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Original Research

Cyanobacterial control in simulated natural water bodies conditions by commercially available ultrasound: biomass reduction and cyanotoxin degradation

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Abstract

Low-frequency and low-intensity commercially available ultrasound to control algal growth in natural water bodies was studied. To evaluate the efficiency of ultrasound on cyanobacteria growth rate reduction and, microcystins release, and degradation, a large-scale lab experiment with 150 L of high-density *Microcystis aeruginosa* suspension simulating natural conditions was conducted at different times of ultrasonication: 0, 15 min, one h, five h, 24 h, and 48 h. The first effect of ultrasonication on biomass reduction was noticed at 24 h of continuous ultrasonication, with the highest reduction rates of 97% and 93% for cell count and chlorophyll-*a*, respectively, at 48 h of continuous ultrasound treatment. The growth inhibition test showed biomass reduction in the samples exposed to ultrasonication for at least one hour with increasing effect from here on. The most efficient in *M. aeruginosa* reduction was the longest tested ultrasound treatment of 48 h with growth inhibition of 96%, followed by 24-h ultrasound treatment with 50%, and 5-h ultrasound treatment with 17% growth inhibition after 9 days of incubation. At five hours of ultrasonication, a sharp increase in dissolved microcystins in the medium was observed as a result of ultrasound-induced stress, followed by a drop of dissolved microcystins under the detection limit at 24 h of continuous ultrasonication. This study showed that commercially available ultrasound devices are highly efficient for cyanobacterial bloom control already at relatively low times of exposure, one to two days, with no health risks due to increased dissolved toxins after continuous ultrasound treatment for 24 h or more.

Keywords

algae control; cyanobacteria; ultrasound; cyanotoxins; *Microcystis aeruginosa*

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Zaviranje rasti cianobakterij v naravnih vodnih telesih s komercialno dostopnim ultrazvokom: zmanjšanje biomase in razgradnja cianotoksinov

Izvleček

V študiji smo raziskovali nizko frekvenčni komercialno dostopni ultrazvok z nizko intenziteto za namen nadzora prekomerne razrasti alg v naravnih vodnih telesih. Za ovrednotenje učinkovitosti ultrazvoka za zmanjšanje biomase cianobakterij ter sproščanja in razgradnje mikrocistinov smo izvedli laboratorijski poskus s 150 L suspenzije potencialno toksičnega seva cianobakterije *Microcystis aeruginosa* z visoko gostoto in simulacijo naravnih pogojev ter različnimi časovnimi intervali uporabe ultrazvoka: 0, 15 min, 1 h, 5 h, 24 h in 48 h. Prvi učinek ultrazvoka na zmanjšanje biomase cianobakterij je bil opazen po 24 urah neprekinjene uporabe ultrazvoka. Najvišje zmanjšanje števila celic (97 %) in koncentracije klorofila-a (93 %) smo ugotovili po 48 urah neprekinjene uporabe ultrazvoka. Študija zaviranja rasti, z devet dnevno inkubacijo, je pokazala zmanjšanje biomase *M. aeruginosa* v vzorcih, ki so bili vsaj eno uro izpostavljeni ultrazvočni obdelavi z naraščajočim učinkom pri daljši izpostavljenosti. Največje zmanjšanje biomase *M. aeruginosa* (96 %) smo ugotovili pri najdaljši testirani ultrazvočni izpostavljenosti kulture in sicer 48 ur. Sledila ji je 24-urna izpostavljenost s 50 % zmanjšanjem biomase in 5-urna izpostavljenost s 17 % zmanjšanjem biomase. Po petih urah ultrazvočne obdelave smo opazili močno povišano koncentracijo raztopljenih mikrocistinov v mediju kot rezultat ultrazvočnega stresa, ki mu je po 24 urah neprekinjene ultrazvočne obdelave sledil padec raztopljenih mikrocistinov pod mejo zaznavnosti. Študija je pokazala, da so komercialno dostopne ultrazvočne naprave zelo učinkovite za nadzor cvetenja cianobakterij že pri relativno kratkih časih izpostavljenosti, enega do dveh dni, brez zdravstvenih tveganj zaradi povečanega sproščanja raztopljenih cianotoksinov po 24 urah ali več neprekinjene uporabe.

Ključne besede

nadzor alg; cianobakterije; ultrazvok; cianotoksini; *Microcystis aeruginosa*

Introduction

Algal blooms can impact the environmental health of water resources and influence water use (Zhu et al., 2021). Toxic cyanobacteria blooms, in particular, due to the production of toxins (cyanotoxins), can have negative impacts on human and animal health, and thus, they can impair the recreational value of the water bodies and drinking water quality (Huisman et al., 2018) and consequently threatens water security and safety in Europe and globally (Ho et al., 2019). Cyanobacterial blooms increase largely due to anthropogenic eutrophication, which is emphasized by climate change, with extreme weather events like heavy rains causing an increase in nutrient runoff from land, further exacerbating eutrophication (Sinha et al., 2017). Global warming and draughts are extending temperature stratification in lakes, strengthening water column stability, which favours buoyant, potentially toxin-forming cyanobacteria over non-buoyant, harmless algae (Paerl & Huisman, 2008). The presence of cyanobacteria in water bodies can cause significant economic losses to businesses, such as water companies, losses to fisheries, and impacts on water-related recreational activities and tourism (Hamilton et al., 2013; Sanseverino et al., 2016).

In case of excessive growth (blooms) some cyanobacteria taxa produce toxins in quantities causing toxicity in mammals, including humans (e.g., Van Apeldoorn et al., 2007), together with volatile organic compounds such as geosmin and 2-methylborneol causing unacceptable taste and odour of drinking water and fish (Jüttner & Watson, 2007; Robin et al., 2006; Yoshinaga et al., 2006). There are many different types of cyanotoxins; however, microcystins (MCs), cylindrospermopsins, anatoxins and saxitoxins are causing the most public health risks (Chorus & Welker, 2021; Qi et al., 2014). Microcystin-LR (MC-LR), the most common MC variant, has been classified as Group 2B, possibly carcinogenic to humans (Lyon, 2010). Once in cells, MCs cause protein phosphatase (PP1, PP2A and PP5) inhibition, resulting in destabilization of the cytoskeleton followed by cellular apoptosis and necrosis (Mackintosh et

al., 1990). High acute doses cause haemorrhage in the liver, but at low doses (<20 µg/kg bw) and with repeated long-term exposure, phosphatase inhibition induces cellular proliferation, hepatic hypertrophy and tumour-promoting activity (Chorus & Welker, 2021). There is a growing body of evidence indicating harmful MC-related neurological and reproductive effects, but the data are not yet robust enough to use as a basis for guideline development (Chorus & Welker, 2021).

Based on available toxicological data, the World Health Organization (WHO) in 2003 established a health safety guideline value for drinking water with lifetime exposure of 1 µg/L for MC-LR equivalents (Chorus & Welker, 2021; WHO, 2003, 2017). In 2021, WHO has upgraded values for the main four groups of cyanotoxins (e.g., 0.7 µg/L for cylindrospermopsins, 1 µg/L for MCs, 3 µg/L for anatoxins and 0.3 µg/L for saxitoxins) and predicted different exposure scenarios; previously mentioned guideline values for lifetime drinking water exposure, short-term drinking water exposure (e.g., 12 µg/L of MCs,) as well as values for recreational exposure (e.g., 24 µg/L of MCs). MC-LR is also included in the revised Drinking Water Directive (Directive (EU) 2020/2184) adopted in December 2020. Also, the Bathing Water Directive (Directive 2006/7/EC) refers to cyanobacteria proliferation as a problem, suggesting appropriate monitoring of bathing water quality and adequate fast managing measures. Exposure to cyanotoxins occurs via drinking contaminated water, swimming (showering) in contaminated water, consumption of fish or shellfish farmed in contaminated water, or consumption of food crops irrigated with contaminated water (Svirčev et al., 2017). According to Testai et al. (2016), contamination of food items with cyanotoxins, for example, through crop spray irrigation, is considered by the European Food Safety Authority (EFSA) as an emerging issue.

