Vol. 24, n.°1, pp. 91-100, 2025
Revista UIS Ingenierías
https://revistas.uis.edu.co/index.php/revistauisingenierias

Cyanotoxin degradation evaluation through low frequency ultrasound

Evaluación de la degradación de cianotoxinas mediante ultrasonido de baja frecuencia

Jinna Marcela Loaiza-González [1a], Ainhoa Rubio-Clemente [1b, 2], Natalia Andrea Herrera-Loaiza [3], Gustavo A. Peñuela-Mesa [1c]

[1] Grupo de Diagnóstico y Control de la Contaminación - GDCON, Facultad de Ingeniería, Universidad de Antioquia, Colombia. Orcid: 0000-0002-5498-686X [a], 0000-0003-1527-260X [b], 0000-0003-3065-0285 [c]. Email: jinna.loaiza@udea.edu.co [a], ainhoa.rubioc@udea.edu.co [b], gustavo.penuela@udea.edu.co [c].
[2] Grupo de Investigación Energía Alternativa – GEA, Universidad de Antioquia, Colombia.
[3] Grupo de Investigación Química Orgánica de Productos Naturales – QOPN, Universidad de Antioquia, Colombia. Orcid: 0000-0002-0291-2252. Email: natalia.herrera@udea.edu.co

Received: 20 November 2024. Accepted: 29 January 2025. Final version: 12 February 2025.

Abstract

Cyanotoxins, such as microcystins (MC) and nodularins (NOD), are highly stable and resistant to conventional physical and chemical degradation, posing a significant risk to human health. In the present work, low frequency ultrasound was used as an advanced oxidation process to degrade cyanotoxins from a Colombian reservoir, evaluating the efficiency of the sonication process, using different powers (10, 30 and 50 W) and exposure times (5, 10, 20 and 30 min) under a frequency of 40 kHz. Ultrasonication proved to be ineffective for MC-LR concentrations up to 2595.42 µg/L, as no significant degradation was observed after 30 minutes of treatment. Additionally, a notable difference was evident in the concentrations of cyanotoxins in the water between sampling campaigns. Thus, risk assessment, implementation of monitoring programs and mitigation efforts in reservoirs deserve greater attention.

Keywords: advanced oxidation process; algal bloom; alternative treatment; cyanotoxins; low frequency; risk assessment; sonication; tropical reservoir; water purification; water quality.

Resumen

Las cianotoxinas, como las microcistinas (MC) y las nodularinas (NOD), son muy resistentes y estables a la degradación química y física convencional, además de representar un mayor riesgo para la salud humana. En el presente trabajo se utilizó el ultrasonido de baja frecuencia como proceso de oxidación avanzada para degradar cianotoxinas presentes en un embalse colombiano, evaluándose la eficiencia del proceso de sonicación, al utilizar diferentes potencias (10, 30 y 50 W) y tiempos de exposición (5, 10, 20 y 30 min) bajo una frecuencia de 40 kHz. Se encontró que el uso de ultrasonido no fue efectivo para concentraciones de hasta 2595,42 µg/L de MC-LR, ya que no se obtuvieron tasas de degradación significativas después de 30 minutos de tratamiento. Además, se evidenció una diferencia notable en las concentraciones de cianotoxinas en el agua entre las campañas de muestreo. En este sentido, la evaluación de riesgos, la implementación de programas de monitoreo y los esfuerzos de mitigación en los embalses merecen mayor atención.

ISSN Online: 2145 - 8456

This work is licensed under a Creative Commons Attribution-NoDerivatives 4.0 License. CC BY-ND 4.0
How to cite: J. M. Loaiza-González, A. Rubio-Clemente, N. A. Herrera- Loaiza, G. A. Peñuela-Mesa, “Cyanotoxin degradation evaluation through low frequency ultrasound,” *Rev. UIS Ing.*, vol. 24, no. 1, pp. 91-100, 2025, doi: https://doi.org/10.18273/revuin.v24n1-2025008

Palabras clave: baja frecuencia; calidad del agua; cianotoxinas; evaluación de riesgos; floración de algas; proceso de oxidación avanzada; purificación de agua; reservorio tropical; sonicación; tratamiento alternativo.

1. Introduction

Algal blooms consist of both toxic and non-toxic strains, often containing a diverse range of toxigenic cyanobacteria that produce various cyanotoxins [1]. Such cyanotoxin mixtures could have several effects on organisms and are commonly found in freshwater [2]. Chemically, cyanotoxins can be peptides, amino acids, alkaloids, and even lipopolysaccharides that act as endotoxins; whose structure is responsible for its pathogenic capacity [3]. Globally, an important factor why the massive cyanobacterial proliferation has a negative environmental impact is ascribed to the possible accumulation of toxins [4]. Indeed, although most toxic biologically active products are confined inside cyanobacterial cells, once cells are naturally or artificially induced death, they can be released into the surrounding water. Thus, in addition to its high toxicity, it is worth highlighting its capacity for bioaccumulation [5], [6] and biomagnification [7].

