluminium-bacteria particles enhancing the flocculation process. This process is in some way hampered in the case of flocculation of electrocoagulated surface water, due to the presence of more hydrophobic compounds as revealed in the higher SUV_{254} value (1.8 L/(m mg)).

The use of the anionic flocculants AN270 and CAA induced lysis of the coagulated cells releasing microcystins (Table 4), which is an undesired effect. The concentration measured in the supernatant exceeded that allowed by WHO several fold (1µg/L). Therefore, in selection of the flocculant, a compromise should be established between the efficiency in the cyanobacteria removal and toxin solubilisation. The structure of the two anionic flocculants used is common in water treatments: anionic polyacrylamide as the case with AN270 and phosphate pregelatinased starch as with CAA. In principle, surfactants carrying other functional groups could be advantageous in water treatment

processes because of their lower production of cyanotoxins, and more economic removal thereof.

3.4. Economic estimates

In the economic evaluation of the process, a clear distinction should be established between the EC process and that of flocculation. The operational parameters for economic estimation with EC are mainly the electrodes, electricity, labour, maintenance, and other fixed costs. Prices for electrical energy and electrode material in May 2019 were 0.120 €/kW h and 1.76 €/kg, respectively. Electricity cost for EC of 1 m^3 of water was estimated by applying a low charge loading (10 C/L) under the operational conditions used herein which yielded a value of 0.443 €/m^3. A lager charge loading was excluded because its electricity cost was higher. No passivation and regeneration of the electrode was included. The cost for electrode material amounted to 0.128 €/m^3. Using a 10% increase in manpower plus maintenance, and other fixed costs for water treatment of 1 m^3 comes to 0.628€.

Prices for flocculants are in the range 1.29-1.97€/kg. The usual recommended rate is 10 mg/L, so that the price for purification of 1 m^3 may reach a value of 0.0197€. Therefore, the price for water treatment of 1 m^3 of water with cyanobacterial bloom by EC, followed by flocculation, will be approximately 0.648€. This price can be lowered by improving the efficiency of the EC process through optimisation of several parameters such as the electrode surface/cell volume ratio, inter-electrode gap, etc. For an estimation of the total cost, further processes related to the intended water use should be included in the calculations.

4. CONCLUSIONS

EC of *Microcystis aeruginosa* at low electrical current densities was advantageous over the use of higher densities because of lower inactivation amounts of cells, avoiding induced cell permeabilisation and release of endotoxins. EC was ineffective in removing extracellular metabolites; however, other concomitant processes during the electrodissolution of the anode occurred such as electroreduction of nitrate accompanied with release of hydroxyls, raising the pH in the bulk suspension. Cells were removed by charge neutralisation with the generated Al species in solution, which sedimented rapidly when using high charge loading. Therefore, the application of commercial flocculants to these electrocoagulated *Microcystis aeruginosa* suspensions were not advantageous in the C/F process. However, the use of anionic flocculants at suspensions generated at low charge loading improved the sedimentation velocity. The efficiency of EC/flocculation applied to cyanobacterial blooming surface water was reduced relative to experiments with axenic cultures due to variations in the chemical composition of the EOM. An economic estimation indicated the feasibility of the combined use of these two techniques for water treatments.

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655

Table 1. pH, ORP, conductivity and nitrate concentration during EC as a function of the charge loading and Microcystis aeruginosa concentration.

Charge loading (C/L)	pH	ORP (mV)	Nitrate concentration (mg/L)	Conductivity (µS/cm)
Cells concentration, 10^5 cells/mL				
0	6.93±0.03	-	12.7±1.6	530±8
4.9	6.98±0.04	-172±32	2.3±0.3	535±24
9.8	7.01±0.02	-257±15	1.9±0.6	501±13
18.4	7.25±0.03	-291±18	1.6±0.1	447±9
31.0	7.34±0.06	-306±9	1.8±0.5	410±6
40.5	7.45±0.05	-308±1	1.7±0.6	391±2
50.5	7.54±0.06	-315±8	1.8±0.3	385±8
Cells concentration, 10^6 cells/mL				
0	6.95±0.02	-	28±2.3	590±12
6.9	6.95±0.32	-217±15	2.3±0.3	604±27
10.0	7.64±0.04	-264±16	1.9±0.7	599±35
18.0	7.77±0.09	-296±18	1.8±0.3	637±3
28.7	8.08±0.03	-352±8	1.6±0.1	577±31
38.4	8.23±0.11	-376±11	1.5±0.3	557±34
51.9	8.12±0.05	-443±19	1.7±0.4	472±9

663 Table 2. Zeta potential and residual turbidity of electrocoagulated suspensions at a
664 charge loading of 10 and 50 C/L as a function of added polymer and its concentration in
665 flocculation tests. The initial concentration of *Microcystis aeruginosa* was 10^6 cell/mL.