Management for preventing or suppressing cyanobacteria blooms can include nutrient-loads reduction, hydrodynamics regulation, chemical-algaecides addition, flocculation and harvesting (Zhou et al., 2018). Chemical-algaecides are usually used in emergencies due to their fast and efficient operation; however, they can have severe side effects on aquatic organisms and the aquatic ecosystem (Huisman et al., 2018). Removing cyanobacteria from water through conventional water treatment processes such as coagulation, flocculation, and filtration is not easy because of their small size and low gravity (Kong et al., 2019). Moreover, water treatment processes that include the use of potassium permanganate or chlorine may even release toxins from the cyanobacteria cells into the water, which can enter the human food chain through drinking water supplies (Rajasekhar et al., 2012) since also cyanotoxins may not effectively be removed and degraded by conventional water treatment techniques, especially during a bloom event (He et al., 2014). Thus, alternative approaches for cyanobacteria bloom prevention and suppression are a necessity.

The application of ultrasonic waves as a non-chemical technique for the reduction of cyanobacterial blooms in fresh water and wastewater has become popular in the last decades (Chorus & Welker, 2021). Several studies reported that ultrasound (US) efficiently reduces the growth rate of cyanobacteria by collapsing the gas vesicles during cavitation, inhibiting cell division, or inflicting immediate damage on photosynthetic activity (e.g., Ahn et al., 2003; Kazunori Nakano et al., 2001; Zhang et al., 2006a). However, the US is known to be able to induce the lysis of cyanobacteria cells, which causes the release of intracellular content into the water (Rajasekhar et al., 2012; Zhang et al., 2006b). Song et al. (2005), among others, reported that the US is also effective in degrading cyanotoxins. An additional positive is that the US proved to have minimal impact on green algae or on algal cells which lack gas vacuoles (Rajasekhar et al., 2012; Tang et al., 2004).

Lab-scale studies on the growth rate reduction efficiency of cyanobacteria by ultrasonication (e.g., Huang et al., 2020; Kong et al., 2019; Li et al., 2019; Lürling et al., 2014; Rumyantsev et al., 2021; Wu et al., 2020) are numerous, while studies on cyanotoxin degradation by ultrasonication (e.g., Chen et al., 2020; Lürling et al., 2014) are a little bit less common. However, there is often a disconnect between the research activities and the commercial products since the research activities mostly focus on high-intensity US devices with cavitation effect, whereas commercially available US devices for controlling algal blooms in natural water bodies are low-intensity for minimizing the risk of non-target organisms and the environment. Moreover, there is a lack of peer-reviewed scientific publications on trials in natural or semi-natural conditions using commercially available US units. Therefore, the aim of our study was to investigate the efficiency of commercially available low-intensity US in controlling cyanobacterial blooms and their toxins in the natural water environment. A large-scale laboratory experiment simulating natural conditions was conducted with a strain of toxic *M. aeruginosa* at high-density conditions (simulating cyanobacterial bloom) in a 150 L volume setting (simulating

shallow reservoir or lake) and with the use of low-intensity and low-frequency commercially available US device. The research questions were a) what is the minimum time of ultrasonication affecting *M. aeruginosa* growth, b) what is the time dynamics after US treatment in the surrounding medium or when to expect the increase and decrease of cyanotoxins in the water column, and c) what happened with cyanotoxin concentrations outside and inside the cyanobacterial cells.

Materials and Methods

Cultivation of *Microcystis aeruginosa*

Microcystis is the most dominant colonial bloom-forming genus responsible for toxic blooms in eutrophic lakes worldwide (Fang et al., 2018). *M. aeruginosa* was used as a test organism in this study. Toxic strain of *M. aeruginosa* (PCC 7806) was purchased as a live culture from the algae bank of the Pasteur Institute (Paris, France) and cultivated according to Stanier et al. (1971) at an ambient temperature of 25 °C (Fang et al., 2018) using nutrient medium BG-11 (Merck, Germany). The starter culture of *M. aeruginosa* was cultivated in aseptic conditions, first in Erlenmeyer flasks (from 100 to 5000 mL) and later in covered glass aquariums. For *M. aeruginosa* cultivation, tubular fluorescent lamps (FLUORA L36W/77, OSRAM, Germany) and a 16 h/8 h light/dark cycle were used. Light irradiance was measured with a light meter (MS-1300, Voltracft, Italy). *M. aeruginosa* culture with low cell concentration (<104 cells/mL) was cultivated for three weeks under low illumination intensity (<40 µmol photon/m^2/s); when *M. aeruginosa* culture reached exponential phase of growth (106 cells/mL), it was further cultivated at higher illumination intensity (60 µmol photon/m^2/s) (Fang et al., 2018) for another two weeks until it reached final density of 108 cells/mL (inoculum).

Experimental design

For the experiment conduction a custom-made polyvinyl chloride (PVC) foam pond (width 70 cm, length 180 cm, height 45 cm) with volume of approximately 350 L coated with PVC foil to ensure water tightness was used (Fig. 2). The experiment was conducted at an ambient temperature of 25 °C with illumination of 60 µmol photon/m^2/s (FLUORA lights 33W/77, Osram, Germany) in 16 h/8 h light/dark cycle.

To obtain the needed culture volume of approximately 150 L, dense *M. aeruginosa* culture (inoculum) was diluted to 10^6 cells/mL by adding fresh nutrient medium BG-11 (Merck, Germany). Diluted *M. aeruginosa* culture was transferred into the PVC pond for US treatment. Commercially available LG Sonic (www.lgsonic.com) US device (power 25 W, frequency output 20-100 kHz) was immersed into the *M. aeruginosa* culture and switched on for the next 48 h.

Monitoring of the system

400 mL of sample was siphoned 10 cm below the water surface at different ultrasonication times (0, 15 min, one h, five h, 24 h, and 48 h) and extracted. This way, *M. aeruginosa* cells, which sank to the bottom of the container due to the destruction of gas vesicles caused by US treatment, were not captured in the sample. The ultrasonication times were selected based on our preliminary experiments (not published) and the findings of Kong et al. (2019). 100 mL of the extracted sample was used for cyanobacterial growth assessment (cell count and chlorophyll-*a* analyses) and MCs analyses. The rest 300 mL of the extracted sample was used for a growth inhibition test. All experiments were performed in triplicates.

Cell count and chlorophyll-*a* concentration

Cell count was performed according to a protocol of Moheimani et al. (2013) by using a light microscope (CX31RBSF, Olympus, Japan) and a Neubauer chamber (Celeromics, France) with a 0.1 mm depth and a 0.0025 mm^2 total counting surface.

Chlorophyll-*a* was analyzed according to Vollenweider (1969). In short, a 5 mL sample was centrifuged at 8000 rpm for 10 min with a UNIVERSAL 320 centrifuge (Hettich Zentrifugen, Germany). The supernatant was discarded, and the sample was re-suspended in 8 mL of methanol (Sigma-Aldrich, USA). The suspension was kept in a water bath at 50 °C for one hour and centrifuged at 4000 rpm for 10 min. Chlorophyll-*a* concentration was determined spectrophotometrically and calculated using the following equation:

$$\text{Chlorophyll } a \left[\frac{\mu g}{mL}\right] = \frac{13.9 \times (E_{665} - E_{750}) \times 8}{V_{sample} \times l}$$

E_{665} and E_{750} are the absorbance of the chlorophyll-*a* suspension in methanol at 665 nm and 750 nm, respectively, and l is the width of the cuvette used.