The potential risk of these toxic metabolites remains mostly unknown [8]. MCs (microcystins) have been studied; especially MC-LR (microcystin-LR) variant [9], [10]. According to the WHO, the exposure of humans to cyanobacteria and their metabolites brings with it effects of varying severity [11], depending on the amount and type of cyanotoxin and the route of exposure [12]. In fact, few episodes of severe or lethal poisoning have been reported in humans after short-term exposure, probably due to the unpleasant appearance and taste of water contaminated by cyanobacteria blooms, preventing its ingestion to a toxic level [13]. However, prolonged exposure to low concentrations could be a critical problem [14].

For drinking water, 1 μg/L total MC-LR has been provisionally recommended by the WHO as a guideline value, being 0.04 μg/kg body weight, the tolerable daily intake suggested [11]. Nevertheless, it is important to note that MC-LR levels in water bodies could exceed this value. Indeed, 0.1-0.3 μg/L limit has been established by the US-EPA [14], adopting its own regulation on the safety of water for consumption, in response to WHO guidelines. Around the world, this problem has increased in frequency and extent [15], largely due to the lack of inclusion of monitoring systems [16], and above all to the lack of studies on cyanopeptides beyond MCs (Janssen, 2019), and the predominance of other types of variants, such as MC-YR and MC-RR [17]. Additionally, the global warming impact and the lack of measures to prevent or reduce discharges to water sources favor optimal conditions for its proliferation [18].

Consequently, over recent decades, harmful algal blooms (HABs) have risen in continental surface waters [19], [20]. Particularly, in the case of non-developed countries and due to the anthropogenic activity intensification and massive use of fertilizers, a pronounced increase has been recorded [21]. As a matter of fact, Wei and collaborators sampled 59 lakes and 37 reservoirs in China. The authors found MCs in 100% and 84% of the lakes and reservoirs, respectively, highlighting the urgent need of strengthening the monitoring and control of these microcystins in water [22]. In the case of Colombia, MCs and HABs have been identified recurrently in several water reservoirs [23], [24], [25], [26]. However, the Colombian environmental legislation has not included the maximum allowable limit of cyanotoxins in water yet [27], since there is a lack of cyanobacterial bloom recording in a systematic way [28]. In 2011, the Instituto Nacional de Salud (INS) published a guide that aims to establish guidelines for sampling them in distribution systems following the decree 1575 of 2007 and its complementary resolution regulations [29]. Nevertheless, the risk ascribed to cyanotoxin presence in water is still unnoticed.

Regarding the water treatment process, conventional purification systems can retain cyanobacterial cells; however, they are not effective in the removal and mineralization of toxins [30]. In fact, chlorination can cause cell lysis and the subsequent intracellular toxin release into the medium [31], [32]. This is why in recent years new techniques have been proposed such as advanced oxidation processes (AOP); among them ultrasound (US) [33], which serves to degrade cyanotoxins and control the proliferation of cyanobacteria [27], [34]. Likewise, it is of utmost importance for future large-scale applications to evaluate whether this technology could be considered an optimal alternative with which water treatment plants operate and if, in fact, it would provide a significant improvement in quality. of the waters, controlling the growth of cyanobacteria in the long term and eliminating the harmful effects caused by these microorganisms. However, it is necessary to consider limitations, such as the range of ultrasound and algal mass. It is essential, therefore, to expand knowledge in this field, and to delve deeper into the effectiveness of the treatment and its optimization.

Under this scenario, this work evaluates the power of low-frequency ultrasound for the elimination of high concentrations of cyanotoxins present in water, such as MC-LR for different time intervals.

2. Methods and materials

2.1. Surface water samples

On September 2, 2022, 40 L of surface water were collected in a Colombian reservoir, where, despite the prolonged rainy season, a cyanobacteria bloom was found. The sonication tests were carried out with this volume of water. Additionally, samples were taken for water quality analysis. For cyanotoxin analyses, the antimicrobial 2-chloroacetamide, ethylenediaminetetraacetic acid and trizma® were added as conservation agents [35]. Previously, a sampling campaign was carried out on November 26, 2021, in which no bloom was evident.

2.2. Reagents and chemicals

Ultrapure water was used for preparing solutions (Millipore Pty Ltd, USA). All chemicals and reagents used were of analytical grade. For the solid phase extraction (SPE) process, HPLC grade methanol (CH_3OH) was used (purity ≥ 99.8%). Additionally, for quantification in the high-efficiency liquid chromatograph, analysis-grade solid ammonium formate ($NH_4\ HCO_2$) (purity > 99.9%), ultrapure gaseous argon (Ar) and nitrogen (N_2) were used, as well as certified standards of cyanotoxins (MC-LR, MC-RR, NOD and MC-YR) (purity > 95%) from Eurofins Abraxis (Warminster, USA).

2.3. Sonochemical reactor

The experimentation was carried out using low-frequency ultrasound equipment (Meinhardt, Germany), which allows operating 5 different powers (10, 20, 30, 40 and 50 W). The reactor is coupled to a transducer and has a 500 mL-capacity cylindrical glass vessel. Ultrasound waves were generated at a fixed frequency of 40 kHz. Approximately 10% of the power produced is lost mainly in the form of heat, which was measured by means of the calorimetric method [36]. For its control, the reactor vessel has a water recirculation that mitigates the increase in the solution temperature, which was maintained around 27 ±3°C, with a cooling temperature of 21 ±1°C. During the experiment, the reactor full capacity was used, and 50 mL aliquots were extracted after the established exposure times (5, 10, 20 and 30 min), in addition to a control (0 min).