Conc.		Zeta potential (mV)		Residual tubidity (%)[a]	
(mg/L)	Flocculant	10 C/L	50 C/L	10 C/L	50 C/L
0	-	-6.2±0.2	-1.5±1.5	-	-
1	AN270	-7.0±0.7	-21.0±1.4	38.0	0.15
	CAA	-10.6±0.9	-1.9±0.7	10.6	0.49
	PA47	10.7±0.8	27.0±3.3	4.12	0.22
	AN870	-4.1±0.5	-5.4±0.6	5.70	0.31
	CS	-6.2±0.7	3.8±0.8	5.01	2.83
10	AN270	-5.2±0.5	-15.9±0.6	6.82	0.26
	CAA	-9.0±0.7	-4.2±2.7	6.73	0.35
	PA47	13.5±2.2	33.4±2.3	25.2	0.20
	AN870	-7.0±0.5	-13.9±0.7	6.02	0.35
	CS	-0.8±0.3	17.3±1.4	4.32	1.22
100	AN270	-6.0±0.4	-16.6±4.8	5.51	0.39
	CAA	-6.5±0.3	-15.8±2.0	8.42	0.72
	PA47	10.4±1.2	32.1±0.5	29.5	0.32
	AN870	-11.0±1.5	n.d.	7.54	0.55
	CS	3.1±0.7	15.7±0.5	8.73	6.51

666 a. Turbidity values after electrocoagulation were 56 and 186 NTU for 10 and 50 C/L
667 charge densities.

668

669 Table 3. Cyanobacteria amounts and their corresponding survival and inactivation
670 percentages in surface water after EC/flocculation as a function of the charge loading
671 and the surfactant used (10 mg/L concentration).

Q (C/L)	Surfactant	Total amount of cyanobacteria (cells/mL)[a]	Alive cells (% of the total)	Inactivated cells (% of the total)
-	-	$4.1x10^6$	94.5±0.7	5.40±0.4
10	-	$3.8x10^6$	89.2±0.3	9.7±0.1
	AN270	$5.5x10^3$	0	100
	CAA	$2.8x10^4$	0	100
50	-	$3.7x10^6$	88.7±0.5	10.7±0.1
	AN270	$8.9x10^3$	0	100
	CAA	$8.0x10^3$	0	100

672 a. Error limits in the determination were ±2%.

673

674 Table 4. Zeta potential, residual turbidity and LR-microcystin concentration of
675 electrocoagulated suspensions of surface water at a charge loading of 10 and 50 C/L as
676 a function of the surfactant used in flocculation tests . Surfactant concentration used was
677 10 mg/L.

Surfactant	Zeta potential (mV)	Residual turbidity	LR-microcystin (µg/L)	Zeta potential (mV)	Residual turbidity	LR-microcystin (µg/L)
		10 C/L			50 C/L	
-	-7.5±1.2	-	4.3±0.7	5.4±1.4	-	6.3±0.9
AN270	-10.2±2.3	16.82	50.1±3.2	-14.9±1.0	0.85	52.2±2.6
CAA	-12.3±1.8	17.39	55.3±4.5	-11.3±0.7	0.99	55.4±1.4

678
679
680
681

682 **Figure captions.**

683 **Fig. 1**. *Microcystis* sp. survival and inactivation as a function of the charge loading
684 under different operational parameters such as the hydraulic retention time (HRT) and
685 total concentration of cyanobacteria. The HRTs used were 1.0 and 2.2 min, and the
686 concentrations were 10^5 and 10^6 cells/mL.

687 **Fig. 2**. Amounts of microcystin-LR detected in EC experiments with cell suspensions
688 of 10^5 and 10^6 cells/mL as a function of the charge loading.

689 **Fig. 3**. Changes in zeta potential and turbidity with charge loading during EC of
690 *Microcystis* sp. suspensions.

691 **Fig. 4.** Theoretical and experimental values of dissolved Al with charge loading for
692 electrocoagulated suspensions of 10^5 and 10^6 cells/mL.

693 **Fig. 5.** Floc size distribution of the electrocoagulated suspension obtained at a charge
694 loading of 10 C/L after treatment with the polyelectrolytes AN870, AN270, CAA, PA47
695 and CS at three doses.

696 **Fig. 6.** Floc size distribution of the electrocoagulated suspension obtained at a charge
697 loading of 50 C/L after treatment with the polyelectrolytes AN870, AN270, CAA, PA47
698 and CS at three doses.