Growth inhibition test

For the growth inhibition test 100 mL of sample taken at each time of ultrasonication (0, 15 min, one h, five h, 24 h, and 48 h) was added to the 100 mL of nutrient medium BG-11 (Merck, Germany) and transferred into a 250 mL Erlenmeyer flask provided with illumination of 60 µmol photon/m^2/s (FLUORA lights 33W/77, Osram, Germany) in 16 h/8 h light/dark cycle for the following 9 days. Continuous mixing was provided by magnetic stirrers (IKA, Germany, 250 rpm). The growth inhibition test was performed in triplicates at an ambient temperature of 25 °C according to the OECD guidelines (OECD, 2011). Chlorophyll-*a* concentration as an indicator of *M. aeruginosa* growth was analysed in each Erlenmeyer flask on days 1, 2, 3, 5 and 9 using a Nanocolor VIS spectrophotometer (Macherey-Nagel, Germany). The relationship between cell count with a Neubauer chamber (Celeromics, France) under a light microscope (CX31RBSF, Olympus, Japan) and measured chlorophyll-*a* concentration was determined. In 200 mL of nutrient medium, 40 mL of *M. aeruginosa* inoculum was added and cultivated for 7 days at the conditions described in Section 2.1. Afterwards, the dilution with the nutrient medium in ranges of 1%, 2.5%, 5%, 7%, 10%, 20%, 30%, 40%, 50%, 60%, 80%, and 100% was performed. A linear relationship (R2=0.9892) between cell count and chlorophyll-*a* was obtained (data not shown). The chlorophyll-*a* concentration of the cyanobacterial suspension was therefore used as a means of monitoring cell concentration and, hence, cell growth (Moheimani et al., 2013). Growth inhibition was calculated according to the following equation:

$$\text{Growth inhibition [\%]} = \frac{(\text{No. of cells at time 0} - \text{No. of cells at specific time of ultrasonication})}{\text{No. of cells at time 0}} \times 100$$

Microcystins concentration

For the analyses of cyclic peptides, a well-established high-performance liquid chromatography (HPLC) method (Sedmak et al., 2008) was used, which is an optimized Harada method (Harada et al., 1988). All the samples were first lyophilised, extracted, and purified. Approximately 100 mL of dense cyanobacterial culture was lyophilised (Alpha Christ, France). Freeze-dried cyanobacteria (exactly 50 mg) were extracted three times with 5% aqueous acetic acid (3 x 20 mL) for 30 min while stirring. The mixture was frozen to increase sedimentation. The extracts were centrifuged at 4000 rpm for 10 min. The combined supernatants were applied to preconditioned 500 mg reversed-phase disposable columns (LiChrolut RP-18, Merck). The columns containing the extract were washed with 20 mL of 10% methanol, and the cyclic peptides eluted with 2 mL methanol (LiChrosolv, Merck), evaporated to dryness under nitrogen stream, and the residues, eluted from the columns dissolved in the buffer for HPLC analysis.

Samples were then analysed using the analytical HPLC method (Waters Corporation), using isocratic elution with methanol: 0.05 M phosphate buffer 58:42 (v/v) pH 3.0. The extracts were separated on an analytical Hibar Pre-Packed RT 125-4 LiCrospher 100 RP-18 (5 µm) column (Merck), flow rate 1 mL/min, using HPLC/PDA (Waters) to visualise cyclic peptides. Millenium 32 software (Ver.3.0, Waters) was used to run the hardware and to process the data.

The cyclic peptides were identified and visualised with a photodiode array detector (PDA). The column eluate was monitored at wavelengths (λmax) 238 in order to locate and distinguish MCs from other biologically active substances from cyanobacteria. From the individual peaks, the amounts of the cyclic peptides were calculated by comparison of the integrated peak areas with the values from the calibration curves that were standardised by previously isolated cyclic peptides in pure form.

Statistical analyses

One-way analysis of variance (ANOVA) with Tukey's post hoc test and a linear correlation were used for statistical analyses. The normality of data was checked using the Lilliefors test; all datasets were distributed normally. The analyses were conducted using the SPSS statistical package.

The algal reduction rate was used to characterize the reduction effect of the US irradiation on *M. aeruginosa* biomass and MCs concentration and was calculated as follows:

$$\text{Reduction rate [\%]} = \frac{Cc - Ct}{Cc} \times 100$$

Cc and Ct are the measured parameters of the control group (time 0; before sonication) and the experimental (test) group at the same time, respectively. The MCs reduction rate was used to characterize the effect of the US irradiation on MCs concentration released from *M. aeruginosa* cells, where 100% reduction represents MCs concentration at the end of the experiment, where no dissolved MCs were detected by HPLC.

Results and Discussion

Impact of ultrasonication on *Microcystis aeruginosa* biomass

M. aeruginosa biomass at different times of ultrasonication (0, 15 min, one h, five h, 24 h, and 48 h) determined as cell count and chlorophyll-*a* concentration are shown in Fig. 1. Initial cell concentration in the water column before the start of the US treatment was 1 x 10^6 cells/mL with initial chlorophyll-*a* concentration of 0.09 µg/L (Fig. 1).

In the first five h of continuous ultrasonication, there was no marked change in the number of *M. aeruginosa* cells or chlorophyll-*a* concentration in the water column (Fig. 1). After 24 h of ultrasonication, cell concentration in the water column decreased by 4-fold to 0.25 x 10^6 cells/mL and chlorophyll-*a* concentration decreased by 2.3-fold to 0.04 µg/L, indicating efficient reduction of *M. aeruginosa* in the water by continuous 24-h US treatment. After 48 h of US treatment, cell concentration in the water column decreased by 28-fold to 4 x 10^4 cells/mL, while chlorophyll-*a* concentration dropped close to the detection limit, showing that the vast majority of the *M. aeruginosa* cells were sedimented. Reduction rates of *M aeruginosa* increased with ultrasonication time (Fig. 2), which is congruent with other studies (Kong et al., 2019; Peng et al., 2020; Rumyantsev et al., 2021). The highest reduction rates reached in our study were 97% for cell count and 93% for chlorophyll-*a* at 48 h of continuous US treatment. However, at 24 h of continuous ultrasonication, reduction rates achieved were 78% and 59% for cell count and chlorophyll-*a*, respectively. Kong et al. (2019) reached an 86% reduction rate of *M.*

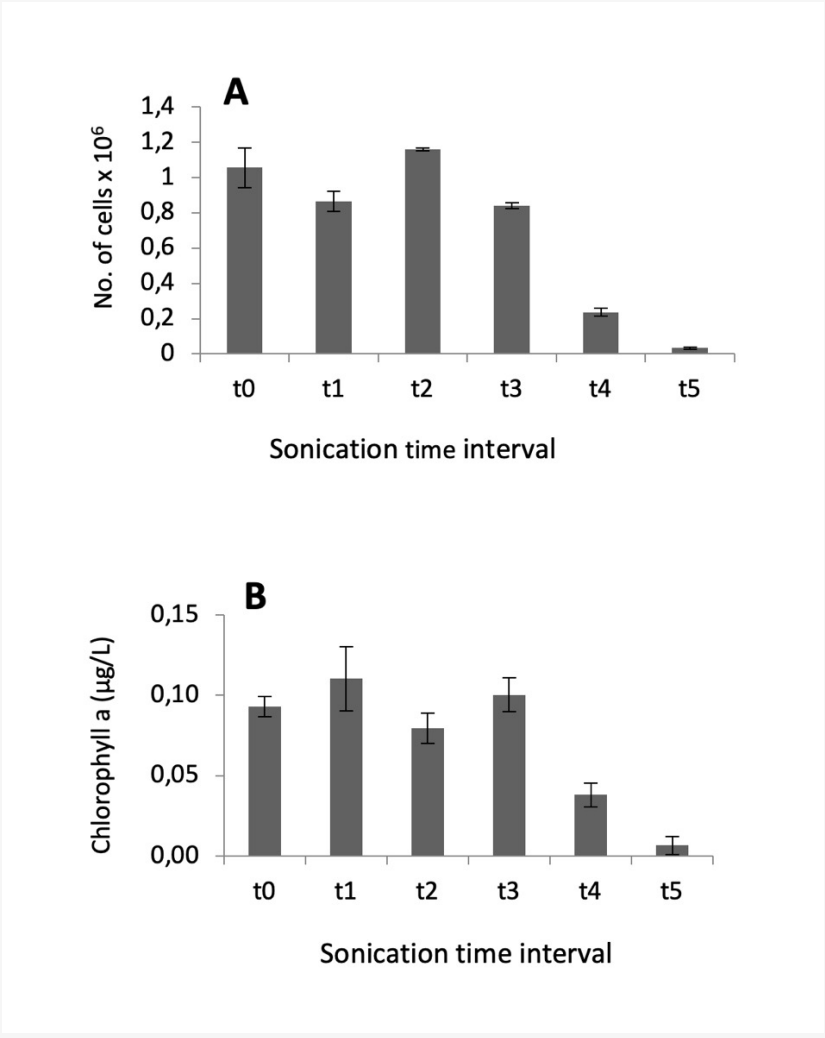

Figure 1. Cell count (A) and chlorophyll-*a* concentration (B) of *Microcystis aeruginosa* at different times of continuous ultrasound treatment: t0=0 min, t1=15 min, t2=1 h, t3=5 h, t4=24 h, and t5=48 h (n=3).

