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Cyanobacteria removal by electroflotation with titanium electrodes

Tiago Burgardt[a,*], Thyara Martins Nonato[a], Fernando Hymnô de Souza[a], Benny Zuse Rousso[b,c], Maurício Luiz Sens[a]

[a]*Federal University of Santa Catarina, Postgraduate Program in Environmental Engineering, Campus Universitário UFSC/CTC Bairro Trindade – CEP 88040-900 Florianópolis – Santa Catarina/Brazil, Tel.: +55 (48) 996371771; emails: tiagoburg@me.com (T. Burgardt), thyaranonato@outlook.com (T.M. Nonato), fernando.hs@outlook.com (F.H. de Souza), mauricio.sens@ufsc.br (M.L. Sens)*
[b]*School of Engineering, Deakin University, 75 Pigdons Rd., Waurn Ponds, VIC 3216, Australia, email: benny.zuserousso@deakin.edu.au (B.Z. Rousso)*
[c]*Institute for Sustainable Futures, University of Technology Sydney, 235 Jones St., Ultimo, NSW 2007, Australia*

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ABSTRACT

Harmful cyanobacteria blooms are a major concern for water managers worldwide because they have negative environmental, social and economic impacts. This study aimed to evaluate cyanobacteria removal performance and water quality by electroflotation using titanium electrodes as a pre-treatment in water treatment plants (WTPs). Water quality, toxicity, cyanobacterial cell integrity and cyanotoxin analysis were performed. An experimental design was carried out to determine the optimum operational parameters (OOP) for cyanobacteria removal. The identified OOP were flow rate of 100.06 $m^3 \cdot m^{-2} \cdot d^{-1}$ and current density of 72.10 $A \cdot m^{-2}$, resulting in an energy consumption of 1.97 $kWh \cdot m^{-3}$. The reactor at OOP, operating for 6h, was able to remove 97% of cyanobacteria in a continuous flow reactor. Furthermore, dissolved organic carbon, turbidity, and apparent color decreased 41%, 55%, and 52%, respectively. Phytotoxicity analyses showed seed germination and radicle growth rates greater than 75% and hypocotyl growth inhibition rates from 3% to 22%. Roots showed no growth inhibition compared with the positive control. Cell permeability analysis revealed cell wall alterations in cyanobacteria after electroflotation; however, cyanotoxins were not detected in treated water. These findings indicate that electroflotation using titanium electrodes is a viable alternative as a pretreatment in WTPs.

Keywords: Electroflotation; Titanium electrodes; Water treatment; Pretreatment; Cyanobacteria; Cyanotoxins; Toxicity

1. Introduction

Population growth, increased industrialization, and inadequate waste disposal have negatively affected the quality of drinking water reservoirs. Intensification of such factors, has led to a growing occurrence of cyanobacterial harmful algal blooms (cyanoHABs) worldwide [1,2]. CyanoHABs have detrimental effects in water treatability from economic and technical points of view. In water treatment plants, mainly in conventional water treatment systems, in water treatment plants, the presence of large concentrations of cyanobacteria may lead to increased consumption of chemicals, and early clogging of filtration beds, which increases water treatment cost. Further, large concentration of cyanobacteria in raw water impact the treatability of other water quality parameters, overall reducing water treatment performance [3,4]. In addition to economic and operational issues, inadequate water treatment can result in the release of harmful intracellular toxins by cyanobacteria, resulting, thus, in technical challenges of how water treatment plans should respond to dynamic cyanoHABs. CyanoHABs also pose a risk to public health and other living organisms, with several reports

* Corresponding author.

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poisonings in human and non-human animals caused by ingestion of cyanobacterial toxins, as well as reports of growth inhibition in food crops irrigated with cyanotoxin-contaminated water [5–7].

Since conventional drinking water treatment has reduced performance for high density of cyanobacteria removal, the use of complementary pretreatments is necessary. For this reason, alternative pretreatment methods to optimize water treatment processes for cyanobacteria laden water have been widely investigated. Among those methods, electroflotation and associated techniques emerge as an alternative. Zhang et al. [8] proposed an electroflotation technique using boron-doped diamond and aluminum electrodes for the removal of *Microcystis aeruginosa*. The authors reported 100% algal removal after 30 min of operation at a current density of 2 $mA \cdot m^{-2}$ [8]. An et al. [9] used a Fenton electrocoagulation system to remove cyanobacteria, achieving 91% cell removal and 15% microcystin removal in 60 min of operation at a current rate of 100 mA. Ghernaout et al. [10] used stainless steel electrodes to remove microalgae. According to the authors, 100% of microalgal removal was achieved in 15 min at a current density of 170 $A \cdot m^{-2}$. Garcia et al. [11] designed an electroflotation technique using aluminum electrodes to remove cyanobacteria. The proposed method afforded 76.3% removal at a current density of 2 $A \cdot m^{-2}$. Nonato et al. [12] treated cyanobacteria-contaminated water by electroflotation using dimensionally stable anodes (DSA) composed of $Ti/Ru_{0.3}Ti_{0.7}O_2$. The authors reported 78% cyanobacteria removal in 60 min of operation at 68.26 $A \cdot m^{-2}$.