Figure 1

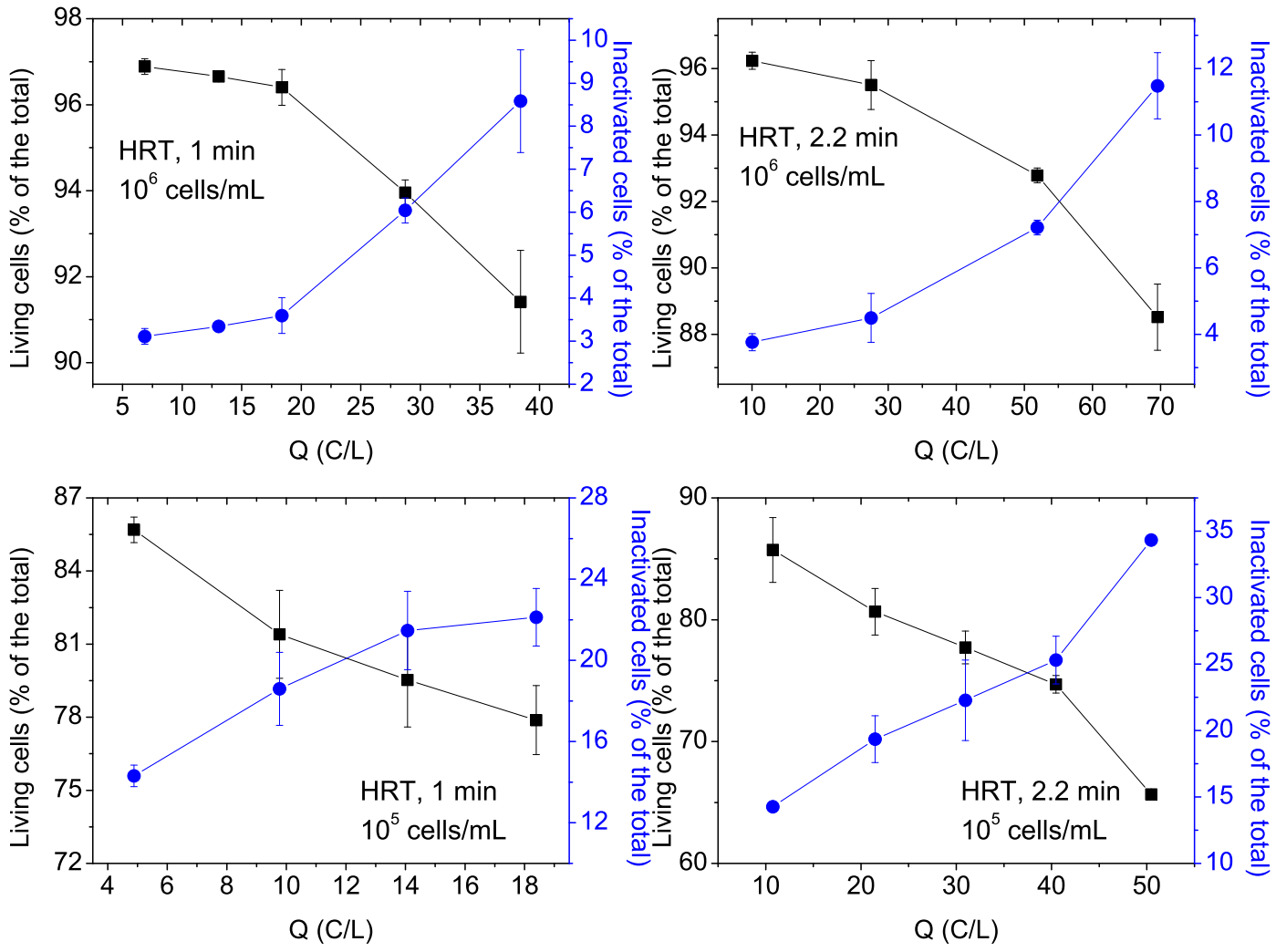

HRT, 1 min
10^6 cells/mL
Living cells (% of the total)
Inactivated cells (% of the total)
Q (C/L)
HRT, 2.2 min
10^6 cells/mL
Living cells (% of the total)
Inactivated cells (% of the total)
Q (C/L)
HRT, 1 min
10^5 cells/mL
Living cells (% of the total)
Inactivated cells (% of the total)
Q (C/L)
HRT, 2.2 min
10^5 cells/mL
Living cells (% of the total)
Inactivated cells (% of the total)
Q (C/L)