Slika 1. Število celic (A) in koncentracija klorofila-*a* (B) pri različnih časih neprekinjene obdelave cianobakterije *Microcystis aeruginosa* z ultrazvokom: t0=0 min, t1=15 min, t2=1 h, t3=5 h, t4=24 h, and t5=48 h (n=3).

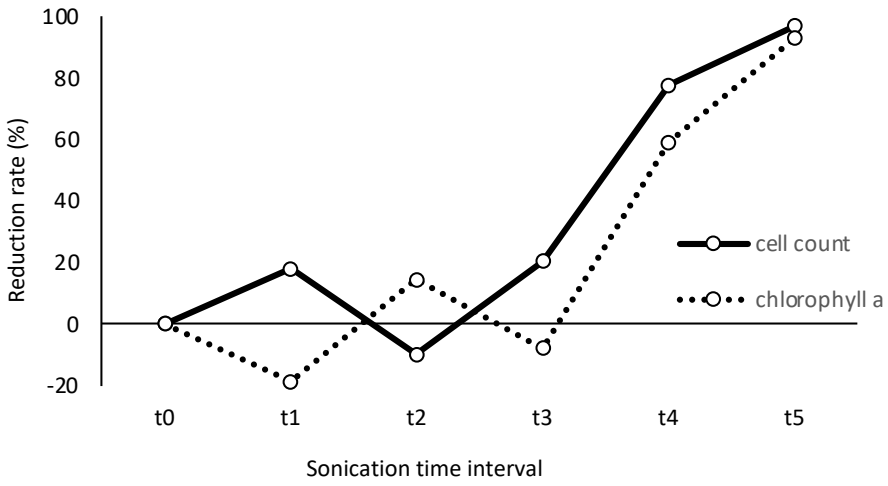

Figure 2. Reduction rate of *Microcystis aeruginosa* at different times of continuous ultrasound treatment: t0=0 min, t1=15 min, t2=1 h, t3=5 h, t4=24 h, and t5=48 h (n=3).

Slika 2. Stopnja zmanjšanja cianobakterije *Microcystis aeruginosa* pri različnih časih neprekinjene obdelave z ultrazvokom: t0=0 min, t1=15 min, t2=1 h, t3=5 h, t4=24 h, and t5=48 h (n=3).

aeruginosa measured as cell count after 20 min of US exposure, while Li et al. (2019) reported on >90% *M. aeruginosa* reduction rate measured as turbidly after only 5 min of US exposure; however, they both studied high-intensity cavitation US units. Acoustic cavitation is a phenomenon where usually low frequency (20–100 kHz) US is causing intense heat of 4,500–7,500 °C accompanied by pressure of around 10,000 Bar (Klemenčič and Krivograd Klemenčič, 2021a). On the other hand, Rumyantsev et al. (2021), who studied low-intensity US (20-200 kHz), found a reduction in cyanobacteria *Synechocystis* biomass after 12 to 15 days of continuous US operation.

The results of ANOVA analysis showed a statistically significant difference ($p<0.05$) in measured parameters of *M. aeruginosa* (cell count, chlorophyll-*a*, reduction rate) in the water column between longer (24 h and 48 h) and shorter ultrasonication times (0, 15 min, one h, and five h). Moreover, there was a statistically significant difference ($p<0.05$) in measured parameters also between 24 h and 48 h of ultrasonication, whereas the difference between shorter ultrasonication times was not statistically significant. Visual inspection showed that after 24 h of continuous ultrasonication, the colour of the water in the experimental pond changed from blue-green to brown-green with foam present on the surface, indicating a shift of *M. aeruginosa* culture to the death phase, while after 48 h of continuous ultrasonication, the water colour turned into brown with even more foam present on the surface, indicating decay of cyanobacteria in the experimental pond.

High-intensity US tend to inactivate *M. aeruginosa* cells due to mechanical destruction and formation of free-radical oxidation as a result of the cavitation effect, which could cause severe damage to the structure and physiological function of algae cells and could damage the cell membrane, wall, and organelle (Kong et al., 2019). On the other hand, the main mechanism of low-intensity US affecting *M. aeruginosa* is, according to Rumyantsev et al. (2021), the US causes stress-state of cyanobacteria, which leads to a sharp increase in the consumption of energy to synthesize exopolysaccharides so that mucous membranes and proteins can grow to restore extracellular protein structures destroyed by US. The two simultaneously working mechanisms of biosynthesis deplete the accumulated and irreplaceable energy, resulting in the death of cyanobacteria.

Cavitation US (high-intensity, high-frequency) is recommended for drinking water treatment purposes (Li et al., 2019). In natural water ecosystems, the use of low-intensity (or low-power) US is widely accepted as an environmentally

friendly method for cyanobacterial control (Rumyantsev et al., 2021) also because it isn't expected to have an impact on non-target organisms such as fish and daphnids (Klemenčič & Krivograd Klemenčič, 2021b). High-frequency US irradiation has a small impact distance within the water; therefore, the ultrasonic frequency should be low for application in large bodies of water such as lakes or reservoirs (Huang et al., 2020). Therefore, low-intensity and low-frequency US units are recommended for controlling algal (cyanobacterial) blooms in natural environments (Klemenčič & Krivograd Klemenčič, 2021b) as is the US unit used in our research.

Impact of ultrasonication on *Microcystis aeruginosa* growth inhibition

The results of the growth inhibition test are shown in Fig. 3. The ANOVA analysis showed a statistically significant difference ($p<0.05$) in *M. aeruginosa* cell count and reduction rate between longer (24 h, 48 h) and shorter times of ultrasonication (15 min, one h). Moreover, there was also a statistically significant difference ($p<0.05$) in measured parameters between 24 h and 48 h of ultrasonication, whereas the difference between shorter ultrasonication times was not statistically significant. Our results showed that up to one hour of US treatment did not affect cell growth at all and that after one hour of ultrasonication, the effect of US treatment on *M. aeruginosa* growth increased with the time of ultrasonication. These results are congruent with the research of other authors (e.g., Li et al., 2019), who also reported that the duration of ultrasonication is among the most important parameters influencing algal reduction. The most efficient in *M. aeruginosa* reduction was the longest tested US treatment of 48 h with a growth inhibition of 96%, followed by 24-h US treatment with 50%, and 5-h US treatment with 17% growth inhibition at the end of the 9-day growth inhibition test (Fig. 3). Huang et al. (2020) who also tested *M. aeruginosa* reduction by low-intensity and low-frequency US observed similar reduction efficiencies; however, at significant lower ultrasonication times up to 10 min which can be the consequence of low volume of 0.5 L algal suspension used compared to 150 L of algal suspension used in our experiment. It is well known that US operational parameters determined from short-term lab tests may not work for the field ultrasonication for algal reduction in a different size of water and in a longer-term operation (Purcell et al., 2013). That is why, in our experiment, a larger volume of algal suspension was applied, simulating shallow water bodies in a semi-natural environmental condition.

Nevertheless, that 48-h of ultrasonication significantly inhibited *M. aeruginosa* growth, the cells were still able to re-grow after US treatment and thus, long-term treatment (>48 h) when using low-intensity US units is required in order to maintain a low number of *M. aeruginosa* cells in the water column. Low-intensity US irradiation (below the cavitation threshold) achieves algal control mainly through mechanical impacts on water; thus the algal reduction rate is relatively low, and the damage to the algal cells can be repaired, as concluded by Huang et al. (2020). *M. aeruginosa* cells complete cell repair within 36–48 hours after low-frequency and low-intensity ultrasonic irradiation. However, even in the case of high-intensity US (above the cavitation threshold), not all *M. aeruginosa* cells break down directly, and the cell wall can keep integrity or suffer some damage, and such cells can recover their activity spontaneously (Kong et al., 2019). It is important to mention that in our re-growth experiment, 250 mL Erlenmeyer flasks were used, and due to continuous mixing, all the cells in the sample were illuminated, which is different from the natural environment. In natural water bodies, US treatment usually causes *M. aeruginosa* cells to sink to the bottom of the water body, where the light intensity is usually too low to support the growth of phototrophic organisms such as cyanobacteria and algae.