Despite its many advantages, electroflotation still has limited applicability because of the low durability of electrode materials. Studies demonstrated that DSA electrodes are particularly efficient in electroflotation for the removal of cyanobacteria and other contaminants, including cyanotoxins. DSA electrodes have high stability, durability, and the ability to produce bubbles measuring approximately 20 μm, which is advantageous for the removal of small contaminants [12–14].

Several studies investigated the efficiency of electroflotation methods in improving water physical and chemical parameters by using different types of electrodes. However, little is known about the effects of electroflotation on cyanobacteria after passage through the system. This study aimed to assess the potential of an electroflotation technique to remove cyanobacteria. Optimum design and operational parameters, besides treatment performance, were assessed through quantification of water physical and chemical parameters, and algal toxin concentrations and conduct a preliminary assessment of cell alterations in cyanobacteria during treatment.

2. Methods

2.1. Study site

This study was conducted in Lagoa do Peri, a water reservoir located in Florianópolis, Santa Catarina State, Brazil. The lagoon has a volume of approximately $21.2 \pm 0.1 \times 10^6$ m^3, average depth of 4.2 m, and maximum depth of 11 m. Lagoa do Peri is susceptible to recurrent filamentous *Cylindrospermopsis raciborskii* and *Pseudanabaena galeata* cyanoHABs, with concentrations ranging from 390,000 to 1,500,000 cells mL^{-1} [11,15,16]. *C. raciborskii* and *P. galeata* are known for their propensity to release toxins.

2.2. Sampling collection

Water samples were collected during the month of July 2021 in two collection campaigns. One of the campaigns was used to carry out preliminary tests to obtain the optimum operational parameters (OOP) for the pilot system. The other collection campaign was used to perform the pilot system. For each campaign, 1000 L of water were collected. Water were collected at the inlet of the Lagoa do Peri Water Treatment Plants following the procedures described by the American Public Health Association [17].

2.3. Pilot system

The experimental electrochemical reactor system consisted of a voltage stabilizer source (ICEL, PS-3010.0223) used to determine current density; a 1/2 HP centrifugal pump used to recirculate water; a metering pump (Grabe, DDM 130-07-PP/TF-1) used to control the inflow of raw water; hydraulic and electric systems; and two reservoirs with a capacity of 500 L each (input/output), as presented in Fig. 1.

2.4. Electrochemical reactor

The electrochemical reactor had an internal diameter of 115 mm and a volume of 2.08 L. Electrodes consisted of titanium cathodes and DSAs ($Ti/Ru_{0.3}Ti_{0.7}O_2$). A total of 10 electrodes were mounted in parallel (5 cathodes and 5 anodes intercalated), with a total effective area of 785 cm^2, and 0.8 cm between electrodes.

2.5. Experimental design and optimum operational parameters

To obtain the OOP, an experimental design (ED) was carried out varying two operational parameters (inflow rate and current density) – independent variables to obtain the best rate of cyanobacterial removal – dependent variable. In this sense, several current density and inflow rate configurations were used. The maximum values of independent variables were based on the maximum operating conditions of the equipment used: maximum dosing pump capacity of 130 $L \cdot h^{-1}$ and maximum allowable current intensity of 13.0 A per anode, that is, the maximum current density that the anodes are able to conduct. The ED included three replications of the center point and four axial points, totaling 11 runs. In this step, all runs were conducted for 90 min at ambient temperature (25°C). Samples of 100 mL were collected at the end of 90 min of each configuration used. An ED matrix containing the values of independent variables is presented in Table 1.

The ED was performed through Central Composite Rotational Design (CCRD) 2^2 in triplicate at the central point and four axial points (–1,68, –1, 0, +1, +1,68). The results obtained in the ED were statistically analyzed using analysis of variance (ANOVA).

2.6. Pilot system operational performance

After obtaining the OOP through ED and validation of the proposed model, the pilot system was performed with the

