

Full Length Research Paper

Potential of *Opuntia stricta* Haw (Mexican elephant ear) in removing cyanobacteria in surface water

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In this study, assays were carried out to evaluate the efficiency of cactus *Opuntia stricta* cladodes as a coagulant for removal of cyanobacteria cells. To carry out the coagulation assays, water from eutrophic reservoir Bodocongó, in Brazilian semi-arid, with cyanobacteria bloom of *Microcystis aeruginosa*, *Plankthotrix isothrix* and *Cylindrospermopsis raciborskii* were sampled. This water was submitted to coagulation with different forms of *O. stricta* cladodes (crude, pulverized and solution), concentration (10; 50 and 100 mg/L) and time (5, 15, 30, 60 and 120 min). In order to assess cell removal, readings were made in aliquots of 5 mL of samples analyzed by triplicates counting on sedimentation chambers using an inverted optical microscope, according to the Utermöhl method. Dosage of 100 mg/L from the solution form of *O. stricta* cladodes gave the best cell and turbidity removal. Colonial species were fast removed than filaments. The most removal occurred in the first 30 min of experiments. No significant differences were observed for pH changes in the experiments. *O. stricta* cladodes gave satisfactory results in reducing cyanobacteria cell in water; however, complete removal was not obtained; further studies are necessary to evaluate the best concentrations and the mechanisms of cyanobacteria removal.

Key words: Eutrophication, biobased coagulants, Cactus.

INTRODUCTION

Eutrophication is considered the Earth's most important water quality problem (Schindler, 2012). It often results in blooms of potentially toxic cyanobacteria that complicate the use of lakes and reservoirs and can cause potential public health risk (Azevedo et al., 2002; Huisman et al., 2018). Cyanobacteria have adapted to survive in a variety of aquatic and terrestrial environments and have been found globally (Rigosi et al., 2014). Harmful cyanobacteria have been increasingly gaining the attention of scientists and government agencies because

they are known to produce various bioactive compounds, and some of them show beneficial therapeutic effects; thus, used as dietary supplements as well as mood enhancers (Jensen et al., 2001). Other cyanobacteria can produce cyanotoxins, which are harmful to humans, animals, and plants and fall into five different types of toxins: (i) hepatotoxins (cylindrospermopsin, microcystins and nodularins); (ii) neurotoxins (anatoxin-a and saxitoxins); (iii) dermatotoxins; (iv) cytotoxins; (v) and irritant toxins [lipopolysaccharides (LPSs)] (Graham et al.,

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2010).

Cyanotoxin can be accumulated into aquatic products via contaminated feeds, direct contact with contaminated water (living environment) and biomagnification through the food web (Ibelings and Chorus, 2007; Vasconcelos et al., 2013). Plants are not usually killed by the environmentally relevant concentration, but their growth and crop yields are affected (Babica et al., 2006; Bittencourt-Oliveira et al., 2016; Svirčev et al., 2017). In addition, the soil may retain toxins when extra water flows through, and it can bioaccumulate toxins during non-bloom seasons (Pflugmacher et al., 2007; Corbel et al., 2014). Humans are exposed to cyanotoxin through drinking water, inhalation, dermal contact and foods (Carmichael et al., 2001; Cheung et al., 2013; Lee et al., 2017).

Thus, controlling eutrophication and mitigating potential toxic cyanobacteria is an essential task (Lürling et al., 2016). Conventional chemicals, like algicides are used to remove cyanobacterial blooms but their application may come with shortcomings such as toxins and nutrient release or unwanted ecotoxicological side effects (Jančula and Maršálek, 2011; Merel et al., 2013). Removal of cyanobacteria from the water column using a combination of coagulant and ballast are a promising technique for controlling cyanobacterial bloom. The flock and lock and flock and sink techniques remove cyanobacteria out of the water column while remaining as intact cells where after the cyanobacteria and their toxins can be degraded on the sediment (Pan et al., 2011; Noyma et al., 2016; Magalhães et al., 2017). For this, the use of local soils, clays or waste products can be a faster, cheaper and easy to handle alternative.