Impact of ultrasonication on microcystins

MCs content was evaluated as a peak surface from an HPLC chromatogram and calculated per dry weight in order to assess the MCs content in different compartments, dissolved MCs in media and MCs in cells (Fig. 4) after different times of US exposure. MCs content (µg/g DW) in the cells (pellet) was more or less constant during the whole experiment, with differences that were not statistically significant (black rectangles in Fig. 4), which is similar to the findings of Lürling et al. (2014). Our results showed a sharp increase in dissolved MC concentrations in aquatic medium starting after 15 min and stopped after five h of ultrasonication. The increase in cyanotoxin concentrations in the media after the application of US has also been reported by other authors (Lürling et al., 2014; Rajasekhar et al., 2012; Rumyantsev et al., 2021; Zhang et al., 2006b). According to Zhang et al. (2006b), the use of

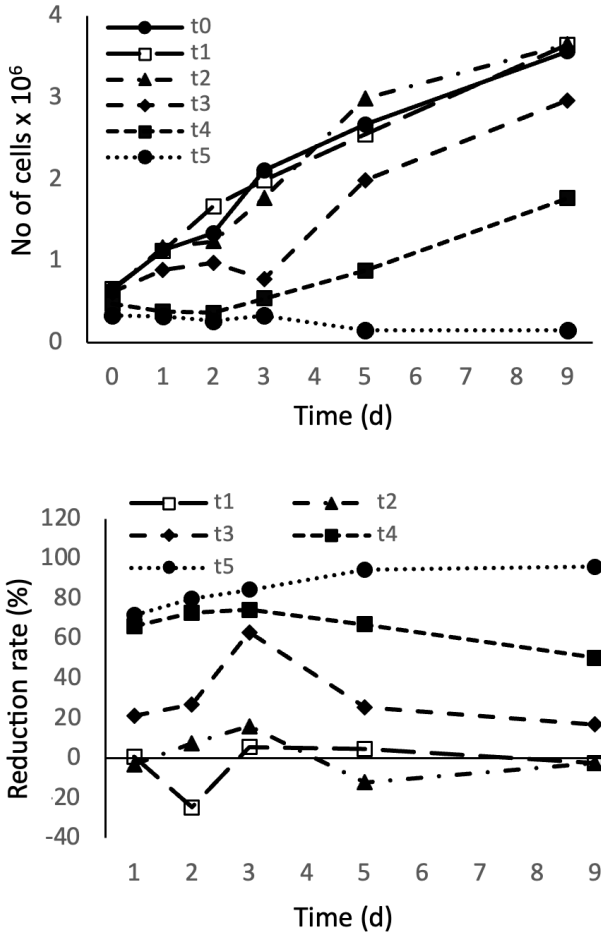

Figure 3. Inhibition of *Microcystis aeruginosa* growth after different times of ultrasound treatment: t0=0 min, t1=15 min, t2=1 h, t3=5 h, t4=24 h, and t5=48 h of ultrasonication (n=3).

Slika 3. Inhibicija rasti cianobakterije *Microcystis aeruginosa* po različnih časih obdelave z ultrazvokom: t0=0 min, t1=15 min, t2=1 h, t3=5 h, t4=24 h, and t5=48 h (n=3).

high-intensity cavitation-producing ultrasound (80 W, 80 kHz) for 5 min increased the extracellular MCs concentration from 0.87 µg/L to 3.11 µg/L in the experiment of *M. aeruginosa* removal by ultrasonication. Lürling et al. (2014) reported on minor release of MCs into the water in the lab-scale experiment of US treatment of *M. aeruginosa* toxic strain using commercial US units (power and frequency of US and time of sonication not reported). Rumyantsev et al. (2021), which used low-intensity US (40–300 kHz) to treat a toxic strain of *Synechocystis* sp., reported a decrease in the concentration of cells in the experimental containers from the 12th to the 15th day of US exposure and on increased synthesis of toxins. Rajasekhar et al. (2012) reported that 5 min sonication (20 KHz) of *M. aeruginosa* suspension at 0.32 W/mL, or for a longer exposure time (>10 min) at a lower power intensity (0.043 W/mL), led to an increase in MCs level in the experimental containers. The increase in cyanotoxin concentration can be the consequence of the damage caused by the US to the algal cells or the collapse of the cells (Zhang et al., 2006b), which is usually the case in high-intensity US applications. However, in some cases, high-intensity US can be so powerful that algal cells are destroyed within minutes with no release of cyanotoxins in the media (Chen et al., 2020). In the case of low-intensity US application, the biosynthesis of toxins can increase after US exposure due to increased stress from cyanobacteria (Rumyantsev et al., 2021) rather than due to cell lysis. This is also indicated by our study since the results of *M. aeruginosa* biomass clearly show no sedimentation of cells (Figs. 1, 2) during the occurrence of a rise in dissolved MCs (from 15 min to 5 h), indicating the absence of major cell damages or cell lysis. According

to Rumyantsev et al. (2021), the cell wall and the mucous membrane of cyanobacteria become thicker under the influence of the US, which is connected with the biosynthesis of toxins. The results of Rumyantsev et al. (2021) indicate that toxigenic cyanobacteria protect themselves against US irradiation by growing mucous membranes and starting the biosynthesis of toxins and their release into the aquatic medium. The reason for the high increase of dissolved MCs in our experiment could be that in the samples also, dissolved MCs from sedimented cells which were not sampled were captured; however, this effect was negligible since the results of cell count and chlorophyll-*a* concentration show almost no sedimentation of *M. aeruginosa* cells after five h of ultrasonication (Figs. 1 and 2). Nevertheless, ultrasonication can temporarily increase the levels of dissolved MCs in the water. The MCs exposed to the US tend to be less toxic, as reported by Hudder et al. (2007), who found that US irradiation of MC-LR effectively reduces hepatotoxicity in mice.

At 24 h of continuous ultrasonication, the concentration of dissolved MCs in the aquatic medium dropped under the detection limit and stayed at that level also after 48 h of continuous ultrasonication. Other authors also reported a decrease in dissolved cyanotoxin concentrations following the initial increase as a result of US treatment (Rajasekhar et al., 2012; Rumyantsev et al., 2021). However, our results are inconsistent with the study of Lürling et al. (2014), where a decrease in cyanotoxin concentrations after US treatment was not observed (continuous operation of commercially available US for 5 days); however, in their study, the levels of dissolved MCs were the same during the experiment indicating no effects of US treatment on MCs synthesis or degradation. In natural water bodies, ultraviolet light (UV) and bacterial degradation are the main mechanisms of MC reduction once MCs are released out of the cells in the water (Edwards et al., 2008; Kaya & Sano, 1998). According to Tsuji et al. (1994), the photochemical breakdown of MCs in full sunlight can take 2 to 6 weeks or even more for a breakdown greater than 90%. A more rapid breakdown under sunlight has been reported in the presence of naturally occurring humic substances, which can act as photosensitisers, with approximately 40% of the MCs degraded per day under summer conditions of insolation (Welker & Steinberg, 1999). In our experiment, tubular fluorescent lamps were employed, which, according to the producer information (OSRAM, Germany), emit

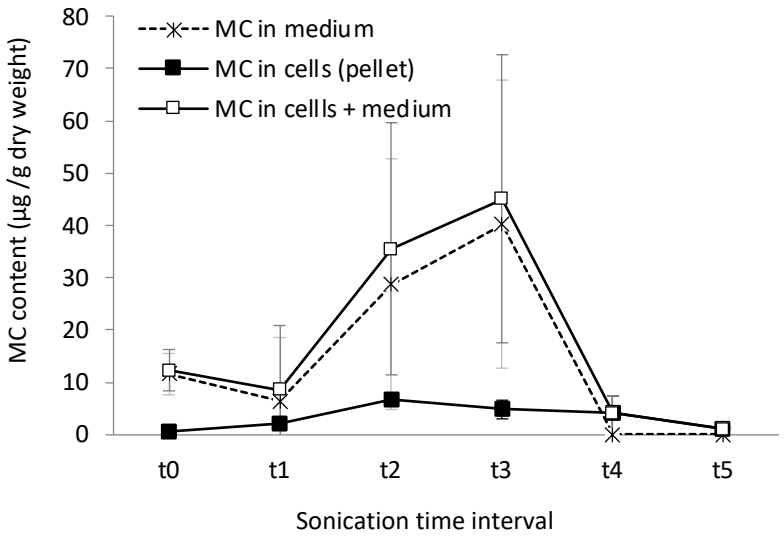

Figure 4. Microcystins (MC) concentration (per dry weight) in the *Microcystis aeruginosa* culture (cells+medium), medium and in the cells (pellet) at different times of continuous ultrasound treatment: t0=0 min, t1=15 min, t2=1 h, t3=5 h, t4=24 h, and t5=48 h of ultrasonication (n=3).