2.4. Experimental design

The natural matrix was subjected to 3 different sonochemical tests. At 40 kHz, the power and exposure time were increased, obtaining samples under a minimum, average and maximum power of 10, 30 and 50 W, at time intervals of 5, 10, 20 and 30 min. in addition to a control or untreated sample (0 min) for each test. The maximum reaction time in each test was 30 min, with an aliquot of 50 mL of sample being taken at time intervals. A total of 15 samples including controls were obtained for each test. All tests were conducted in the natural water matrix. The concentration of cyanotoxins was determined at the beginning and at the end of each treatment.

Given the difficulty of working with natural samples and obtaining equal experimental units, of 500 mL each, a control without treatment (0 min) was carried out for each test, given that shaking does not guarantee its homogeneity. This is why some of the differences seen between one replicate and another may be due to the disparate content of cyanotoxins present in the natural water samples, which significantly influences the standard deviation of the values obtained after the analyses. Additionally, it should be noted that each treatment was carried out in triplicate.

2.5. Methods of analysis

An ACQUITY UPLC H-Class liquid chromatograph coupled to Xevo TQD UPLC/MS/MS (Waters, USA) was used for the analysis of cyanotoxins, prior to the preparation of the samples by means of the extraction and concentration of the analyte of interest, through the SPE technique. The above, based on the internal protocol of the GDCON research group (GE-PA-089-GDCON v03) [35]. All experimentation was carried out at room temperature 20 ±2°C.

Since we worked with a natural matrix, and in order to cause the rupture of the cyanobacteria cell wall, each sample was subjected to a process of 3 freeze-thaw cycles. In this way, the aim was to obtain the greatest possible amount of extracellular toxins. The quantification limit (LQ) of the chromatographic analysis method is 0.1 µg/L. Additionally, filtration of each of the samples was necessary, due to the high content of algal biomass (Figure 1), for which 0.45 µm glass fiber filters were used. It should be noted that the sonication process was carried out in the natural matrix without filtering. The filtration was carried out later to be able to carry out the SPE process.

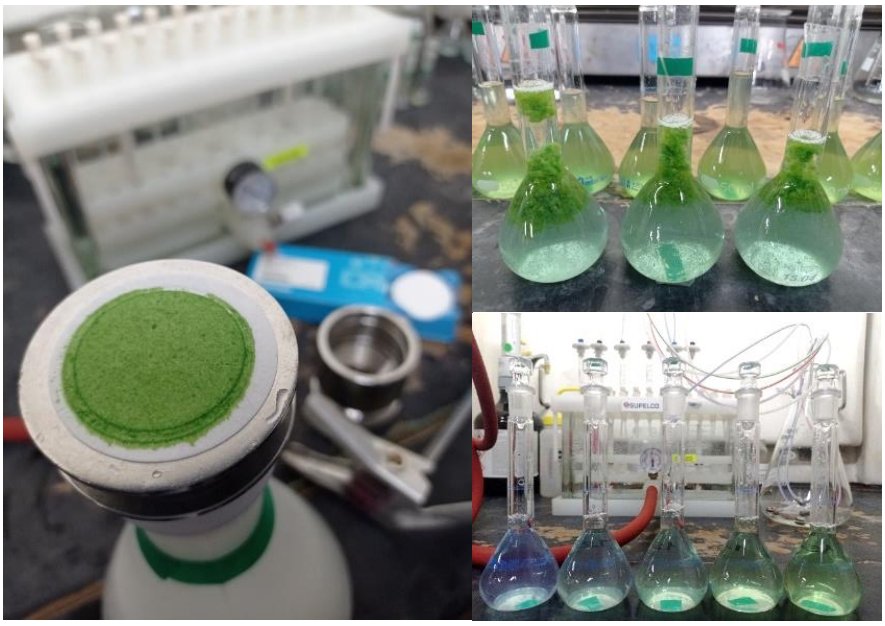

Figure 1. Sample filtration process.

2.5.1. Extraction and concentration

Subsequently, the samples were subjected to SPE process, for which Oasis 60 mg/3 mL HLB extraction cartridges (Waters, USA) were utilized. Before starting the process, the manifold hoses were washed with the use of syringes, passing a 90:10 water-methanol solution, to eliminate possible interferences. The cartridge is conditioned by rinsing and without letting it dry, with 10 mL of methanol (HPLC grade), by gently dripping through the manifold, followed by 10 mL of ultrapure water. Then, each previously filtered water sample contained in 50 mL volumetric flasks is introduced under vacuum through the conditioned cartridge at a flow rate of 5 mL/min. Afterwards, the analytes are eluted with 10 mL of methanol, to 25 mL vials. Additionally, the sample is dried using a gentle flow of air (O_2) for approximately 2 h under constant observation, until a reduced volume of approximately 1 mL is obtained. This is taken to a 5 mL volumetric flask, washing the vial with small amounts of 90:10 methanol-water solution and making up the volume with this same solution. Finally, the solution is taken to a 2 mL amber vial with a UPLC-certified slotted septa cap. In this way, a concentration factor of 10 is obtained. As an analytical control, a blank, a control of 1 and/or 5 µg/L, an enriched sample and a replica of this were used for each batch of 20 samples, following the requirements of the Quality Management System of the GDCON group.