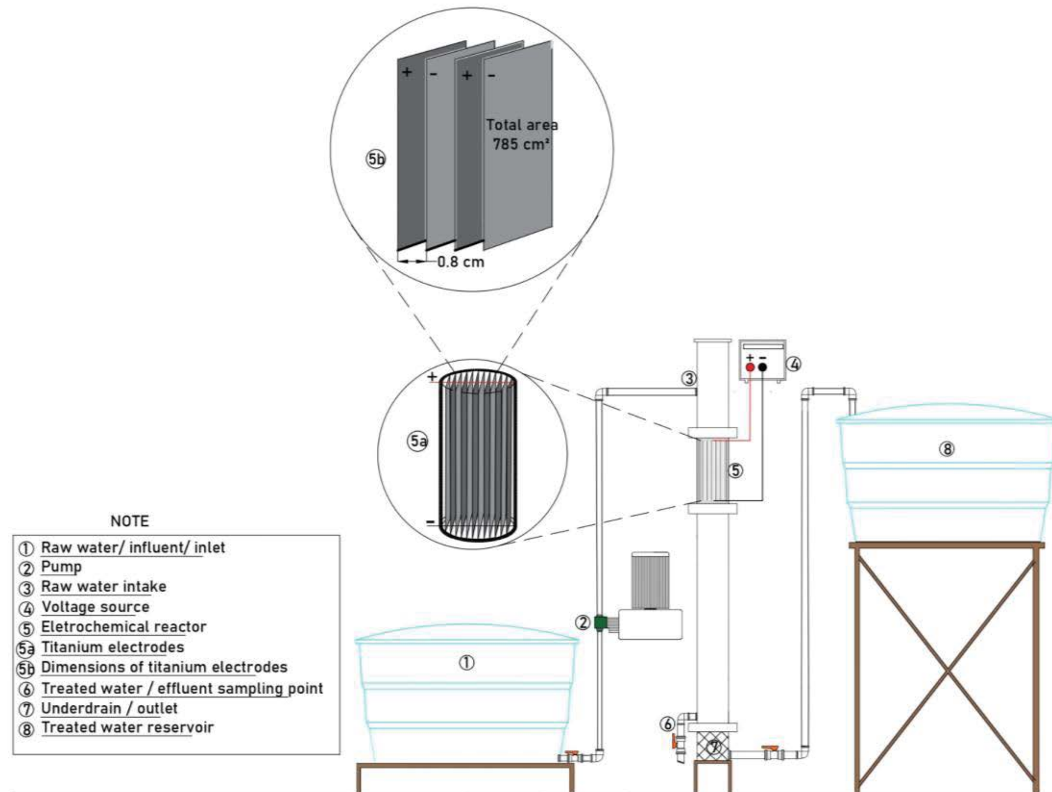

Fig. 1. Schematic diagram depicting dimensionally stable anodes electrodes and structural components of the experimental electroflotation system.

Table 1
Experimental design matrix describing values independent variables and experimental conditions with three replications of center point runs and four axial points

Run	Experimental conditions	
	q_1	q_2
1	130	38.21
2	70	101.91
3	130	101.91
4	49.6	70.06
5	150.4	70.06
6	100	16.56
7	100	125.56
8	100	70.06
9	100	70.06
10	100	70.06
11	130	38.21

Note: q_1, inflow rate ($m^3 \cdot m^{-2} \cdot d^{-1}$); q_2, current density ($A \cdot m^{-2}$).

best experimental conditions. In this sense, electroflotation was performed for 6 h under the optimum conditions identified. Raw and treated water samples (100 mL) were collected every 1 h and analyzed for physical and chemical parameters and other analysis that will be presented in the methods, according to ASTM methods, as described in Table 2.

2.7. Cyanobacterial count

For analysis of cyanobacteria and filaments, water samples were preserved in 2% Lugol iodine solution (3–5 drops of Lugol solution to every 10 mL of sample). A 1 mL aliquot was placed in a Sedgewick–Rafter counting chamber and observed under an Olympus BX40 optical microscope at 200 × magnification. Ten subsections of the chamber (1 μL) were randomly counted and used to calculate the total number of individuals in all subsections (1,000 μL). Results are reported in number of individuals per milliliter (ind mL^{-1}). Cell number was estimated based on the mean number of *C. raciborskii* cells detected, given that this species was the predominant type of filamentous cyanobacteria in the samples [16].

For analysis of filament and cell lengths, samples were observed at 200× (filaments) and 1,000× (cells) magnification. Images were acquired using an Olympus BX40 microscope with QCapture Pro 6.0 software. Then, cells and filaments lengths, respectively, were measured using ImageJ software (v. 1.52i). The mean number of cells was calculated from the ratio of average filament length to average cell size.

2.8. Phytotoxicity assay

The toxicity of water produced by the pilot system was analyzed using *Lactuca sativa* seeds [18]. Phytotoxicity assays provide insight into acute (germination index) and chronic (root and hypocotyl growth) effects of toxic compounds

Table 2
Analytical methods and equipment used for determination of physical and chemical parameters

Parameter	Analytical method	Equipment
Dissolved organic carbon	5310 B (APHA, 2012)	Shimadzu TOC-5000A Analyzer
Turbidity	2130 B (APHA, 2012)	Hach 2100P Turbidimeter
Apparent color	2120 C (APHA, 2012)	Hach DR 2800 Spectrophotometer
Cyanotoxins (microcystin, cylindrospermopsin, and saxitoxin)	EPA 815-R-15-009	Agilent G6410A LC/MS-MS System
Cyanobacterial count	10900 C (APHA, 2012)	Optical Microscope and Sedgewick–Rafter Chamber
pH	4500 H+ (APHA, 2012)	Hach HQ40D Multimeter
Temperature	2550 B (APHA, 2012)	Hach HQ40D Multimeter

on seeds during germination and initial development. Autoclaved borosilicate Petri dishes (5 cm diameter) were lined with Whatman No. 1 filter paper, which served as a support for seed fixation. Each filter paper was saturated with 3 mL of raw or treated water, and plates were sealed to retain moisture. After preparation, plates were incubated for 120 h at 25°C in the absence of light. This procedure was carried out using inlet and outlet triplicate water samples collected every 1 h until the end of 6 h operation. Germination was assessed as percentage of radicle emergence in relation to the positive control, calculated using Eq. (1). Root [Eq. (2)] and hypocotyl [Eq. (3)] growth inhibition percentages were determined by comparison of radicle and hypocotyl lengths with the positive control.