In this perspective, natural plant-based coagulants have been tested (Miller et al., 2008; Nishi et al., 2011; Camacho et al., 2017). The main advantages of using this for water treatment are apparent; they are cost-effective, unlikely to produce treated water with extreme pH and highly biodegradable (Daza et al., 2016). These advantages are especially augmented if the plant from which the coagulant is extracted is indigenous to a rural community (Yin, 2010). In the era of climate change, depletion of the earth's natural resources and widespread environmental degradation, the application of these coagulants is a vital effort, aligned with global sustainable development initiatives.

Application of cacti species for water treatment is rather recent compared to other natural coagulants such as common bean (*Phaseolus vulgaris*) (Antov et al., 2010) nirmali seed (*Strychnos potatorum*) (Babu and Chaudhuri, 2005) or *Moringa oleífera* (Muthuraman and Sasikala, 2014; Oladoja and Pan, 2015). The most commonly studied cactus genus for water treatment is *Opuntia*, which represents one of the most diverse and distributed genera of plants (Zhang et al., 2006; Miller et al., 2008; Ortiz et al., 2013; Oladoja, 2015). It has since been introduced all over the world and can be found in

temperate, subtropical and tropical regions (Izuegbuna et al., 2019). Besides *Opuntia*, other cactus species including *Cactus latifaria* have also been successfully used as natural coagulants (Diaz et al., 1999).

The high coagulation capability of *Opuntia* is most likely attributed to the presence of mucilage which is a viscous and complex carbohydrate stored in cactus inner and outer pads that has great water retention capacity (Saenz et al., 2004). Previous studies have established that mucilage in *Opuntia cactis* contains carbohydrates such as l-arabinose, d-alactose, l-rhamnose, d-xylose, and galacturonic acid (Trachtenberg and Mayer, 1981). Miller et al. (2008) reported that galacturonic acid is possibly the active ingredient that affords the coagulation capability of *Opuntia spp.* though it should be noted that it only accounts for only 50% of turbidity removal.

Among *Opuntia* species, only a few works are found in literature using *O. stricta* for water treatment (Zhang et al., 2006), however, this species is resistant to the carmine cochineal pest (*Dactylopius spp.*) being widely cultivated, especially in the Brazilian semiarid (Santos et al., 2018). It is noteworthy that no study was carried out to evaluate the coagulation potential of *Opuntia* species for the removal of cyanobacteria. Therefore this study analyzed the potential efficiency of *O. stricta* cladode as a coagulant for the removal of cyanobacteria cells. These coagulants are not only naturally reproducible but may also offer many other advantages like local availability, adaptability, and lesser health hazards than residual mineral coagulants or synthetic polymers.

MATERIALS AND METHODS

Study site

Bodocongó reservoir is located in Campina Grande, Brazilian semiarid (7°13'11" S, 35°52'21" W), at an altitude of 548 m above de sea level. The climate conditions are a warm semiarid (BSwh in the Köppen system). The temperature of annual mean is between 25 and 31°C and rainfall of 700 mm/year. The reservoir is part of Paraíba river basin; it has a surface area of 371897 m², a mean and maximum depth of 3.5 and 7.0 m, respectively, a mean total water volume of 1019830 m³, which may vary considerably depending on climate conditions. The urbanization processes around the reservoir promote frequently sewerage and wastewater discharge into Bodocongó's waters, and also a decline in riparian vegetation. It is a hipereutrophic reservoir with mean concentration of a total Potassium of 396.0 µg/L (Abilio et al., 2006).

For the assays, water samples were taken from different sites in the reservoir on January, 2019. At this time there was a cyanobacterial bloom, with contributions of *Microcystis aeruginosa* (Kützing) Kützing 1846, *Sphaerocavum brasiliense* De Azevedo & C.L. Sant' Anna 2003, *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya & Subba Raju in Desikachary 1997 and *Plankthotrix isoethrix* (Skuja) Komárek and Komárková 2004.