2.5.2. Extraction and concentration

30 L samples were injected into the equipment. A separation column Kinetex C18, 1.7 µm particle size, 2.1 mm x 50 mm, was used to separate the analytes. The operating conditions of the UPLC equipment were: 15 °C (sample temperature), 0.25 mL/min (flow), 30 °C (column temperature), 10 min (run time); aqueous mobile phase: water-methanol 95:5, 5 mM ammonium formate, and organic mobile phase: methanol-water 95:5, 5 mM ammonium formate. On the other hand, the operating conditions for the mass spectrometer were the following: 350 °C (desolvation temperature) and 150 °C (source temperature), desolvation gas flow (N_2) and cone gas flow (N_2) of 650 L/h and 50 L/h, respectively, capillary voltage of 3.5 kV and ESI ionization (+).

The working range of the calibration curve was 1-50 µg/L (1, 5, 10, 20, 30, 40 and 50 µg/L). To prepare the standard solutions, a cyanotoxin mixture of 200 µg/L in HPLC grade methanol was used. The LQ was 1 µg/L. The values detected in the samples below this value are reported as less than the LQ (<LQ).

3. Results and discussion

In total, four cyanotoxins were analyzed: three variants of MCs (MC-YR, MC-RR and MC-LR) and nodularin (NOD), all of them hepatotoxins. In the previous sample from November 2021 (Table 1), the presence of MCs was detected, except for MC-YR; However, in the sampling carried out in September 2022, the three MC variants

were detected in high concentrations. In none of the cases, the presence of NOD was detected. From this last sample, the sonication tests were carried out. In general, the MC-LR concentrations were considerably higher than the other MCs. Specifically, in order of magnitude the concentration of cyanotoxins was MC-LR>MC-RR>MC-YR, reaching a value above 6 mg/L of MC-LR. The other MCs were found in much lower concentrations, below 1 mg/L.

Table 1. Concentration of cyanotoxins in the reservoir (photic zone)

Sampling date	Cyanotoxins (µg/L)			
	MC-LR	MC-RR	MC-YR	NOD
11-26-2021	11.97	1.44	<LQ	<LQ
09-02-2022	6773.66	118.84	4.27	<LQ

Figures 2, 3 and 4 show the results obtained regarding the cyanotoxins removal throughout the sonication tests; however, no significant degradation percentages were achieved. Indeed, when using natural samples, the initial concentration of cyanotoxins varied for each assay, so normalized values are presented to facilitate their interpretation. Additionally, although the tests were done in triplicate, it was necessary to omit the atypical values observed in the first run, since initially the samples were not filtered prior to the SPE process, which did not allow adequate extraction and concentration. of the analyte, observing values very far from the average compared to the other two runs.

It is also evident that sometimes the concentration of cyanotoxins increase with respect to the initial concentration, which could be due to the fact that certain cells managed to remain intact after the freeze-thaw cycle and cell lysis occurred during the sonication process, and only until then, the release of intracellular cyanotoxins occurs.

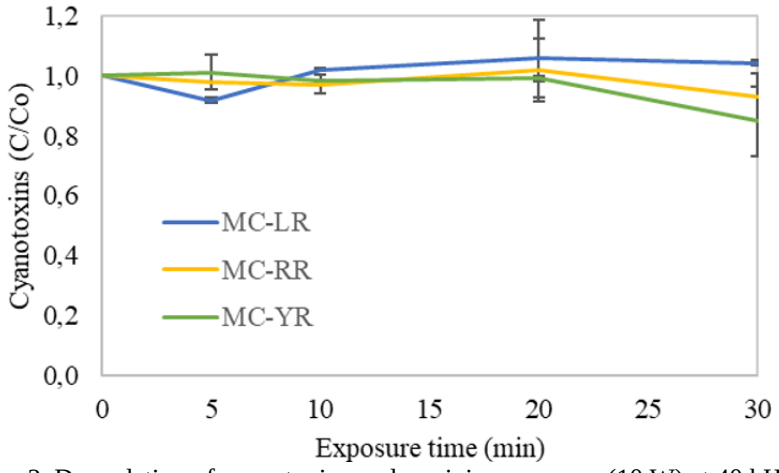

Figure 2. Degradation of cyanotoxins under minimum power (10 W) at 40 kHz.

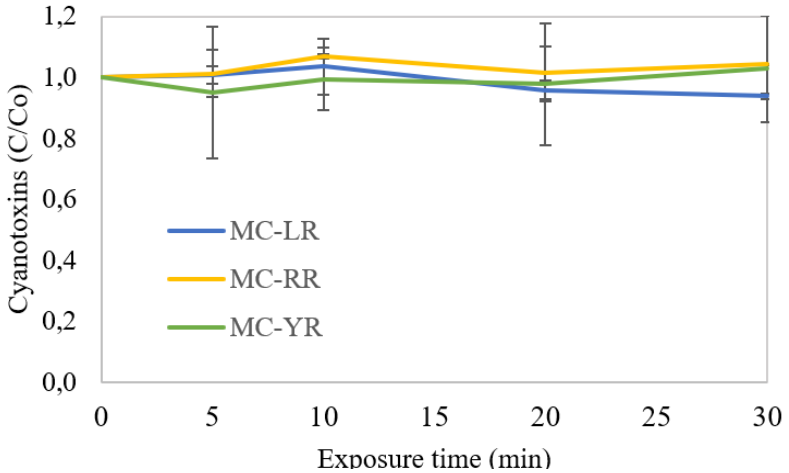

Figure 3. Degradation of cyanotoxins under medium power (30 W) at 40 kHz.