$$\text{Germination percentage} = \frac{\text{Number of germinated seeds}}{\text{Number of seeds}} \times 100 \quad (1)$$

$$\text{RHGI} = \frac{\text{MHL}_c - \text{MHL}_s}{\text{MHL}_c} \times 100 \quad (2)$$

$$\text{RRGI} = \frac{\text{MRL}_c - \text{MRL}_c}{\text{MRL}_c} \times 100 \quad (3)$$

where RHGI is the relative hypocotyl growth inhibition (%), MHL_c is the mean hypocotyl length of the positive control, MHL_s is the mean hypocotyl length of the sample, RRGI is the relative radicle growth inhibition (%), MRL_c is the mean radicle length of the positive control, and MRL_s is the mean radicle length of the sample.

Germination was assessed on 60 seeds per treatment (raw and treated water samples collected after 0, 60, 120, 180, 240, 300, and 360 min of operation) in triplicate. Seeds with a visible radicle were considered germinated.

2.9. Optical microscopy and cell viability analysis

Samples were subjected to different pre-preparation methods to choose the best conditions for staining with LIVE/DEAD BacLight Bacterial Viability Kit (BVK) (Invitrogen). Regardless of the pre-preparation method used, raw water samples exhibited sufficient cellular material for analysis. The minimum cell count for *C. raciborskii* observation was 30 filaments [19]. For treated samples, centrifugation was used to obtain sufficient cellular concentration for visualization (55 times). After centrifugation, samples were prepared according to the method of de Souza et al. [16], and 0.1 mL aliquots were mounted on glass slides with coverslips for visualization [16]. After staining with BVK, samples were observed under the WG cube (green emission) with red filter and FITC/Texas red (blue emission) with green and red filters (simultaneously). The natural fluorescence of cyanobacteria and microalgae was also tested, given that chlorophyll has natural fluorescence [19]. For this, samples were observed using the WG cube with red filter.

BVK contains the DNA markers SYTO 9 (green) and propidium iodide (red), which have different permeabilities in bacterial cells. SYTO 9, having the ability to penetrate cells, binds to DNA, causing bacteria to be stained green. Propidium iodide, by contrast, is not permeable, only being able to bind to the DNA of cells with damaged walls. Thus, viable bacteria (those with intact cell walls) are stained green (SYTO 9), whereas unviable cells (those with damaged walls) are stained green (SYTO 9) and red (propidium iodide). The BVK kit was successfully used to assess *C. raciborskii* cell integrity [19–21]. Raw water, treated water samples after electroflotation at 30 min (E30) and 180 min (E180), and flocs were analyzed microscopically.

3. Results and discussion

3.1. Experimental design and statistical analysis

The ED, variables, configurations and results are described in Table 3. Raw water used in the ED had a cell density of 4.5 × 10^5 cells·mL^{-1}. Cyanobacteria removal ranged from 25.14% to 68.54%, according to variables and their levels.

Experimental results were analyzed statistically, and the following model was proposed [Eq. (4)]:

$$R = 66.95 + 0.49X_1 - 14.83X_2 + 1.63X_1^2 - 12.56X_2^2 - 6.63X_1X_2 \quad (4)$$

where R is the percentage of cyanobacteria removal, X_1 is the water inflow rate (m^3·m^{-2}·d^{-1}), X_2 is the electric current density (A·m^{-2}), and X_1X_2 is the interaction between factors.

Table 3
Central composite rotatable design matrix with three replicates of the center point and four axial points for optimization of cyanobacteria removal (%) from water by a 90 min electroflotation process

Run	Inflow rate ($m^3·m^{-2}·d^{-1}$)	Current density ($A·m^{-2}$)	Removal (%)
1	70	38.21	29.81
2	130	38.21	43.35
3	70	101.91	45.32
4	130	101.91	32.33
5	49.6	70.06	25.14
6	150.4	70.06	27.65
7	100	16.56	29.42
8	100	125.56	33.45
9	100	70.06	67.89
10	100	70.06	68.54
11	100	70.06	66.71

Statistical analysis revealed an adequate fit of the model to experimental data, with R^2 = 0.99 (Table 4). ANOVA results are described in Table 4.

ANOVA results presents that the predicted model is valid at 95% confidence interval (Table 4). The *F*-value (111.82) was greater than the *F*-critical (5.05), demonstrating that the model adequately predicts the data. The residual value (26.20) was low compared with the regression value (2,930.27), and the pure error was 1.72 (Fig. 2).

Optimal removal efficiency was estimated to be achieved using the following operating conditions: water inflow flow rate of 100.06 $m^3·m^{-2}·d^{-1}$ and current density of 72.10 $A·m^{-2}$ (Fig. 3).

Current density values higher than those used in center point runs did not exert significant changes in cyanobacteria removal efficiency (Fig. 3). Thus, increasing the current density above the center point resulted in an increase in power consumption but not in removal efficiency. Values below 72.10 $A·m^{-2}$, however, led to a reduction in removal efficiency. This finding can be explained by the large amount of chlorine bubbles produced, which induce coalescence, a phenomenon that negatively affects removal efficiency [10,22,23].