Figure 2

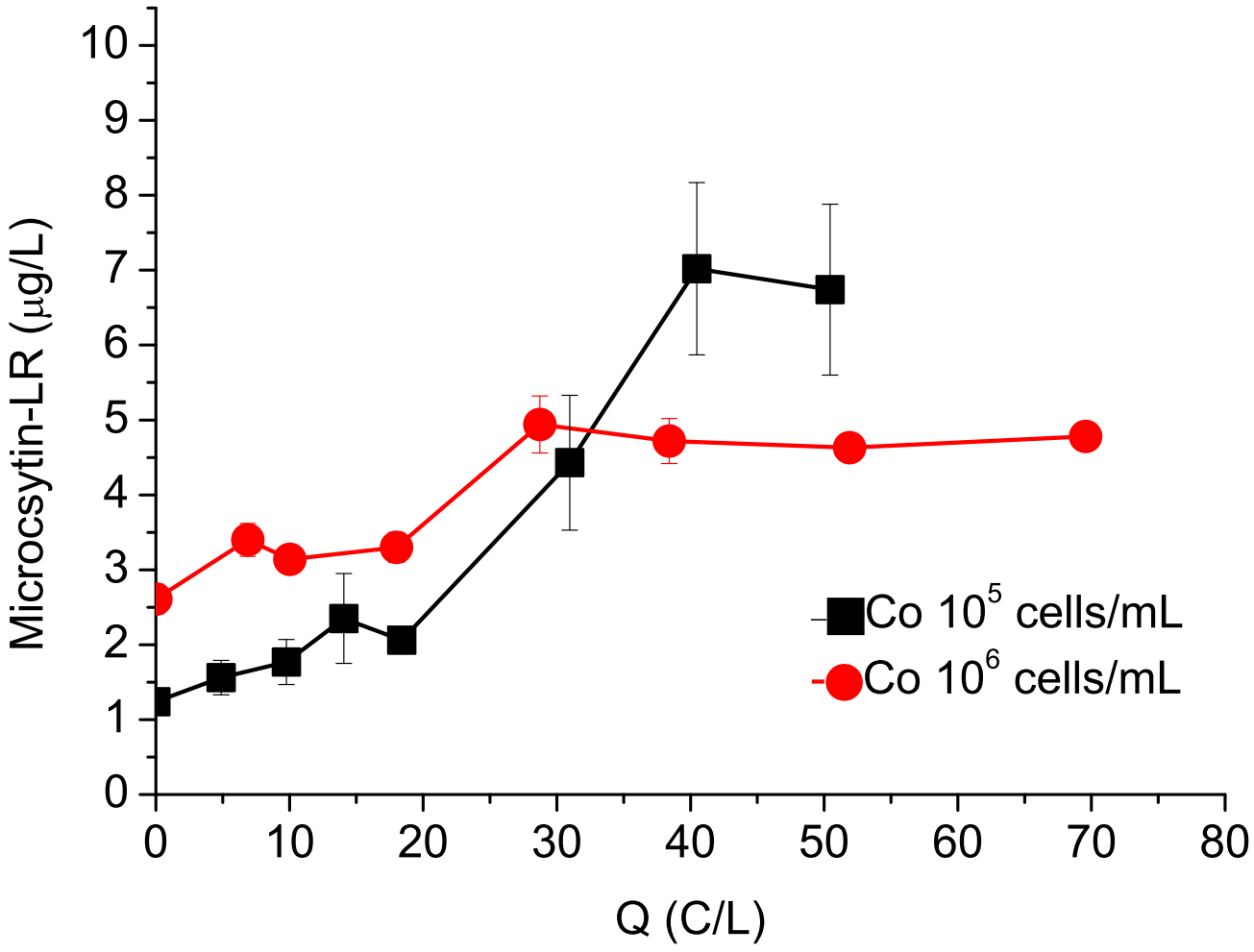
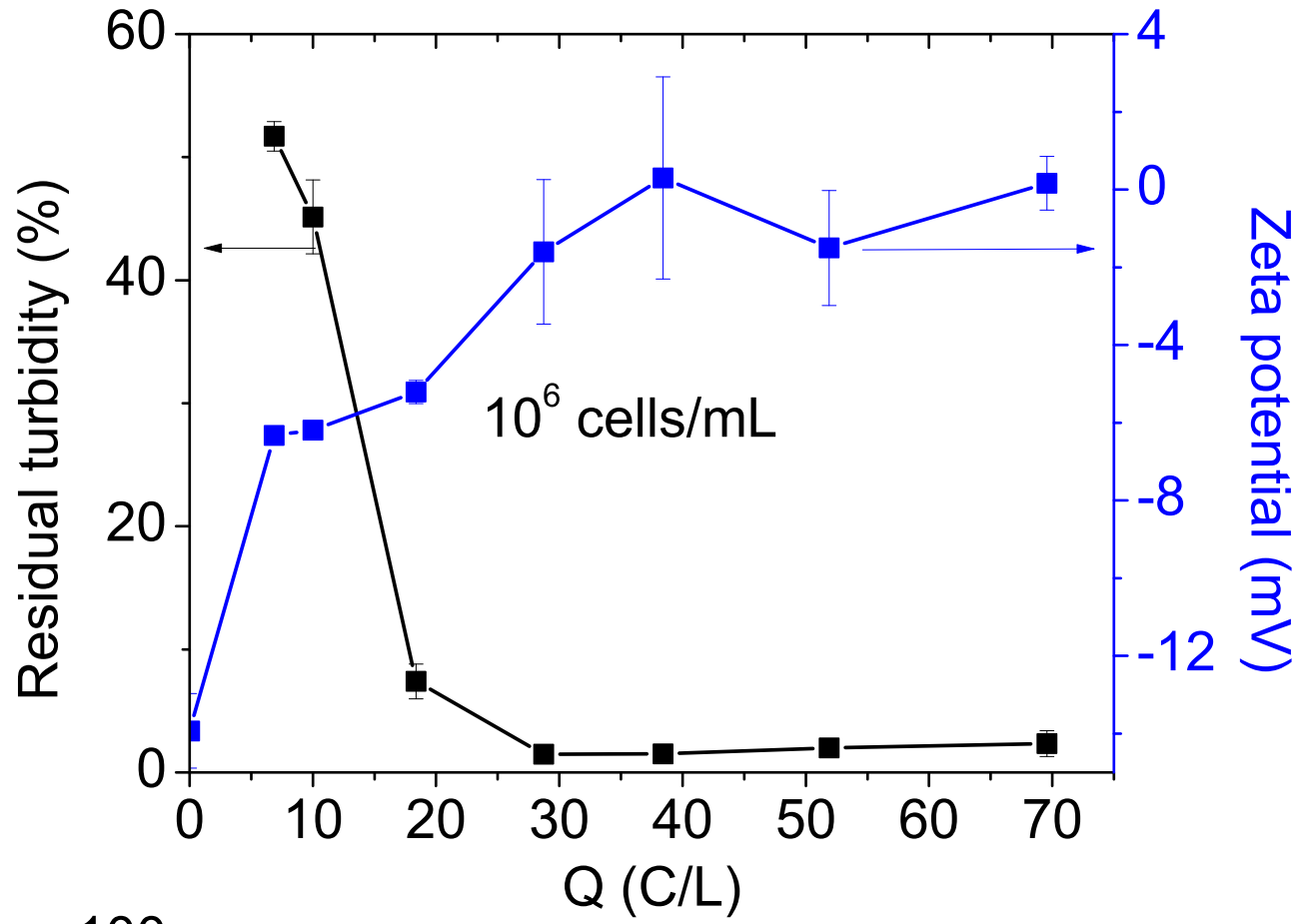