However, in the tests under the maximum power of 50 W, a slight decrease, approximately 10%, in the concentration of MCs is observed.

It has been reported that ultrasound is effective for the elimination of cyanotoxins, such as MCs generated by *Microcystis aeruginosa* [34]. However, as mentioned, the effectiveness of the sonication process will be given by the adjustment of operating factors such as the duration of exposure to ultrasound waves and the frequency, intensity (or power) [37]. In this sense, [38] tested various frequencies (410, 150 and 20 kHz at 30 W) obtaining a reduction of MCs by 70.6% and 65.2% at 150 and 410 kHz, after 20 min of sonication. However, when evaluating the effect of power intensities (90, 60 and 30 W at 20 kHz), 63.6%, 50.2%, and 18.1% of the MCs were degraded, respectively, after 20 min of sonication. This indicates that intermediate frequencies produced a relatively better efficiency in terms of the degradation of MCs, while at low frequencies it was necessary to increase the sonication powers to obtain a considerable degradation rate [38]. In this regard, the removal rate accelerates with increasing ultrasonic intensity.

In this study, although it has been worked with a very similar frequency range and powers (10, 30 and 50 W at a fixed frequency of 40 kHz), degradation percentages were as significant as reported by Ma et al., who suggests that the high concentrations of cyanotoxins in water [38], as proposed by Chen et al. [39], greatly influences the degradation efficiency. In this way, when evaluating the effect of the initial concentration on the degradation of MCs, they obtained lower removal percentages as the initial concentration of cyanotoxins increased, even using a power of up to 1200 W and a maximum concentration of 136.25 µg/L (about 70% in 15 min). It should be noted that in the current research, even though the tests carried out had different initial concentrations as it was a natural matrix, each of the samples started with considerably higher concentrations, from a minimum concentration of 925.41 µg/L to a maximum concentration of 2595.42 µg/L for MC-LR. Additionally, Chen et al. also pointed out that the MC elimination rate can reach its maximum in a short time and then tends to stabilize. In this way, they achieved a removal rate of 81% of MCs, in just 5 min, starting from a concentration of 12.43 µg/L under a power of 1200 W; and managing to almost completely eliminate MCs, up to 99%, after 15 min [39]. Therefore, MCs can be rapidly degraded under ultrasound treatment depending on their concentration in water.

On the other hand, it is important to highlight that, although cyanotoxins are mostly confined to the interior of cells, they are finally within the water after cell death. Likewise, turbulence in water bodies can increase the production and release of cyanotoxins [40], increasing the risk of exposure to MCs during cyanobacteria bloom periods. Consequently, it is possible to find high concentrations of cyanotoxins in aquatic environments, because of biotic and abiotic factors. As was evident in the reservoir, whose concentrations reached magnitudes even mg/L, instead of µg/L as is common. Therefore, it is important that the sonication process tends not to increase the release of toxins into the environment. This is why Zhang et al. recommended the use of ultrasonic power lower than 48 W at 80 kHz [41], finding that higher power under prolonged irradiation caused an undesirable effect, in terms of the release of MCs that significantly increased extracellular concentrations. Likewise, Ma and coworkers indicated that ultrasonic irradiation of less than 5 min might not introduce the rise of dissolved MCs [38].

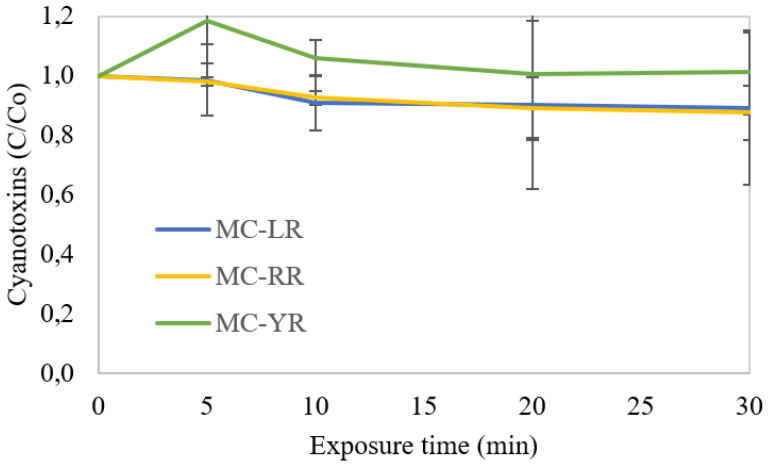

Figure 4. Degradation of cyanotoxins under maximum power (50 W) at 40 kHz.

Additionally, cyanotoxin concentrations in water have significant spatio-temporal variation, and episodes of high-level peaks may be missed in the traditional monitoring scheme. This was evidenced, in the sampling campaigns carried out, by finding much lower concentrations of MCs during pre-sampling, compared to the concentrations detected months later, which probably could have dissipated in the volume of water due to the action of wind and rain. Thus, Caly et al. [23] detected cyanotoxins in 6 of the 7 stations monitored, although at minimum concentrations, which did not exceed the guide-value established by the WHO. However, it is required to incorporate representative and sensitive alternatives that allow the detection of cyanotoxins even at trace concentrations [41], [42].