3.2. Physical and chemical analyses

3.2.1. Cyanobacteria removal and size

Raw water had a cell density of 4.5 × 10^5 cells·mL^{-1}. The highest removal rates were observed in the first 2 h of operation, with a mean removal of 70% (3.15 × 10^5 cells·mL^{-1}) (Fig. 4). A similar pattern was observed in the run that afforded the highest removal rate. After 6 h of operation, the system showed a total removal of 97% (4.36 × 10^5 cells·mL^{-1}). These findings agree with those of previous studies using the electroflotation technique, which achieved removal efficiencies between 70% to 100% [8–12]. In the referred studies, the authors used electrical density rates of 0.002–170 $A·m^{-2}$.

The electroflotation technique is efficient for cyanobacteria removal, given that, in aqueous medium, cyanobacteria behave as colloidal particles. Therefore, application of electric charges leads to particle stabilization. Formation of oxygen and hydrogen bubbles on electrodes further contributes to cyanobacteria removal because of the size and physical characteristics of these microorganisms, affording efficient separation from the liquid medium [11].

Filament size reduce during operation. At first, raw water had a predominance of medium- (37%) and large-sized (34%) individuals. Over time, there was a gradual increase

Table 4
Analysis of variance (p < 0.05) for a regression model predicting cyanobacteria removal from water by electroflotation

Source of variation	Sum of squares	Degrees of freedom	Mean squares	F	$F_{critical}$	p
Regression	2,930.27	5	586.05	111.82	5.05	0.05
Residual	26.20	5	5.24			
Lack of fit	24.49	3	8.16			
Pure error	1.72	2	0.86			
Total	2,956.47	10				

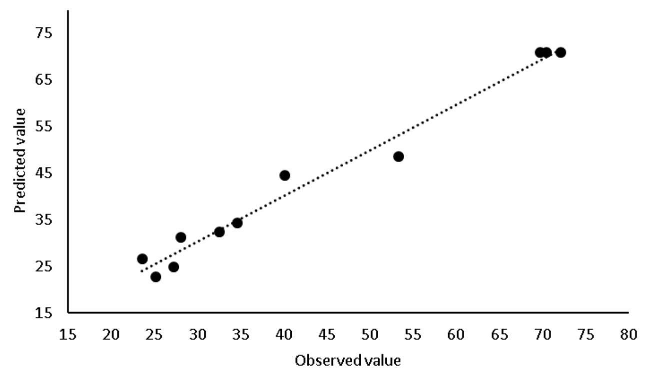

Fig. 2. Correlation between observed and predicted values of cyanobacteria removal (%) from water by electroflotation.

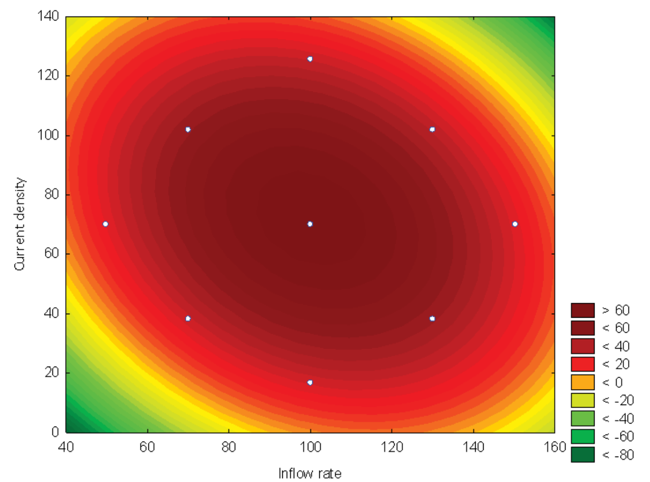

Fig. 3. Response surface plot showing the optimum conditions of independent variables for cyanobacteria removal (%) from water by electroflotation.

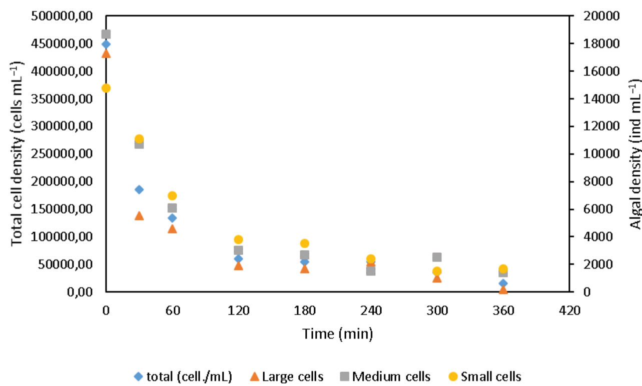

Fig. 4. Cyanobacteria removal from water during 6 h of electroflotation. The main vertical axis represents the total removal of cyanobacteria cells (blue diamond) during the 360 min of operation, while the secondary axis represents the removal of individuals (square, circle and triangle) classified by size during the 360 min of operation.

in the proportion of medium- and small-sized individuals. At the end of the 6 h of operation, medium (42%) and small (52%) individuals predominated. This finding may be due to the fact that, throughout electrolysis, larger filaments are ruptured, resulting in smaller filaments. Another hypothesis is that larger filaments are removed with greater efficiency. One of the concerns regarding filament breakage is the release of intracellular toxins, a topic that will be addressed below [11].