Microcystin-LR (μg/L)
Q (C/L)
Co 10^5 cells/mL
Co 10^6 cells/mL
0
1
2
3
4
5
6
7
8
9
10
0
10
20
30
40
50
60
70
80

Figure 3


Residual turbidity (%)
Zeta potential (mV)
Q (C/L)
10^6 cells/mL
0
20
40
60
0
10
20
30
40
50
60
70
4
0
-4
-8
-12

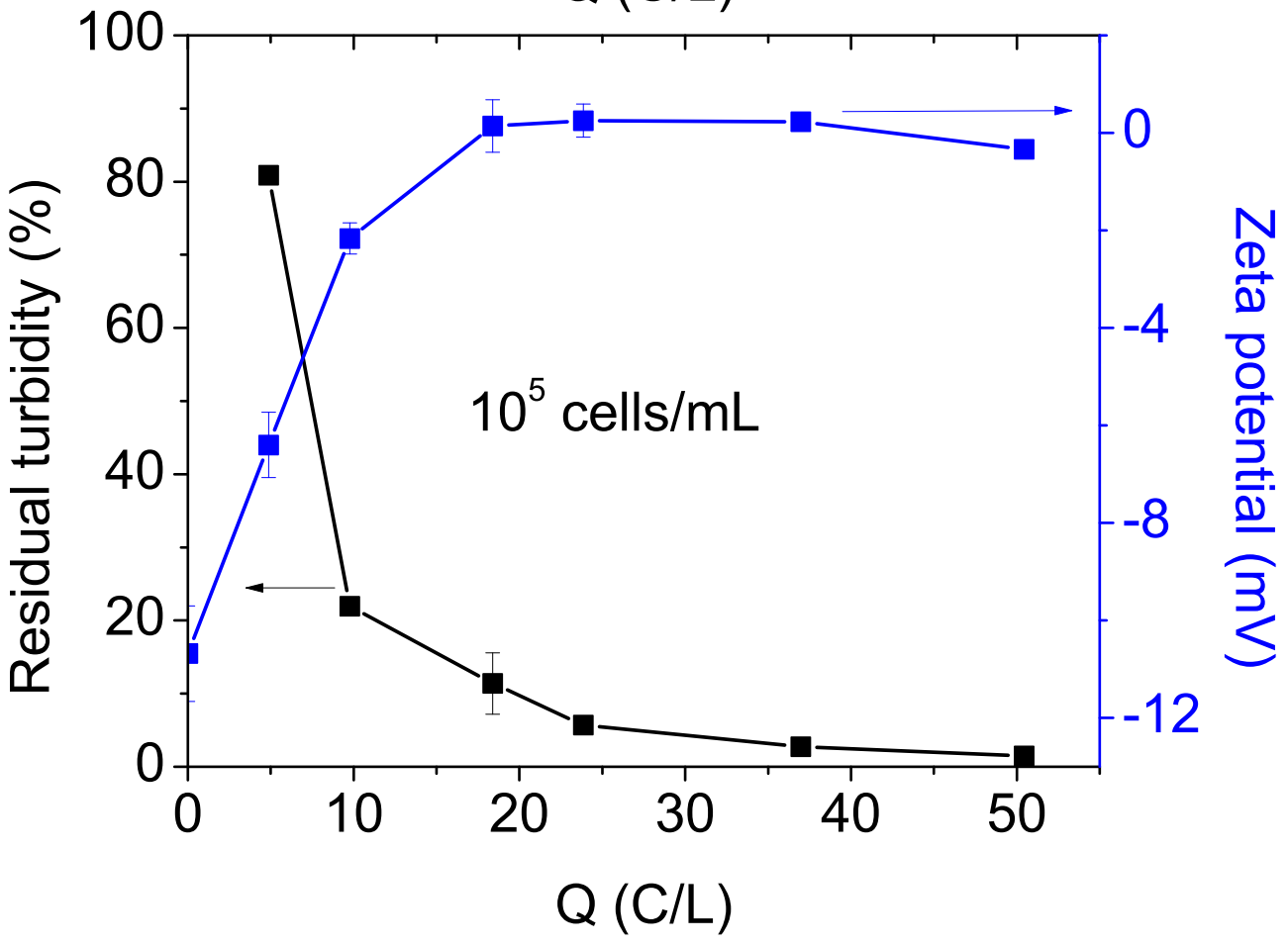

Residual turbidity (%)
Zeta potential (mV)
Q (C/L)
10^5 cells/mL
0
20
40
60
80
100
0
10
20
30
40
50
0
-4
-8
-12