Preparation of coagulant

The cactus *O. stricta* were collected from experimental campus of Brazilian National Semiarid Institute (INSA, for this acronym in

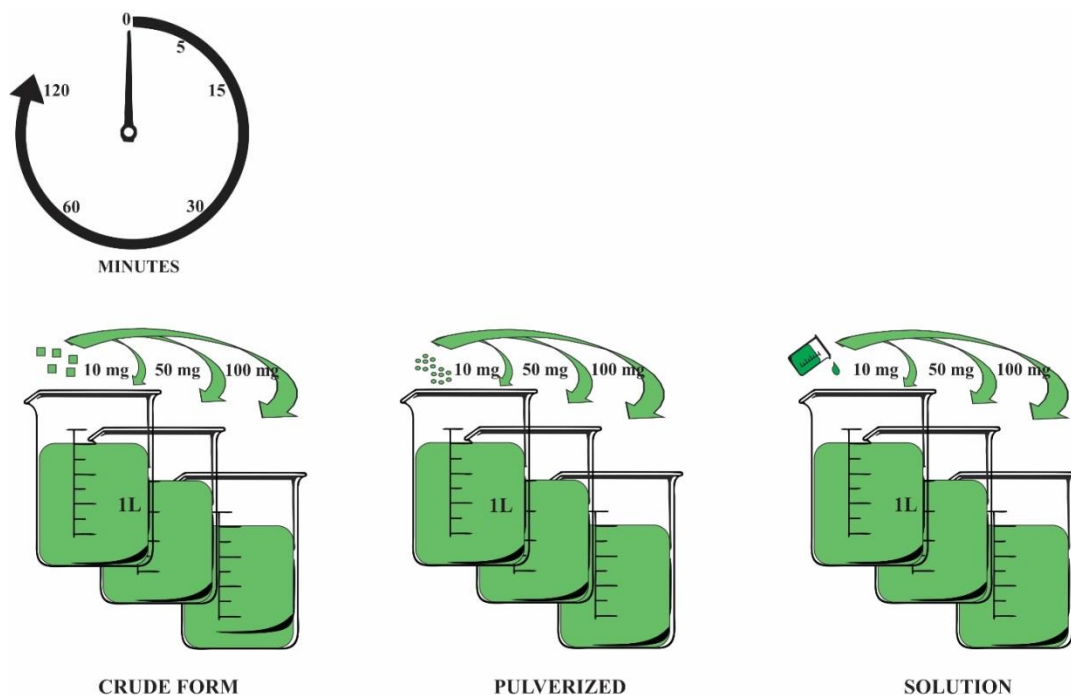


Figure 1. Schematic representation of experimental design.

Portuguese). To assays, the cladodes were used in three forms: Crude form, pulverized and pulverized solution. The collected cladodes were washed with ultrapure water, spines were removed and then stored in refrigerator at 4°C, to assays with crude form. Other part of cactus was sliced into strips 1 cm wide, freeze and lyophilized at -80°C. The dried cactus was milled using a Wiley mill and sieved with the aid of sieves (ABNT, 2010); a powder was obtained with particles of approximately 100 µm in diameter (Miller et al., 2008). A part of this is used in pulverized assays and others were diluted to 1% stock solution (concentration = 1000 mg/L) to pulverized solution assays. The solution of the lyophilized was used because studies of Subramonian et al., (2014) pointed out different absorption sites between the solid and diluted strata.

Experimental design

The efficiency of *O. stricta* to remove cyanobacteria was tested in a laboratory scale. The experiments were composed by three treatments (form, concentration and time) with 3 to 5 levels repeated three times (Figure 1).

Aliquots of 1000 mL water of Bodocongó reservoir were transferred to 2000 mL graduated beakers. Water was treated with designated treatment (final concentration of 10, 50 and 100 mg/L) or left untreated (controls), at 25°C. After 5, 15, 30, 60 and 120 min, pH, turbidity and total dissolved solids (TDS) were measured with HORIBA U52 multiparameter. Samples with 5 mL were fixed with 2% lugol solution for quantitative analyzes using inverted microscope (Zeiss Axiovert), as described by Utermöhl (1958).

Data analyzes

Results were examined by analysis of variance (ANOVA), followed by Fisher's multiple comparison test for all measured parameters (pH, turbidity, TDS and cyanobacteria cells), for each treatment.