3.2.2. Turbidity and apparent color

Raw water showed initial turbidity and apparent color of 12.51 NTU and 179 uH, respectively. Over the 6 h of treatment, a 55% reduction in turbidity (5.61 NTU) and a 52% reduction in apparent color (86 uH) were achieved. The highest reductions in these parameters occurred within 180 min of operation, with little variation thereafter, as depicted in Fig. 5. Previous studies presented similar results using electroflotation, with 50%–90% removal of turbidity and 45%–92.5% removal of color [11,12,24,25]. In the referred studies, treatment was carried out using current densities of 2–915 $A \cdot m^{-2}$.

3.2.3. Dissolved organic carbon

Raw water had a dissolved organic carbon (DOC) of 11 $mg \cdot L^{-1}$. Following treatment, the DOC was 6.11 $mg \cdot L^{-1}$, representing a removal of 41%. The highest DOC removal was achieved at the end of treatment (360 min) (Fig. 6). Hakizimana et al. [26] achieved DOC removals of 29.0%–63.1% by electroflotation with current densities of 20–200 $A \cdot m^{-2}$ and flow rates of 0.25–1.2 L min^{-1}. Ahmed et al. [27], by using a current density of 19.3 $A \cdot m^{-2}$ and flow rates of 2.72–3.33 10^{-3} $m^3 \cdot h^{-1}$, achieved a DOC removal of about 50%.

DOC removal by electroflotation can be explained by the formation of metal hydroxides that adsorb organic matter and/or neutralize the organic matter charge of water. Hydrogen bubbles formed during the process positively influence hydrodynamics, increasing mass transfer and collision rates between hydroxide species and organic matter [26]. The electric current further contributes to removal by promoting oxidation of organic matter [26].

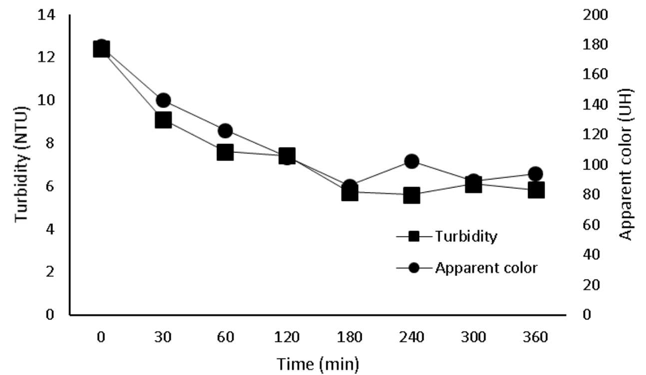

Fig. 5. Turbidity and apparent color removal from water during 360 min of electroflotation.

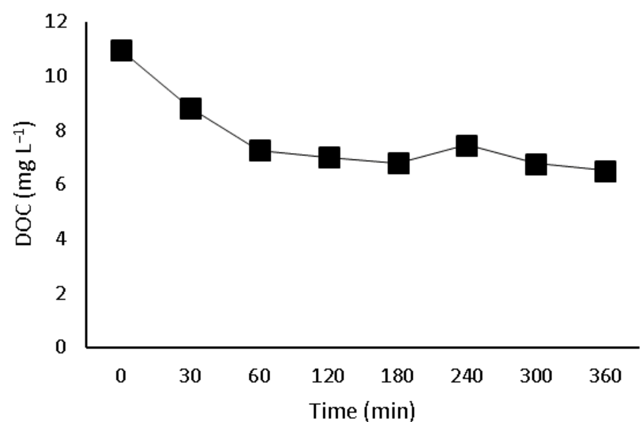

Fig. 6. Dissolved organic carbon removal from water during 360 min of electroflotation.

3.2.4. pH and temperature

As demonstrated in Fig. 7, the pH of the liquid medium increased from 7 to 7.6 during the 360 min of treatment under a fixed current density. The variation in pH during electroflotation depends on the formation of products at the cathode and anode. Hydrogen gas is generated at the cathode, and the pH increases due to the formation of hydroxyl anions (OH^-). In turn, at the anode, H^+ species are formed, resulting in a reduction in pH. Given that the formation of hydroxyl ions surpasses that of H^+, the pH of the solution increases after electroflotation [22].

With the current density fixed at 72.10 $A \cdot m^{-2}$, the temperature increased from 26°C to 27.5°C likely due to energy dissipation into the aqueous medium via the Joule effect. Heating is explained by friction of molecules during agitation, formed by the movement of charged molecules in the material, and the transfer of energy to the aqueous medium [28,29].