Figure 4

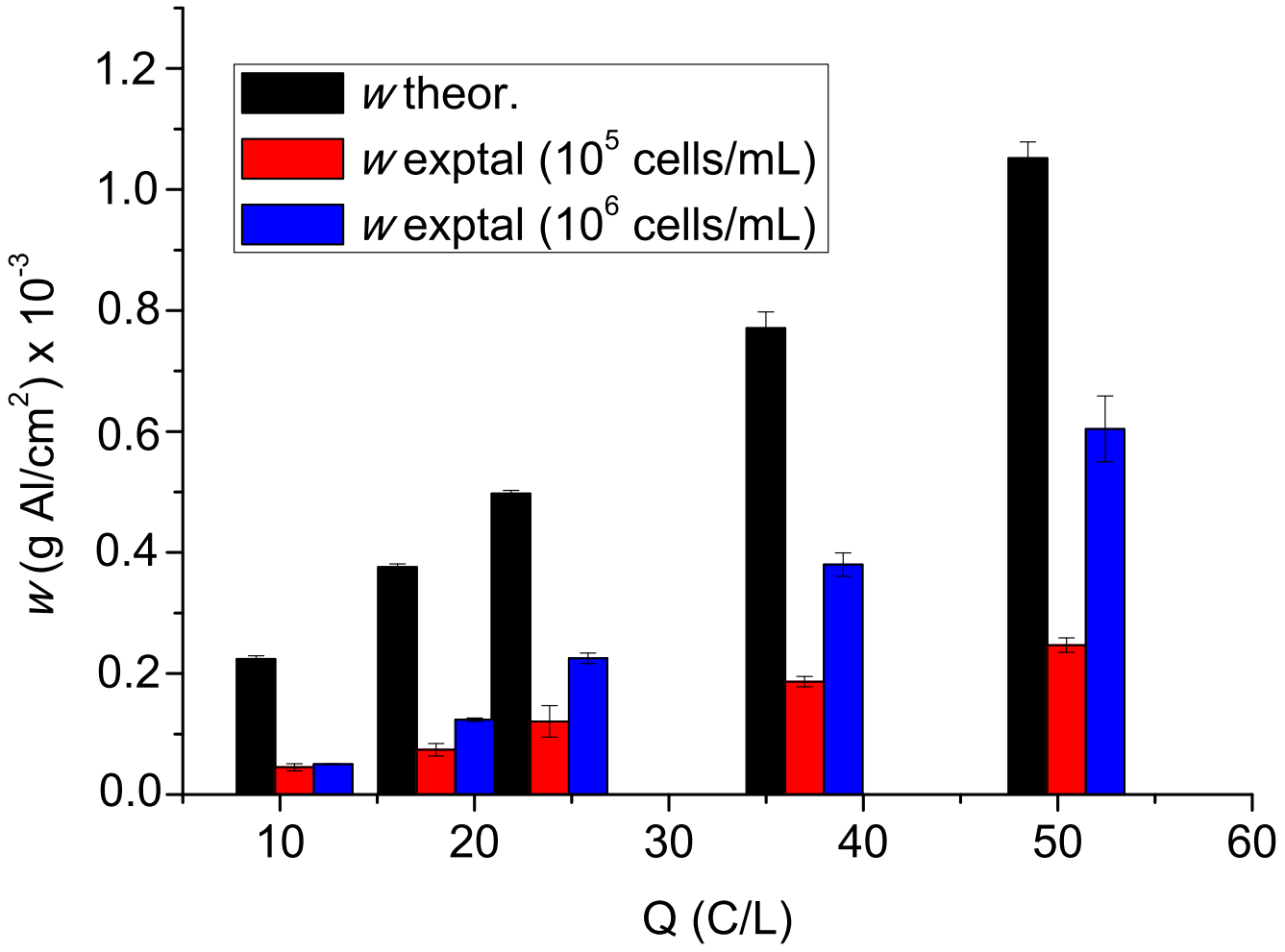

w theor.
w exptal (10^5 cells/mL)
w exptal (10^6 cells/mL)
w (g Al/cm^2) x 10^-3
1.2
1.0
0.8
0.6
0.4
0.2
0.0
10
20
30
40
50
60
Q (C/L)