Normality was assessed by Kolmogorov-Smirnov test and the homoscedasticity by Fisher's test. The effects of combined treatments were estimated by two-way ANOVA. For this statistical analyzes, values with $p < 0.05$ were considered significant. The program 'Statistica' version 7.0/2004 (Statsoft) was used.

RESULTS

Significant differences were observed in the density of cyanobacteria, TDS and turbidity, when compared to the forms of *O. stricta* used after 120 min exposure. The best results occurred when the solution was used (Table 1). Among the concentrations tested, significant differences were observed in all forms used, for all variables except pH and TDS. The best coagulant effects were observed at dose C2 (50 mg/L), except when solution was used, when C3 dosage were more effective, however, not significantly different. The effects of the interaction between form and concentration were observed only for the turbidity variable (Table 1).

Significant statistical interaction was observed regarding exposure time and *O. stricta* forms to all variables, except pH ($F_{\text{cyanobacteria cell}} = 8.3$, $p < 0.01$; $F_{\text{TDS}} = 40.9$, $p < 0.01$; $F_{\text{Turbidity}} = 5.9$, $p < 0.01$).

For all treatments were observed Cyanobacteria cell removal, that ranged from $30(\pm 4.3)$ to $70(\pm 2.7)$. Considering the time factor, significant differences were observed to coagulation process, however cell removal occurred majorly in the first 30 min of the experiments (Figure 2A). Colonial species were fast removed in all

Table 1. Mean and standard deviation of Cyanobacteria cell, pH, total dissolved solids (TDS) and turbidity for different forms and concentration of *O. stricta* treatments after 120 min.

Variable	Control	Crude form			Pulverized			Solution			Form*	Interaction (Form x Concentration)
		C1	C2	C3	C1	C2	C3	C1	C2	C3		
Cyanobacterial cell (Cell/mL)	106570 (±1150) ^a	61668 (±1100) ^b	56486 (±920) ^c	76220 (±930) ^b	58944 (±543) ^b	34592 (±341) ^c	60475 (±568) ^b	50510 (±786) ^b	31998 (±552) ^c	30994 (±540) ^c	CF=P≠S	**
pH	8.0 (±0.1)	8.04	7.92	7.7	8.1	8.1	8.0	7.8	7.8	7.7	**	**
TDS (g/L)	1.7(±0.1) ^a	1.5(±0.1) ^a	1.4(±0.1) ^a	1.5(±0.1) ^a	1.7(±0.1) ^a	1.6(±0.1) ^a	1.7(±0.1) ^a	1.5(±0.1) ^a	1.2(±0.1) ^b	1.0(±0.1) ^a	CF=S≠P	**
Turbidity (NTU)	58.1 (±1.5) ^a	31.7 (±2.0) ^b	28.7 (±2.2) ^c	30.6 (±1.5) ^b	36.8 (±2.7) ^b	48.8 (±7.2) ^b	47(±7.0) ^b	28.7 (±2.8) ^b	25.9 (±1.7) ^c	23.0 (±2.6) ^c	CF=S≠P	<0.05

Data with same letter did not differ significantly ($p>0.05$) among concentrations applied. *ANOVA for Form treatment – CF (crude form), P (Pulverized), S (Solution)

** Not differ significantly ($p>0.05$)

treatment. Filaments were optimally removal after 30 min of experiment.

pH in water increased in pulverized treatments and decreased in crude form and pulverized solution treatments, but no significant differences were observed (Table 1). These results indicated that the use of *O. stricta* does not alter pH of water and this efficiency is not strictly dependent on pH.

The optimal conditions to remove TDS of water occurred with pulverized solution in C3 concentration (Figure 2B), however the efficiency were lower than 30%. In pulverized treatments an increase in TDS were observed in the first 5 min of the experiment to C2 and C3 concentration.

Turbidity removal ranged from 19(±10.9) to 52(±5.8)%. The optimal results were obtained to solution treatment at C3 concentration (Figure 2C). Significant effect was observed in the interaction between form and concentrations, and form and time of experiment in the efficiency of turbidity removal (Table 1). In Pulverized treatments, a gradual increase in turbidity occurred after the first 15 min of experiments when C3 dosage was applied. Significant turbidity removals were observed after 30 min of the experiments

using crude form and 60 min using solution treatments (Figure 2C).