3.2.5. Electroflotation time effect on parameters

As shown in previous results, the removal performance of the system varies with the electroflotation operating time. Therefore, the operating time must be considered as a key variable when assessing the performance of the electroflotation in a continuous flow reactor. With the application of the OOP – inlet water flow rate of 100.06 $m^3·m^{-2}·d^{-1}$ and current density of 72.10 $A·m^{-2}$, the system required 120 min to stabilize and reach its optimal removal conditions. The best removal performances for most parameters occurred between 120 and 180 min of operation. After that time, removal performance either slightly decreased for turbidity and apparent color, slightly increased for cyanobacteria cell counts, or showed no incremental increases for DOC. This is due to the complexity of the continuous flow interactions with the electroflocculation mechanisms, which increased efficiency of bubble and floc formation and foam accumulation over time. One of the possible causes for the decrease in the system's performance after this time has been associated with supernatant foam formation during operation [10]. Thus, it is recommended to remove the supernatant material to minimize performance loss during prolonged operation. The temporal performance of the system, in terms of all evaluated parameters, is depicted in Fig. 8.

3.3. Optical microscopy and cell viability analysis

Raw water, treated water, and flocs were analyzed microscopically. All samples contained diatoms and cyanobacteria (Fig. 9α). In raw water (Fig. 9Aγ), microorganisms showed natural fluorescence in cells due to the presence of chlorophyll [19]. However, this was not observed in treated samples after electroflotation at 30 min (E30) and 180 min (E180) (Fig. 9Bγ), indicating that electroflotation altered pigmentation.

Fluorescence was observed in raw water samples labeled with BVK (Fig. 9Aβ). Visualization under FITC/Texas red and WG filters produced different results for both *C. raciborskii* and *Aulacoseira* sp. The fluorescence of raw water stained with BVK indicated the presence of other bacteria.

The fluorescence of treated samples E30 and E180 (Fig. 9B) differed from that of raw water (Fig. 9A).

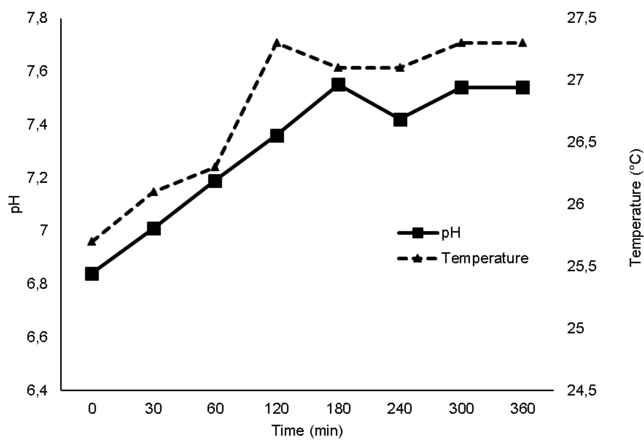

Fig. 7. Water pH and temperature during 360 min of electroflotation.

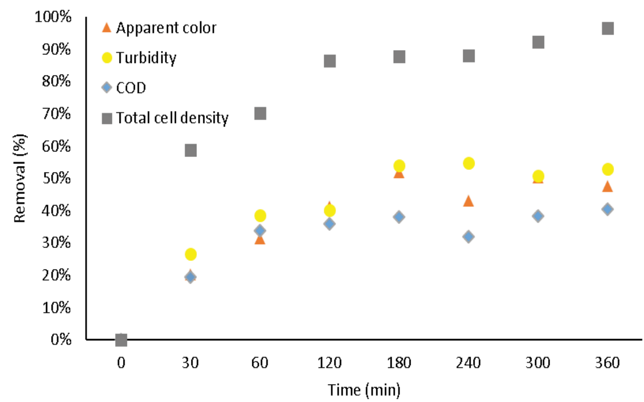

Fig. 8. Turbidity, apparent color, chemical oxygen demand and total cell density removal throughout the 360 min of the electroflotation process.

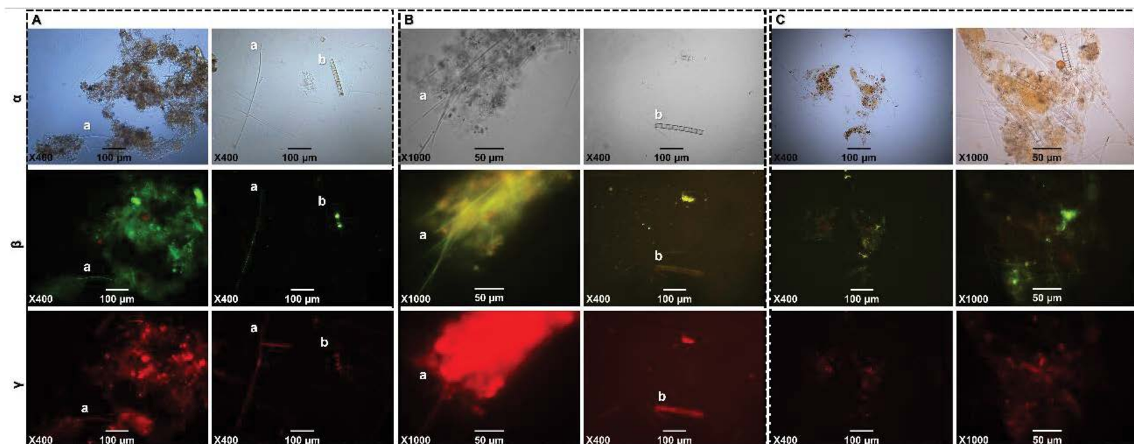

Fig. 9. Optical microscopy images of (A) raw water, (B) electroflotated water, and (C) flocs. Legend: α, bright field; β, Bacterial Viability Kit with FITC/Texas red (blue emission) and simultaneous use of green and red filters; γ, WG cube (green emission) with red filter; (a) *Cylindrospermopsis raciborskii* and (b) *Aulacoseira* sp.