Figure 5

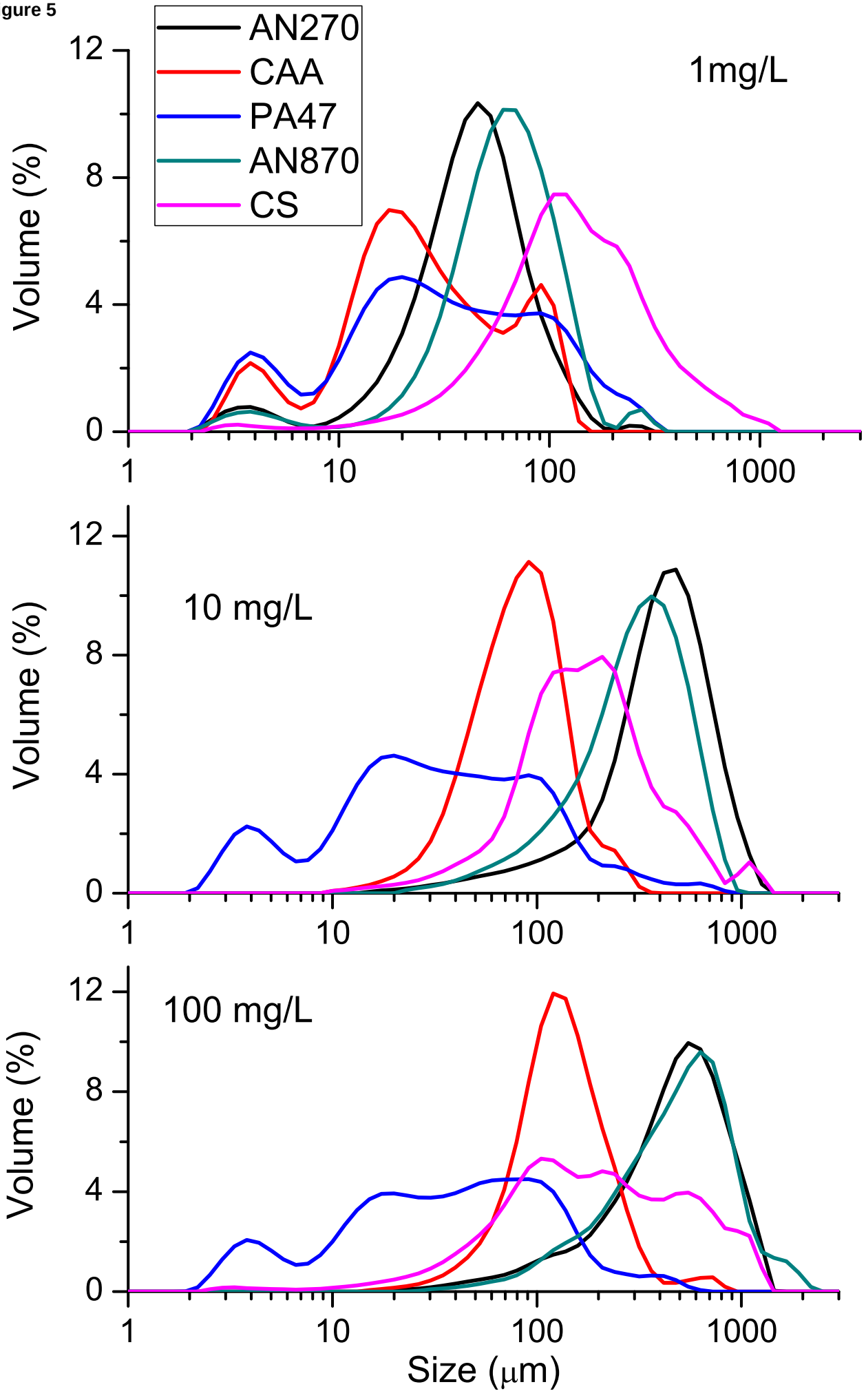
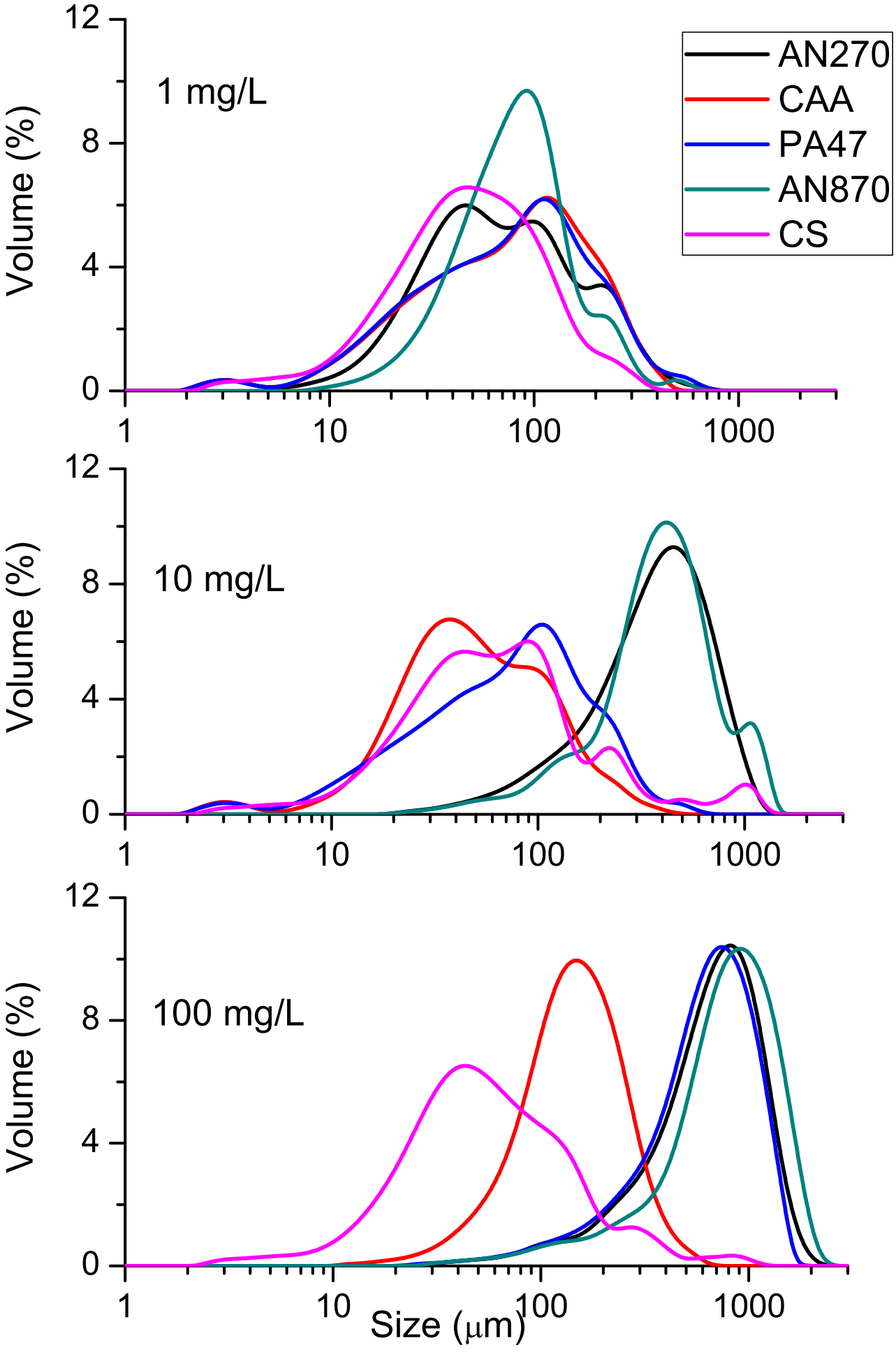