DISCUSSION

O. stricta is a viable alternative for the removal of cyanobacteria in water. Considerable cyanobacteria cell (30-70%) and turbidity (19-52%) were removed after 120 min of the experiments. In this case the coagulation activity was qualified as present, especially in pulverized solution form. Miller et al. (2008) considered coagulation activity qualified as “absent” if turbidity removal is below 30%.

The predominant coagulation mechanism for *Opuntia* spp. is adsorption and bridging, whereby clay particles do not directly contact one another but are bound to a polymer-like material from *Opuntia* spp. It was also thought that adsorption may occur through hydrogen bonding or dipole interactions and this possibility was ascribed to the likelihood that natural electrolytes from within the *Opuntia* spp. pad, particularly the divalent cations, which are known to be important for coagulation with anionic polymers facilitated by

the adsorption (Oladoja, 2015).

The findings from studies on the screening of green biobased materials as coagulants for water and wastewater purification showed that these evolving type of coagulants hold a lot of potential as substitute to the conventional synthetic metal or polymer based coagulants in water and wastewater treatment operations (Yin, 2010). Regarding cyanobacteria removal by coagulation, good results have been reported, depending on the characteristics of organic matter present in water, the prevalent cyanobacteria species, and the type and concentration of coagulant (Heng et al., 2009; Henderson et al., 2010; Shen et al., 2011). The results demonstrated a good potential of *O. stricta* to remove colonial and filamentous organisms. According to Li et al., (2018), colonial species are first removed because they are free of protruding appendages, or have mucilage (polymeric substances), while, filamentous could not be wrapped by coagulants.

Natural coagulants exhibit highly effectual turbidity removal capabilities, with some of them removing up to 99% of initial turbidity (Nish et al., 2011; Oladoja and Pan, 2015). In the results, turbidity removal was about 52%, not exceeding