4. Conclusions

MC-LR, MC-RR, MC-YR and NOD hepatotoxins were analyzed. In the reservoir, the three MC variants were detected in high concentrations. On the contrary, NOD recorded <LQ in both sampling campaigns. In order of magnitude, the cyanotoxin concentration was MC-LR>MC-RR>MC-YR. Particularly, MC-LR was found in considerably higher concentrations than the other MCs. However, all of them exceeded the WHO recommended guideline value of 1 µg/L for MC-LR, reaching a value of up to 6.77 mg/L. In this regard, risk assessment and mitigation efforts for cyanobacterial blooms in reservoirs deserve greater attention, considering the potential danger of exposure to cyanotoxins, regardless of their use. Additionally, a notable difference was evident in the concentrations of cyanotoxins in the water between sampling campaigns, so possibly the episodes of high concentrations may be lost in the traditional monitoring scheme, as they have a significant spatio-temporal variation.

Regarding the sonication tests, no significant degradation rates were obtained after 30 min of treatment, which indicates that at such high concentrations of MCs, up to 2595.42 µg/L for MC-LR, the use of powers in the range of 10-50 W under 40 kHz, therefore, an increase in the ultrasonic intensity is necessary in consideration of the initial concentration of cyanotoxins, in order to obtain much higher degradation percentages. Nonetheless, for field applications, its effect on cyanobacterial cells and the release of cyanotoxins to the aquatic environment must also be considered. Thus, although it has been demonstrated in recent years that the application of ultrasound is effective for the elimination of cyanotoxins, such as MCs, the effectiveness of the sonication process will be given by the adjustment of the operating parameters, including frequency, intensity (or power) and the exposure duration to ultrasound waves.

Funding and acknowledgments

The authors would like to thak the financial support provided by the Grupo de Investigación Diagnóstico y Control de la Contaminación – GDCON of Universidad de Antioquia. Additionally, this research work was supported by the Ministry of Science, Technology and Innovation [grant numbers 831-2019].

Autor Contributions

J.M. Loaiza-González: Data curation, formal analysis, investigation, conceptualization, methodology, writing original draft. A. Rubio-Clemente: Conceptualization, formal analysis, methodology, writing original draft, writing – review & editing. N. A. Herrera-Loaiza: writing original draft, writing -review & edition. G. A. Peñuela-Mesa: writing -review & edition, funding acquisition, project manager.

Conflicts of Interest

The authors declare that there is no conflict of interests of any kind regarding the publication of the results of our research work.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

References

[1] M. Jablonska, L. Cerasino, A. Boscaini, C. Capelli, C. Greco, A.K. Klemenčič, R. Kurmayer, "Distribution of toxigenic cyanobacteria in Alpine lakes and rivers as revealed by molecular screening," *Water Res.*, vol. 258, pp. 121783, 2024, doi: https://doi.org/10.1016/j.watres.2024.121783

[2] K.M. Stroski, D.L. Roelke, C.M. Kieley, R. Park, K.L. Campbell, N.H. Klobusnik, B.W. Brooks, "What, how, when, and where: Spatiotemporal water quality hazards of cyanotoxins in subtropical eutrophic reservoirs," *Environ. Sci. Technol.*, vol. 58(3), pp. 1473-1483, 2024, doi: https://doi.org/10.1021/acs.est.3c06798

[3] J.S. Yunes, "Cyanobacterial Toxins", In *Cyanobacteria: From Basic Science to Applications*. Academic Press, 2019, doi: https://doi.org/10.1016/C2017-0-01395-2

[4] T.L. Pham, M. Utsumi, "An overview of the accumulation of microcystins in aquatic ecosystems," *J. Environ. Manage*, vol. 213, pp. 520–529, 2018, doi: https://doi.org/10.1016/j.jenvman.2018.01.077

[5] D. Glidewell, J.E., Beyer, K.D. Hambright, "Microcystins bioaccumulate but do not biomagnify in an experimental aquatic food chain," *Harmful Algae*, vol. 141, pp. 102768, 2025, doi: https://doi.org/10.1016/j.hal.2024.102768

[6] A. Umehara, T. Takahashi, T. Komorita, R. Orita, J.W. Choi, R. Takenaka, R. Mabuchi, H.D. Park, H. Tsutsumi, "Widespread dispersal and bio-accumulation of toxic microcystins in benthic marine ecosystems," *Chemosphere*, vol. 167, pp. 492–500, 2017, doi: https://doi.org/10.1016/j.chemosphere.2016.10.029

[7] M. Crettaz-Minaglia, D. Sedan, L. Giannuzzi, "Bioacumulacion y biomagnificacion de cianotoxinas en organismos acuaticos de agua dulce," In Cianobacterias como determinantes ambientales de la salud, pp. 171–186, Ministerio de Salud de la Nación, departamento de Salud Ambiental, 2017.

[8] E.M.L. Janssen, "Cyanobacterial peptides beyond microcystins – A review on co-occurrence, toxicity, and challenges for risk assessment," *Water Res.*, vol. 151, pp. 488–499, 2019, doi: https://doi.org/10.1016/j.watres.2018.12.048

[9] G. Kaur, "Freshwater Cyanotoxins", *In Biomarkers in Toxicology*. Elsevier Inc. pp. 601-613, 2019.