AN270
CAA
PA47
AN870
CS
1mg/L
10 mg/L
100 mg/L
Volume (%)
Size (μm)
0
4
8
12
1
10
100
1000

Figure 6


1 mg/L
10 mg/L
100 mg/L
AN270
CAA
PA47
AN870
CS
Volume (%)
Size (μm)
0
4
8
12
1
10
100
1000

Supplementary data

Table S1. Primers used for PCR amplification.

Target	Primer set	Sequence (5'-3')	DNA length	Reference
Generic	CYA359F CYA781 R	GGGGAATYTTCCGCAATGGG GACTACAGGGGTATCTAATCCCTTT	470 pb	Nübel et al. 1997
mcyB	mcyB2959F mcyB3278R	TGGGAAGATGTTCTTCAGGTATCCAA AGAGTGGAAACAATATGATAAGCTAC	320 pb	Nonneman & Zimba, 2002

Nonneman, D., Zimba, P. 2002. A PCR-based test to assess the potential for microcystin occurrence in channel catfish production ponds. J. Phycol. 38, 230-234.

Nübel, U., García-Piche, F., Muyzer, G. 1997. PCR primers to amplify 16s rRNA genes from cyanobacteria. Appl. Environ. Microbiol. 63, 3327-3332.

Table S2. Physico-chemical properties of the surface water used and after electrocoagulation (10 and 50 C/L charge loadings).

Parameter	Raw water	10 C/L	50 C/L
ORP (mV)	+174±8	-321±29	-339±35
Dissolved O_2 (g/L)	3.6±0.5	5.2±1.0	4.7±0.7
Conductivity (µS/cm)	1305±32	1049±14	841±83
pH	8.9±0.2	8.8±0.2	9.0±0.2
DQO (mg/L)	119±12	142±51	122±28
NO_3^- (mg/L)	29.5±1.6	13.1±2.1	5.8±0.5
Turbidity	45.9±2.2	71.9±8.5	224.7±17.0

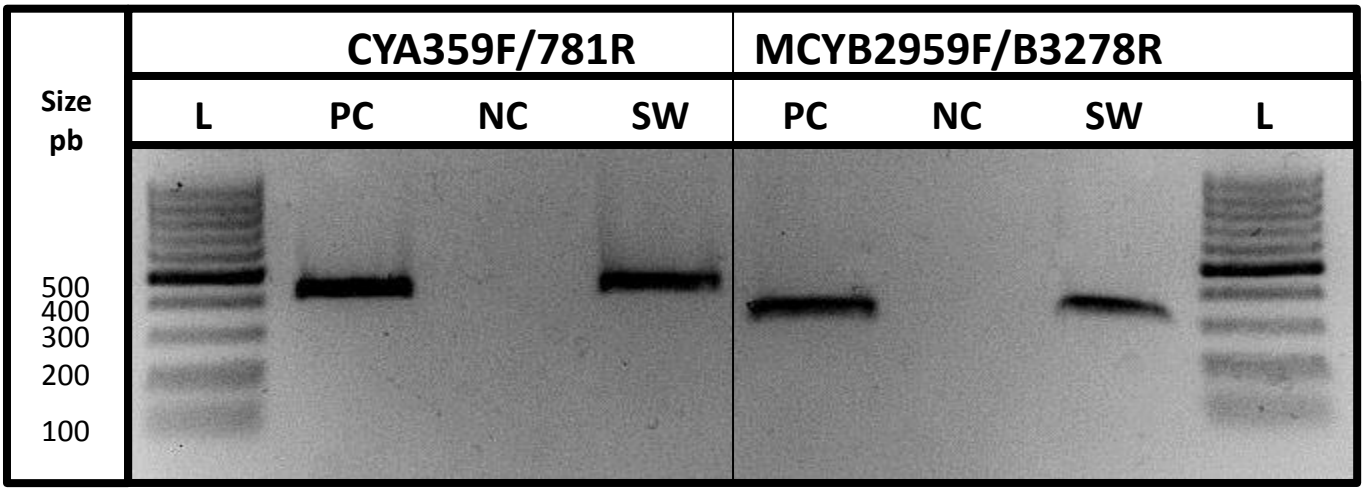


Figure S1. Negative image of an ethidium bromide-stained electrophoresis gel separation pattern of 6 PCR samples originated by amplification of cyanobacteria 16S rDNA with generic and *mcyB* specific primers in the surface water sample (SW). For 16S rDNA amplifications, a sample containing no DNA was used as the negative control (NC), and positive DNA extracted from an axenic culture of *M. aeruginosa* (PC) was used as positive control. L: DNA ladder, sizes of DNA ladder bands are indicated.