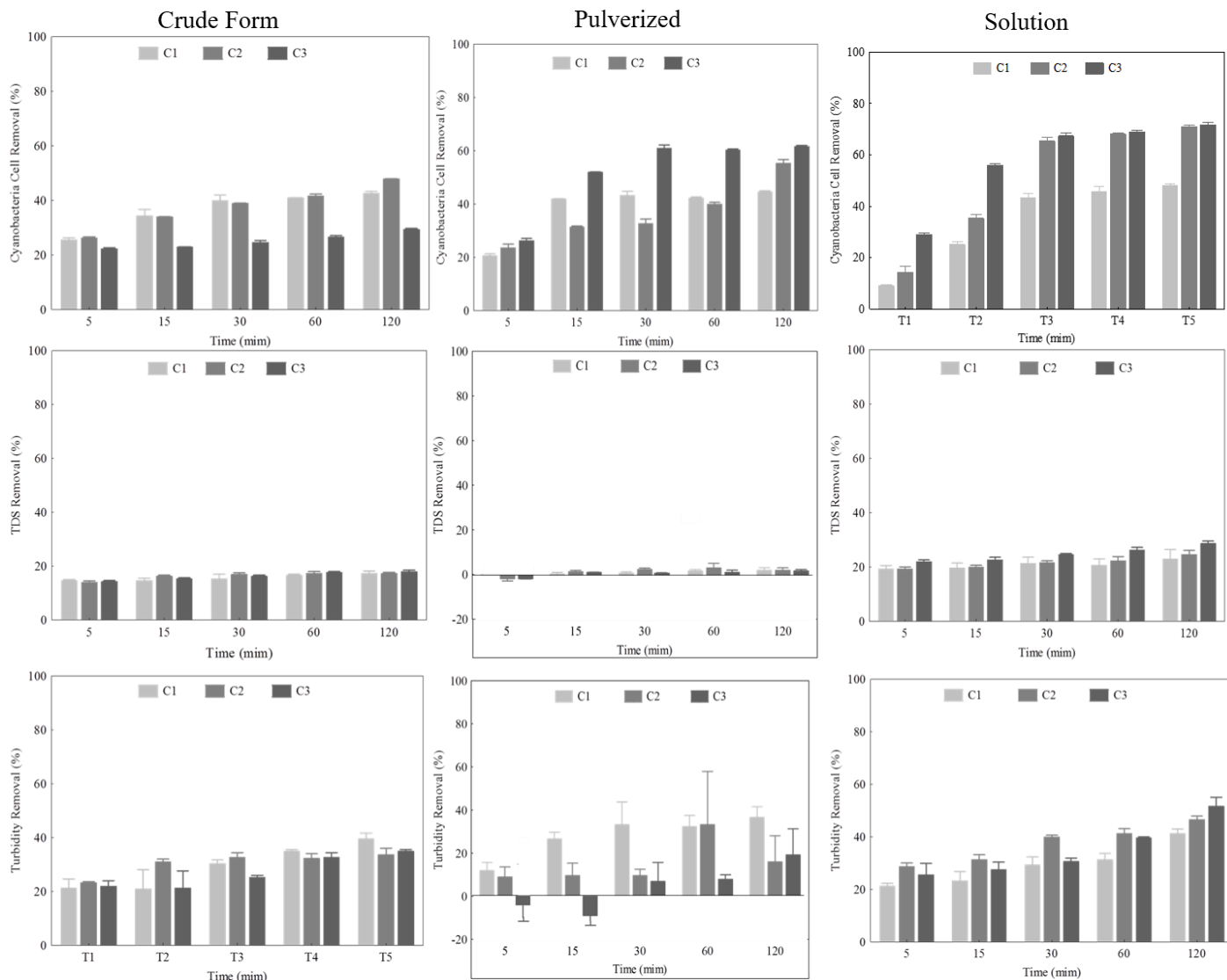


Figure 2. Removal efficiency of cyanobacteria cells, TDS and turbidity at different concentrations and forms of *O. stricta* along time.

21 NTU.

The absence of removal and increase in water turbidity observed may have occurred due to the addition of the *O. stricta* in pulverized form, increasing the organic load. Similar increases in color and turbidity in water treated with green biobased coagulants have been observed in other studies, using *M. oleifera*, particularly when the initial color and turbidity are relatively low (Ndabigengesere and Narasiah, 1998).

It was observed that coagulation activity reduced when the *O. stricta* dose is too low or too high, with best results at intermediate dosage (50 mg/L); this is consistent with a bridging removal mechanism. The bridging mechanism required a stoichiometric relationship between particle concentration and coagulant dose (Oladoja, 2015). Such efficiencies are certainly comparable to the established chemical coagulants (e.g. aluminum). Optimum dosages

are generally within the range of 10 to 60 mg/L. Natural coagulants are most effective at basic waters as evident by the optimum pH values from 7 to 10 (Zhang et al., 2006; Miller et al., 2008). Furthermore, the pH of the water is not affected during coagulation and the pH adjustment may not be necessary for subsequent treatment processes.

The presence of humic substances in natural surface water may significantly alter *Opuntia* dose for optimal coagulation (Zhang et al., 2006). Therefore, *O. stricta* may prove useful as primary coagulant for subsequent treatment through slow sand filters; however, its efficiency should also be further investigated. Analysis of size and nature of flocks achieved through coagulation using *O. stricta* is coherent with the previous suppositions that potential mechanism of coagulation through *Opuntia* is adsorption and inter-particle bridging (Oladoja, 2015).

One of the snags of the use of green biobased coagulants is the substantial increase in the organic load of the treated water, which may result in the possibility for undesired and increased microbial activities. Organic matter is regarded as the source of odor, color, and taste, and a precursor of disinfection by-products in drinking water treatment, so, considering our results, we suggest the use of *O. stricta* to water before irrigation practice.

This is the first record of use of *O. stricta* cladodes to removal cyanobacterial cell. Other studies are necessary to evaluate the performance of *O. stricta* to remove cyanotoxins as well as to improve its efficient and compare it to inorganic coagulants.

Conclusion

Cactus *O. stricta* was an abundant natural product, cost effective, safe for human health that can be used to remove cyanobacteria in water used for irrigation. The potential to remove Cyanobacterias can be explored for water treatment for consumption associated with other coagulants-flocculants.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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