Slika 4. Koncentracija mikrocistinov (MC) (podana kot suha teža) v kulturi cianobakterije *Microcystis aeruginosa* (celice+medij), medij in v celicah (peleti) pri različnih časih neprekinjene obdelave z ultrazvokom: t0=0 min, t1=15 min, t2=1 h, t3=5 h, t4=24 h, and t5=48 h of ultrasonication (n=3).

significantly less UV than sun-light does (wavelength range 380 nm and higher) and thus, it can be hypothesised that the effect of photo-oxidation on MCs was minimal. Bacterial degradation of MCs is often characterised by an initial lag phase lasting from 2 days to more than several weeks with little loss of MCs (Edwards et al., 2008). Once the bacterial biodegradation process commences, the reduction of MCs can be fast, with half-lives of 0.2–5 days for different MCs (Tsuji et al., 2006). Therefore, we can conclude that deterioration and complete cessation of dissolved MCs in the aquatic medium after 24 h of ultrasonication in our experiment is the consequence of US irradiation with photooxidation and bacterial degradation having only minimal impact. According to Song et al. (2005), the principle of US degradation for algal toxins is to degrade MC-LR by attacking the benzene ring and the cracks in the peptide bonds.

Our results are also presented as the reduction rate of dissolved MCs (Fig. 5, where 100% reduction represents MCs concentration where no dissolved MCs were detected). Negative reduction is interpreted as an increase of dissolved MCs in the medium, which is a consequence of ultrasonication-caused reduction or leaking of MCs from the *M. aeruginosa* cells (Fig. 5).

Visual observation of *Microcystis aeruginosa* cells exposed to ultrasonication

M. aeruginosa cells were visually observed under a light microscope (Nikon Eclipse TE 300, Japan) at 600x magnification, and their diameter was measured in order to detect cell size changes after US treatment. According to Turner et al. (2000), cell size change is a direct indicator of cell disruption, and smaller-sized cells can indicate disruption of cells as the effect of US treatment. However, no visual changes or changes in the diameter of the cells were observed. Nevertheless, we cannot conclude, based on these results, that *M. aeruginosa* cells were not damaged by the US because, according to Kong et al. (2019), the US can break up the colloidal sheath outside the cell wall without cell disintegration. In the research of Kong et al. (2019), damaged algal cells released the cytoplasm through a broken breach, but most cells retained an intact peripheral structure, although the internal structure was destroyed. Moreover, *M. aeruginosa* cells are very small, with a diameter of up to 8 µm and for more precise visual observation of such small organisms, electronic microscopy or flow cytometer analyses would be more suitable.

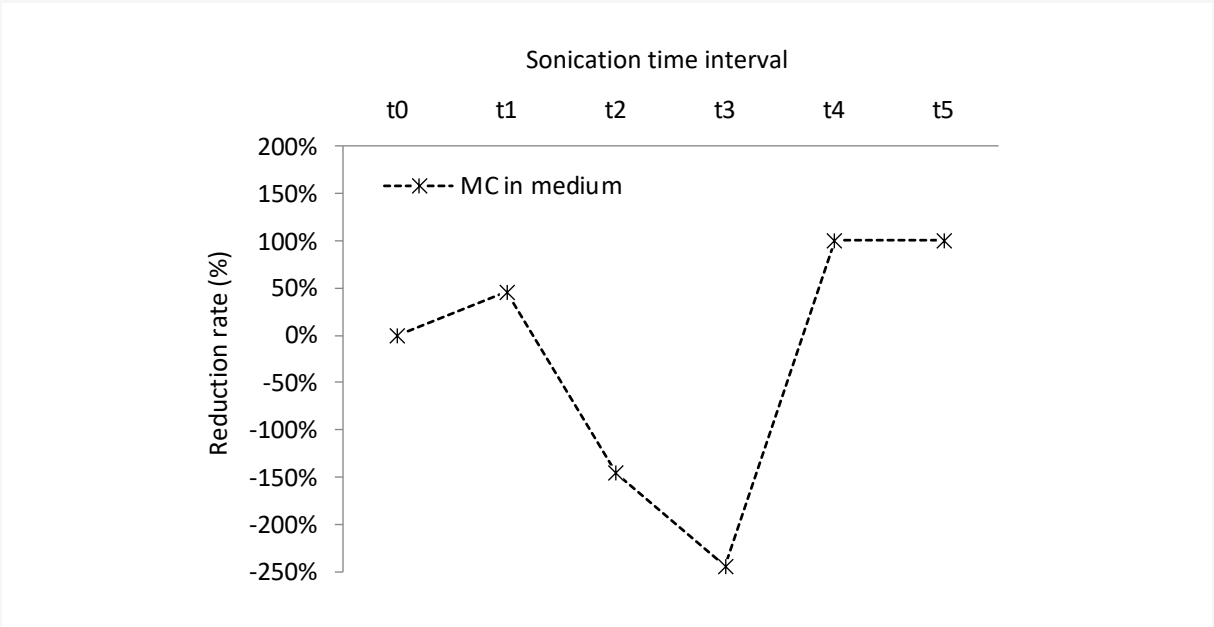

Figure 5. The reduction rate of dissolved microcystins (MC) at different times of continuous ultrasound treatment: t0=0 min, t1=15 min, t2=1 h, t3=5 h, t4=24 h, and t5=48 h (n=3).

Slika 5. Stopnja zmanjšanja raztopljenih mikrocistinov (MC) pri različnih časih neprekinjene obdelave z ultrazvokom: t0=0 min, t1=15 min, t2=1 h, t3=5 h, t4=24 h, and t5=48 h (n=3).

Conclusions

In this research, we studied the effect of commercially available low-frequency and low-intensity ultrasound (US) devices to control algal blooms in natural water bodies regarding cyanobacterial biomass reduction and microcystins (MCs) release and degradation at different times of ultrasonication. In order to come close to natural conditions, a volume of 150 L of dense *Microcystis aeruginosa* suspension was used as a test field. The results showed no biomass reduction in the first five hours of US treatment; however, at 24 hours of continuous US treatment, the reduction in *M. aeruginosa* biomass achieved was 78% and 59% for cell count and chlorophyll-*a*, respectively, with the highest reduction rates of 97% for cell count and 93% for chlorophyll-*a* at 48 h of continuous US treatment. The results of the growth inhibition test showed that the effect of US treatment on *M. aeruginosa* growth increased with the time of ultrasonication and that *M. aeruginosa* cells can repair themselves from the damage caused by ultrasonication. Ultrasonication up to one hour did not affect cell growth at all, while the most efficient in *M. aeruginosa* reduction was the longest tested US treatment of 48 h with growth inhibition of 96%, followed by 24-h US treatment with 50%, and 5-h US treatment with 17% growth inhibition at the end of the 9-day re-growth period.

There are still reservations about using US technology to control cyanobacterial blooms due to the possible release of toxins in the water and related health risks. The results of our study indeed showed an increase in dissolved MCs in the water during the first five hours of ultrasonication, which was related to US-induced stress since no cell lysis was observed; however, after the initial increase, the dissolved MCs concentration dropped under the detection limit at 24 h of continuous US operation and stayed low till the end of the experiment. This indicates the high effectiveness of US in MCs degradation since photooxidation and bacterial degradation, which are the main mechanisms of dissolved MCs reduction in a natural environment, are usually much more time-consuming processes.