C. raciborskii was identified by a yellowish color in treated samples; filaments had a lower intensity and different distribution than in the raw water sample. Raw water samples analyzed under the WG filter also exhibited a different fluorescence pattern than treated samples. The yellowish tones of treated samples result from the mixture of green and red fluorescence, without one overlapping the other (Fig. 9Bβ). This finding indicates that cell integrity was affected, given that propidium iodide was able to penetrate cells [19–21].

In treated samples, the nucleus of *Aulacoseira* sp. (Fig. 9Bβ, b) was no longer defined by SYTO 9 (green), as was observed in raw water (Fig. 9Aβ,b). Treated samples also lacked areas of chlorophyll accumulation (Fig. 9Aγ,b), demonstrating that DNA and chlorophyll were scattered in *Aulacoseira* sp. cells after electroflotation.

Sludge exhibited a mixture of the characteristics of raw water and treated samples (E0 and E3); however, yellowish tones predominated (Fig. 9Cβ). It was also possible to observe long filaments in flocs present in the sludge (Fig. 9Cα), corroborating that larger filaments were removed at higher rates, even after stabilization (120 min) (Fig. 4).

3.4. Cyanotoxins (microcystin, cylindrospermopsin, and saxitoxin) in treated water

The breakage of filaments and consequent exposure of cells to oxidative stress and electric current can lead to the release of intracellular toxins [10,30,31]. We assessed the presence of microcystin, cylindrospermopsin, and saxitoxin in water treated by the pilot system. For this, raw water and water treated for 2 h and 6 h were evaluated in triplicate. This analysis time was chosen because the highest removal rate and change in cell size were observed within 120 min of treatment, as shown in Fig. 4. However, none of the samples contained cyanotoxins.

It can be said that, despite the change in filament size (Fig. 4) and disruption of cell wall integrity (Fig. 9), no traces of cyanotoxins were found in treated water, which can be attributed to the fact that electroflotation causes cell inactivation [34]. Another hypothesis is that, if intracellular toxins were released to aqueous medium, they were fully mineralized or converted into byproducts via oxidation, particularly by the action of OH^- [32,33].

3.5. Phytotoxicity

Cyanotoxins may impair terrestrial plant development by inhibiting protein phosphatases 1 and 2A, causing oxidative stress, reducing photosynthetic activity, and promoting cell apoptosis [5,34]. The toxicity assay showed that raw water provided an absolute germination percentage of 80%. The highest percentage of seed germination (90%) occurred with water samples treated for 120 min. For samples treated for 360 min, the germination percentage was like that of raw water (Table 5), thus indicating that electroflotation did not lead to an increase in toxicity.

Overall, the findings demonstrate that absolute germination was not affected by electroflotation. Germination rates greater than 80% were achieved, except with E3. According to Young et al., a germination of 80% is the minimum threshold to disregard any possible negative effects on germination [35].

Table 5
Germination percentage, radicle growth rate, and hypocotyl growth rate of *Lactuca sativa* seeds grown in water treated by electroflotation

Sample	Germination rate (%)	Standard deviation	Inhibition hypocotyl growth rate (%)
CP	95	0.75	–
AB	80	1.16	4.74
E0	85	2.20	15.33
E1	90	1.83	5.47
E2	80	1.47	2.55
E3	75	0.81	10.58
E4	80	0.75	21.90
E5	80	1.50	14.96
E6	85	1.86	19.34

The hypocotyl length of the positive control was 27.4 mm (standard deviation of 0.73). Inhibition of hypocotyl development was low compared with the control, ranging from 3% to 22% in E2 and E4, respectively. The mean hypocotyl inhibition was 12%, with a standard deviation of 0.66 to 1.14. Regarding radicle development, the radicle length of the positive control was 20.13 mm, with a standard deviation of 1.17. Root development was not inhibited by treatment.

4. Conclusion

Statistical analysis of experimental data allowed determining optimal operating parameters for cyanobacteria removal by electroflotation. The optimized method was tested over 6 h, resulting in maximum cyanobacteria cell removal of 97% with an energy consumption of 1.97 $kWh \cdot m^{-3}$. It can be seen that the removal performance varied during the operational system time and showed the best removal performances between 120 and 180 min of system operation for the evaluated parameters. Throughout treatment, there was a reduction in filament size, indicating filament breakage or more efficient removal of larger filaments. Cell integrity assessments revealed changes in the cell wall of microorganisms. Treated water was found not to be toxic, lacking traces of cyanotoxins. Therefore, the technique proved to be efficient for removing cyanobacteria without affecting treated water quality under the operating conditions of the pilot system and the characteristics of the raw water source used in this study. Future studies should focus on different concentrations of cyanobacteria to assess whether the electroflotation technique is feasible with different concentrations and formation and identification of possible by-products originated during the electroflotation treatment since the formation of toxic by-products, in general, was not observed in this study, nor the release of cyanotoxins.

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