[10] D.M.M. Caramés, "Tecnologías de control de floraciones de cianobacterias y algas nocivas en cuerpos de agua, con énfasis en el uso de irradiación por ultrasonido," *Innotec*, vol. 12, no. 12, pp. 54–61, 2016.

[11] WHO, "Guidelines for drinking-water quality, 4th edition," The World Health Organization. 2011.

[12] H. Yang, Y. Yao, W. Chen, X. Gu, H. Chen, Q. Zeng, T. Xiang, "Occurrence and risk assessment of different cyanotoxins and their relationship with environmental factors in six typical eutrophic lakes of China," *Environ. Res*., pp. 121184, 2025, doi: https://doi.org/10.1016/j.envres.2025.121184

[13] L.M. Grattan, S. Holobaugh, J.G. Morris, "Harmful algal blooms and public health," *Harmful Algae*, vol. 57, pp. 2–8, 2016, doi: https://doi.org/10.1016/j.hal.2016.05.003

[14] F.M. Buratti, M. Manganelli, S. Vichi, M. Stefanelli, S. Scardala, E. Testai, E. Funari, "Cyanotoxins: producing organisms, occurrence, toxicity, mechanism of action and human health toxicological risk evaluation," *Archives Toxicol*., vol. 91, no. 3, pp. 1049–1130, 2017, doi: https://doi.org/10.1007/s00204-016-1913-6

[15] B.W. Brooks, J.M. Lazorchak, M.D.A. Howard, M.V.V. Johnson, S.L. Morton, D.A.K. Perkins, E.D. Reavie, G.I. Scott, S.A. Smith, J.A. Steevens, "Are harmful algal blooms becoming the greatest inland water quality threat to public health and aquatic ecosystems?," *Environ. Toxicol., Chem*., vol. 35, no. 1, pp. 6–13, 2016, doi: https://doi.org/10.1002/etc.3220

[16] V. Gaget, M. Lau, B. Sendall, S. Froscio, A.R. Humpage, "Cyanotoxins: Which detection technique for an optimum risk assessment?," *Water Res*., vol. 118, pp. 227–238, 2017, doi: https://doi.org/10.1016/j.watres.2017.04.025

[17] J. Li, R. Li, J. Li., "Current research scenario for microcystins biodegradation – A review on fundamental knowledge, application prospects and challenges, " *Sci. Total Environ.*, vol. 595, pp. 615–632, 2017, doi: https://doi.org/10.1016/j.scitotenv.2017.03.285

[18] E.U. Alum, "The role of toxicology in climate change: Understanding the risks of novel environmental toxins," *Sustain. Environ*., vol. 11, no. 1, pp. 2467485, 2025, doi: https://doi.org/10.1080/27658511.2025.2467485

[19] H.W. Paerl, M.A. Barnard, "Mitigating the global expansion of harmful cyanobacterial blooms: Moving targets in a human- and climatically-altered world," *Harmful Algae*, vol. 96, pp. 101845, 2020, doi: https://doi.org/10.1016/j.hal.2020.101845

[20] P.M. Glibert, "Harmful algae at the complex nexus of eutrophication and climate change," *Harmful Algae*, vol. 91, pp. 101583, 2020, doi: https://doi.org/10.1016/j.hal.2019.03.001

[21] X. Hou, L. Feng, Y. Dai, C. Hu, L. Gibson, J. Tang, Z. Lee, Y. Wang, X. Cai, J. Liu, Y. Zheng, C. Zheng, "Global mapping reveals increase in lacustrine algal blooms over the past decade," *Nature Geosci*., vol. 15, no.2, pp. 130–134, 2022, doi: https://doi.org/10.1038/s41561-021-00887-x

[22] H. Wei, Y. Jia, Z. Wang, "Microcystin pollution in lakes and reservoirs: A nationwide meta-analysis and assessment in China," *Environ. Pollut.*, vol. 309, pp. 119791, 2022, doi: https://doi.org/10.1016/j.envpol.2022.119791

[23] L.F. Caly, D.C. Rodríguez, G.A. Peñuela, "Monitoring of cyanobacteria and cyanotoxins in a Colombian tropical reservoir," *Environ. Sci. Pollut. Res*., vol. 29, no. 35, pp. 52775–52787, 2022, doi: https://doi.org/10.1007/s11356-022-19216-9

[24] C. León, G.A. Peñuela, "Detected cyanotoxins by UHPLC MS/MS technique in tropical reservoirs of northeastern Colombia," *Toxicon*, vol. 167, pp. 38–48, 2019, doi: https://doi.org/10.1016/j.toxicon.2019.06.010

[25] J.M. Loaiza-González, A. Rubio-Clemente, M.C. León-Salazar, D.C. Rodríguez Loaiza, G.A. Peñuela Mesa, "Recurrencia de toxinas cianobacterianas en cuerpos de agua eutrofizados," In *Prácticas y herramientas de sostenibilidad*. pp. 153–181, Tecnológico de Antioquia Institución Universitaria, 2021.