We can conclude that low-frequency and low-intensity commercially available US units are very effective in controlling cyanobacterial blooms already at relatively low exposure times of one to two days. The minimal recommended treatment time with such US devices, based on our results, is one day or 24 h to allow ultrasonic technology to degrade dissolved toxins and to avoid possible toxin-related health issues.

Author Contributions

Conceptualization, A.K.K. and T.E.; methodology, A.K.K. and T.E.; investigation, A.K.K. and T.E.; resources, A.K.K.; data curation, A.K.K. and T.E.; writing—original draft preparation, A.K.K.; writing—review and editing, A.K.K. and T.E.; project administration, A.K.K.; funding acquisition, A.K.K. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

References

Ahn, C.-Y., Park, M.-H., Joung, S.-H., Kim, H.-S., Jang, K.-Y., Oh, H.-M., 2003. Growth Inhibition of Cyanobacteria by Ultrasonic Radiation: Laboratory and Enclosure Studies. Environmental Science and Technology, 37(13), 3031–3037. https://doi.org/10.1021/ES034048Z

Chen, G., Ding, X., Zhou, W., 2020. Study on ultrasonic treatment for degradation of Microcystins (MCs). Ultrasonics Sonochemistry, 63, 104900. https://doi.org/10.1016/j.ultsonch.2019.104900

Chorus, I., Welker, M., 2021. Toxic Cyanobacteria in Water. In Toxic Cyanobacteria in Water. CRC Press. https://doi.org/10.1201/9781003081449/

Directive (EU) 2020/2184 of the European Parliament and of the Council of 16 December 2020 on the quality of water intended for human consumption (recast). Off. J. Eur. Union (L 435/1).

Directive 2006/7/EC of the European Parliament and of the Council of 15 February 2006 concerning the management of bathing water quality and repealing Directive 76/160/EEC. Off. J. Eur. Union (L 64/37).

Edwards, C., Graham, D., Fowler, N., Lawton, L.A., 2008. Biodegradation of microcystins and nodularin in freshwaters. Chemosphere, 73(8), 1315–1321. https://doi.org/10.1016/J.CHEMOSPHERE.2008.07.015

Fang, F., Gao, Y., Gan, L., He, X., Yang, L., 2018. Effects of different initial pH and irradiance levels on cyanobacterial colonies from Lake Taihu, China. Journal of Applied Phycology, 30(3), 1777–1793. https://doi.org/10.1007/S10811-018-1394-5

Hamilton, D.P., Wood, S.A., Dietrich, D.R., Puddick, J., 2013. Costs of harmful blooms of freshwater cyanobacteria. In Cyanobacteria: An Economic Perspective (pp. 245–256). Wiley Blackwell. https://doi.org/10.1002/9781118402238.ch15

Harada, K., Suzuki, M., Dahlem, A.M., Beasley, V.R., Carmichael, W.W., Rinehart, K.L., 1988. Improved method for purification of toxic peptides produced by cyanobacteria. Toxicon, 26(5), 433–439. https://doi.org/10.1016/0041-0101(88)90182-1

He, X., Zhang, G., De La Cruz, A.A., O'Shea, K.E., Dionysiou, D.D., 2014. Degradation mechanism of cyanobacterial toxin cylindrospermopsin by hydroxyl radicals in homogeneous UV/H2O2 process. Environmental Science and Technology, 48(8), 4495–4504. https://doi.org/10.1021/ES403732S

Ho, J.C., Michalak, A.M., Pahlevan, N., 2019. Widespread global increase in intense lake phytoplankton blooms since the 1980s. Nature, 574(7780), 667-670. https://doi.org/10.1038/s41586-019-1648-7

Huang, H., Wu, G., Sheng, C., Wu, J., Li, D., Wang, H., 2020. Improved Cyanobacteria Removal from Harmful Algae Blooms by Two-Cycle, Low-Frequency, Low-Density, and Short-Duration Ultrasonic Radiation. Water, 12(9), 2431. https://doi.org/10.3390/w12092431

Hudder, A., Song, W., O'Shea, K.E., Walsh, P.J., 2007. Toxicogenomic evaluation of microcystin-LR treated with ultrasonic irradiation. Toxicology and Applied Pharmacology, 220(3), 357–364. https://doi.org/10.1016/J.TAAP.2007.02.004

Huisman, J., Codd, G.A., Paerl, H.W., Ibelings, B.W., Verspagen, J.M.H., Visser, P.M., 2018. Cyanobacterial blooms. Nature Reviews Microbiology, 16(8), 471–483. https://doi.org/10.1038/s41579-018-0040-1

Jüttner, F., Watson, S.B., 2007. Biochemical and ecological control of geosmin and 2-methylisoborneol in source waters. Applied and Environmental Microbiology, 73(14), 4395–4406. https://doi.org/10.1128/AEM.02250-06

Kaya, K., Sano, T., 1998. A photodetoxification mechanism of the cyanobacterial hepatotoxin microcystin-LR by ultraviolet irradiation. Chemical Research in Toxicology, 11(3), 159–163. https://doi.org/10.1021/TX970132E

Nakano, K., Lee, T.J., Matsumura, M., 2001. In Situ Algal Bloom Control by the Integration of Ultrasonic Radiation and Jet Circulation to Flushing. Environmental Science and Technology, 35(24), 4941–4946. https://doi.org/10.1021/ES010711C

Klemenčič, P., Krivograd Klemenčič, A., 2021a. Effect of ultrasonic algae control devices on non-target organisms : a review. Acta Biologica Slovenica, 64, 5–17. https://doi.org/10.14720/abs.64.1

Klemenčič, P., Krivograd Klemenčič, A., 2021b. The effect of ultrasound for algae growth control on zooplankton. Acta Hydrotechnica, 34(60), 1–9. https://doi.org/10.15292/ACTA.HYDRO.2021.01

Kong, Y., Peng, Y., Zhang, Z., Zhang, M., Zhou, Y., Duan, Z., 2019. Removal of *Microcystis aeruginosa* by ultrasound: Inactivation mechanism and release of algal organic matter. Ultrasonics Sonochemistry, 56, 447–457. https://doi.org/10.1016/J.ULTSONCH.2019.04.017

Li, Y., Shi, X., Zhang, Z., Peng, Y., 2019. Enhanced coagulation by high-frequency ultrasound in *Microcystis aeruginosa*-laden water: Strategies and mechanisms, 55, 232-242. https://doi.org/10.1016/j.ultsonch.2019.01.022

Lürling, M., Meng, D., Faassen, E.J., 2014. Effects of hydrogen peroxide and ultrasound on biomass reduction and toxin release in the cyanobacterium, *microcystis aeruginosa*. Toxins, 6(12), 3260–3280. https://doi.org/10.3390/toxins6123260

Lyon, F., 2010. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans VOLUME 94 Ingested Nitrate and Nitrite, and Cyanobacterial Peptide Toxins.

Mackintosh, C., Beatti, K.A., Klumpp, S., Cohen, P., Codd, G.A., 1990. Cyanobacterial microcystin-LR is a potent and specific inhibitor of protein phosphatases 1 and 2A from both mammals and higher plants. FEBS letters, 264(2), 187-192.

Moheimani, N. R., Borowitzka, M. A., Isdepsky, A., & Sing, S. F. (2012). Standard methods for measuring growth of algae and their composition. In: Borowirzka, M.A., Moheimani, N.R. (Eds.), Algae for biofuels and energy, pp. 265-284. Dordrecht: Springer Netherlands. https://doi.org/10.1007/978-94-007-5479-9_16

OECD, 2011. Test no. 201. Freshwater Alga and Cyanobacteria, Growth Inhibition Test. https://search.oecd.org/env/test-no-201-alga-growth-inhibition-test-9789264069923-en.htm

Paerl, H.W., Huisman, J., 2008. Climate: Blooms like it hot. Science, 320(5872), 57–58. American Association for the Advancement of Science. https://doi.org/10.1126/science.1155398

Peng, Y., Zhang, Z., Wang, M., Shi, X., Zhou, Y., Zhou, Y., Kong, Y., 2020. Inactivation of harmful *Anabaena flos-aquae* by ultrasound irradiation: Cell disruption mechanism and enhanced coagulation. Ultrasonics Sonochemistry, 69, 105254. https://doi.org/10.1016/J.ULTSONCH.2020.105254

Purcell, D., Parsons, S., Jefferson, B., 2013. The influence of ultrasound frequency and power, on the algal species *Microcystis aeruginosa, Aphanizomenon flos-aquae, Scenedesmus subspicatus* and *Melosira* sp. Environmental Technology, 34(17–20), 2477–2490. https://doi.org/10.1080/09593330.2013.773355

Qi, Y., Rosso, L., Sedan, D., Giannuzzi, L., Andrinolo, D., Volmer, D.A., 2014. Seven new microcystin variants discovered from a native *Microcystis aeruginosa* strain-unambiguous assignment of product ions by tandem mass spectrometry. Rapid Commun. Mass Spectrom, 29(2), 220–224. https://doi.org/10.1002/rcm.7098

Rajasekhar, P., Fan, L., Nguyen, T., Roddick, F.A., 2012. Impact of sonication at 20kHz on *Microcystis aeruginosa, Anabaena circinalis* and *Chlorella* sp. Water Research, 46(5), 1473–1481. https://doi.org/10.1016/J.WATRES.2011.11.017

Robin, J., Cravedi, J. P., Hillenweck, A., Deshayes, C., Vallod, D., 2006. Off flavor characterization and origin in French trout farming. Aquaculture, 260(1–4), 128–138. https://doi.org/10.1016/j.aquaculture.2006.05.058

Rumyantsev, A.V.A., Rybakin, V.N., Rudskii, I.V, Korovin, A.N., 2021. The Effects of Low-Intensity Ultrasound on Toxigenic Cyanobacteria. Geoecology, 498, 441-443. https://doi.org/10.1134/S1028334X21050147

Sanseverino, I., Conduto, D., Pozzoli, L., Dobricic, S., Lettieri, T., 2016. Algal bloom and its economic impact. Publications Office of the European Union, Luxembourg. doi:10.2788/660478

Sedmak, B., Eleršek, T., Grach-Pogrebinsky, O., Carmeli, S., Sever, N., Lah, T.T., 2008. Ecotoxicologically relevant cyclic peptides from cyanobacterial bloom (*Planktothrix rubescens*) - A threat to human and environmental health. Radiology and Oncology, 42(2), 102–113. https://doi.org/10.2478/V10019-008-0001-9

Sinha, E., Michalak, A. M., Balaji, V., 2017. Eutrophication will increase during the 21st century as a result of precipitation changes. Science, 357(6349), 405–408. https://doi.org/10.1126/science.aan2409

Song, W., Teshiba, T., Rein, K., O'Shea, K.E., 2005. Ultrasonically Induced Degradation and Detoxification of Microcystin-LR (Cyanobacterial Toxin). Environmental Science and Technology, 39(16), 6300–6305. https://doi.org/10.1021/ES048350Z

Stanier, R. Y., Kunisawa, R., Mandel, M., Cohen-Bazire, G., 1971. Purification and properties of unicellular blue-green algae (order Chroococcales). Bacteriological Reviews, 35(2), 171. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC378380/

Svirčev, Z., Drobac, D., Tokodi, N., Mijović, B., Codd, G.A., Meriluoto, J., 2017. Toxicology of microcystins with reference to cases of human intoxications and epidemiological investigations of exposures to cyanobacteria and cyanotoxins. Archives of Toxicology, 91(2), 621–650. https://doi.org/10.1007/s00204-016-1921-6

Tang, J., Wu, Q., Hao, H., Chen, Y., Wu, M., 2004. Effect of 1.7 MHz ultrasound on a gas-vacuolate cyanobacterium and a gas-vacuole negative cyanobacterium. Colloids and Surfaces B: Biointerfaces, 36(2), 115–121. https://doi.org/10.1016/J.COLSURFB.2004.06.003

Testai, E., Scardala, S., Vichi, S., Buratti, F. M., Funari, E., 2016. Risk to human health associated with the environmental occurrence of cyanobacterial neurotoxic alkaloids anatoxins and saxitoxins. Critical Reviews in Toxicology, 46(5), 385–419. https://doi.org/10.3109/10408444.2015.1137865

Tsuji, K., Asakawa, M., Anzai, Y., Sumino, T., Harada, K., 2006. Degradation of microcystins using immobilized microorganism isolated in an eutrophic lake. Chemosphere, 65(1), 117–124. https://doi.org/10.1016/J.CHEMOSPHERE.2006.02.018

Tsuji, K., Naito, S., Kondo, F., Ishikawa, N., Watanabe, M.F., Suzuki, M., Harada, K., 1994. Stability of microcystins from cyanobacteria: effect of light on decomposition and isomerization. Environmental Science and Technology, 28(1), 173–177. https://doi.org/10.1021/ES00050A024

Turner, K., Porter, J., Pickup, R., Edwards, C., 2000. Changes in viability and macromolecular content of long-term batch cultures of *Salmonella typhimurium* measured by flow cytometry. Journal of Applied Microbiology, 89(1), 90–99. https://doi.org/10.1046/J.1365-2672.2000.01086.X

Van Apeldoorn, M.E., Van Egmond, H.P., Speijers, G.J.A., Bakker, G.J.I., 2007. Toxins of cyanobacteria. Molecular Nutrition and Food Research, 51(1), 7–60. https://doi.org/10.1002/mnfr.200600185

Vollenweider, R.A., 1969. Primary Production in Aquatic Environments. In: Internal Biology Program Handbook (Vol. 12). Blackwell Scientific Publications, Oxford. https://doi.org/10.4319/LO.1970.15.1.0168A

Welker, M., Steinberg, C., 1999. Indirect photolysis of cyanotoxins: one possible mechanism for their low persistence. Water Research, 33(5), 1159–1164. https://doi.org/10.1016/S0043-1354(98)00318-2

WHO, 2003. Guidelines for safe recreational water environments, Volume 1, Coastal and fresh waters, The World Health Organization's (WHO) new Guidelines for Safe Recreational Water.

WHO, 2017. Guidelines for drinking-water quality, 4th edition, incorporating the 1st addendum. http://www.who.int/water_sanitation_health/publications/drinking-water-quality-guidelines-4-including-1st-addendum/en/

Wu, X., Xu, G., Wang, J., 2020. Ultrasound-assisted coagulation for *Microcystis aeruginosa* removal using Fe3O4-loaded carbon nanotubes, RSC Advances, 10(23), 13525-13531. https://doi.org/10.1039/d0ra01530j

Yoshinaga, I., Hitomi, T., Miura, A., Shiratani, E., Miyazaki, T., 2006. Cyanobacterium *Microcystis* bloom in a eutrophicated regulating reservoir. Japan Agricultural Research Quarterly, 40(3), 283–289. https://doi.org/10.6090/jarq.40.283

Zhang, G., Zhang, P., Liu, H., Wang, B., 2006a. Ultrasonic damages on cyanobacterial photosynthesis. Ultrasonics Sonochemistry, 13(6), 501–505. https://doi.org/10.1016/J.ULTSONCH.2005.11.001

Zhang, G., Zhang, P., Wang, B., Liu, H., 2006b. Ultrasonic frequency effects on the removal of *Microcystis aeruginosa*. Ultrasonics Sonochemistry, 13(5), 446–450. https://doi.org/10.1016/J.ULTSONCH.2005.09.012

Zhou, T., Zheng, J., Cao, H., Wang, X., Lou, K., Zhang, X., Tao, Y., 2018. Growth suppression and apoptosis-like cell death in *Microcystis aeruginosa* by H2O2: A new insight into extracellular and intracellular damage pathways. Chemosphere, 211, 1098–1108. https://doi.org/10.1016/J.CHEMOSPHERE.2018.08.042

Zhu, X., Dao, G., Tao, Y., Zhan, X., Hu, H., 2021. A review on control of harmful algal blooms by plant-derived allelochemicals. Journal of Hazardous Materials, 401, 123403. https://doi.org/10.1016/j.jhazmat.2020.123403