[26] K. Palacio, E. Hernández, G. Peñuela, N. Aguirre, F. Vélez, "Características morfológicas de las cianobacterias y fitoplancton dominante en tres embalses de Antioquia: un enfoque basado en la forma y el biovolumen," *Revista U.D.C.A.*, vol. 22, no. 2, pp. e1306, 2019, doi: https://doi.org/10.31910/rudca.v22.n2.2019.1306

[27] M. Munoz, S. Cirés, Z.M. de Pedro, J.A. Colina, Y. Velásquez-Figueroa, J. Carmona-Jiménez, A. Caro-Borrero, A., Salazar, M.C. Santa María Fuster, D. Contreras, D., E. Perona, A. Quesada, J.A. Casas, "Overview of toxic cyanobacteria and cyanotoxins in Ibero-American freshwaters: Challenges for risk management and opportunities for removal by advanced technologies," *Sci. Total Environ*., vol. 761, pp. 143197, 2021, doi: https://doi.org/10.1016/j.scitotenv.2020.143197

[28] S. Salomón, C.A. Rivera-Rondón, A.M. Zapata, "Cyanobacterial blooms in Colombia: State of knowledge and research needs in the context of climate global change," *Revista de La Academia Colombiana de Ciencias Exactas, Fisicas y Naturales*, vol. 44, no. 171, pp. 376–391, 2020, doi: https://doi.org/10.18257/raccefyn.1050

[29] INS, "Manual de instrucciones para la toma, preservación y transporte de muestras de agua de consumo humano para análisis de laboratorio," *Instituto Nacional de Salud. Colombia*, 2011.

[30] S. Park, Y.H. Kim, J.W. Lee, S. Jang, J.E. Kim, G. Kang, Y.K. Choi, "Adsorptive performance of rice husk-derived biochar for nodularin cyanotoxin from aqueous solution: Isotherm, kinetic, regeneration, and column studies," *J. Water Process Eng.*, vol. 70, pp. 106866, 2025, doi: https://doi.org/10.1016/j.jwpe.2024.106866

[31] X. Xian, C. Luo, G. Lian, X. Yu, L. Zhu. "Cyanobacteria in late lag and exponential phases suit different pre-chlorination and coagulation strategies", *Environ. Pollut.*, vol. 366, pp. 125427, 2025, doi: https://doi.org/10.1016/j.envpol.2024.125427

[32] H. Walker, *Cyanotoxins in Drinking Water: Fundamental Concepts and Solutions*. *CRC Press*, 2025.

[33] Y. Yao, Y Pan, S. Liu, "Power ultrasound and its applications: A state-of-the-art review", *Ultrasonics Sonochem*, vol. 62, pp. 104722, 2020, doi: https://doi.org/10.1016/j.ultsonch.2019.104722

[34] J. Park, J. Church, Y. Son, K.T. Kim, W.H. Lee, "Recent advances in ultrasonic treatment: Challenges and field applications for controlling harmful algal blooms (HABs)," *Ultrasonics Sonochem.*, vol. 38, pp. 326–334, 2017, doi: https://doi.org/10.1016/j.ultsonch.2017.03.003

[35] U.S. EPA, "Single Laboratory Validated Method for Determination of Microcystins and Nodularin in Ambient Freshwaters by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)", United States Environmental Protection Agency, 2017.

[36] C. Parra-Enciso, B.S. Avila, A. Rubio-Clemente, G.A. Peñuela, "Degradation of diclofenac through ultrasonic-based advanced oxidation processes at low frequency," *J. Environ. Chem. Eng.*, vol. 10, pp. 108296, 2022, doi: https://doi.org/10.1016/j.jece.2022.108296

[37] J.M. Loaiza-González, A. Rubio-Clemente, G.A. Peñuela-Mesa, "Aplicación de ultrasonido para el control de cianobacterias y la degradación de cianotoxinas," *Revista UIS Ingenierías*, vol. 22, no. 4, pp. 51-60, 2023, doi: https://doi.org/10.18273/revuin.v22n4-2023005

[38] B. Ma, Y. Chen, H. Hao, M. Wu, B. Wang, H. Lv, G. Zhang, "Influence of ultrasonic field on microcystins produced by bloom-forming algae," *Colloids Surfaces B*, vol. 41, pp. 2–3, pp. 197–201, 2005, doi: https://doi.org/10.1016/j.colsurfb.2004.12.010

[39] G. Chen, X. Ding, W. Zhou, "Study on ultrasonic treatment for degradation of Microcystins (MCs)", *Ultrasonics Sonochem*., vol. 63, pp. 104900, 2020, doi: https://doi.org/10.1016/j.ultsonch.2019.104900

[40] Q. He, Z. Liu, M. Li, "Effects of aeration induced turbulence on colonial morphology and microcystin release of the bloom-forming cyanoabcterium Microcystis," *J. Oceanol. Limnol*., pp. 1-12, 2024, doi: https://doi.org/10.1007/s00343-024-4096-7

[41] P. Wang, B. Du, J. Smith, W. Lao, C.S. Wong, E.Y. Zeng, "Development and field evaluation of the organic-diffusive gradients in thin-films (o-DGT) passive water sampler for microcystins," *Chemosphere*, vol. 287, pp. 132079, 2022, doi: https://doi.org/10.1016/j.chemosphere.2021.132079

[42] J.M. Loaiza-González, A. Rubio-Clemente, G.A. Peñuela, "Cyanotoxin monitoring and detection using passive sampling application," *Water Air Soil Pollut.*, vol. 235, no. 7, pp. 423, 2024, doi: https://doi.org/10.1007/s11270-024-07195-1