

USE OF IONIZING RADIATION FOR THE INHIBITION AND REMOVAL OF CYANOTOXINS IN THE WATER: A REVIEW

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ABSTRACT

Cyanobacteria blooms have been observed in the aquatic systems in different continents, frequently with the production of cyanotoxins that negatively alter water potability. This work provides a small review of the state of the art of the use of advanced oxidation processes in the degradation of cyanobacteria and their toxins. It is divided into 3 major sections: the first part focuses on cyanobacteria and the mechanisms of production related to the environmental conditions. The second part we exposed the guidance values of the evaluation in drinking water for the control of cyanotoxins worldwide, and Brazil regulations. In the third and last part, we present some studies about the use of the advanced oxidation processes for the inhibition and degradation of cyanotoxins, focuses on ionizing radiation: gamma-ray and electron beam irradiation. In conclusion, the ionizing radiation is an efficient and economically viable alternative on the remediation of areas contaminated by cyanobacteria blooms and their toxins, mainly in the reservoirs destined to the water treatment and supply. As well, some suggestions are provided for additional studies about the use of this technology for the treatment of drinking water.

1. GENERAL CHARACTERISTICS OF CYANOBACTERIA

Cyanobacteria, also known as blue algae or cyanophytes, are Gram-negative bacteria [1] and are commonly found in all ecosystems, such as lakes, ponds, hot springs, ultraoligotrophic open rivers and ocean, tropical forests and subsurface soils [1-4]. The fossil record of these prokaryotic organisms occurs through sedimentary rocks dating to about 3.5 billion years ago, and they are probably the first primary producers of organic matter to release elemental oxygen into the primitive atmosphere [5;6].

These microorganisms contain the chlorophyll a, carotene and xanthophyll pigments. The cyanobacteria are distinguished from the other groups due to the presence of phycoerythrin (red) and phycocyanin (blue) pigments [1;4].

The cyanobacteria comprise different forms, being found as unicellular, colonial and multicellular filaments. In the unicellular form, they have spherical, ovoid or cylindrical cells. Nevertheless, the cells can aggregate in irregular colonies, being united by the viscous matrix secreted during the growth of the colonies. Ordered colonies can be produced, combined with sheath secretions by means of regular series of cell division [7-9].

In contrast to eukaryotic microalgae, cyanobacteria do not have defined nuclei, thus resembling bacteria. Furthermore, they do not have membrane-associated photosynthesis organelles, as the photosynthetic pigments are in the free thylakoids in the cytoplasm. Cellular forms are simple

and species that form colonies are usually protected by a well-developed mucilaginous sheath [1].

2. THE RELATION BETWEEN EUTROPHICATION AND CYANOBACTERIA BLOOMS

Eutrophication is the enrichment of rivers, lakes, and reservoirs, caused by increased levels of nutrients, usually phosphorus and nitrogen compounds [8], and thus enhancing the growth of phytoplanktonic organisms.

This enrichment of water influences the formation of cyanobacteria blooms. However, these blooms are also currently related to the discharge of anthropogenic phosphorus inputs into surface waters by sewage disposal, industrial activities, and extensive agricultural practices. Studies have suggested that blooms are also caused by a complex interaction of high concentrations of nutrients, sunlight, temperature, turbidity, pH, conductivity, salinity, carbon availability and water flow [1;10].

The climatic conditions of a region play an important role in the time and duration of the flowering of cyanobacteria. In temperate regions, cyanobacteria blooms arise during late summer and early fall, lasting between 2 and 4 months. In contrast, the flowering season may begin earlier and persist for longer in regions with the well-defined Mediterranean or subtropical climates. In tropical or subtropical areas, such as China, Brazil, and Australia, under favorable environmental conditions, the flowering of cyanobacteria can occur during most of the year [10]. As a matter of fact, the significant presence of cyanobacteria blooms raised some concerns about its concentration in various environments, such as marine and aquatic. These concerns are based on previous investigations showing that these blooms may be toxic. In fact, it is estimated that between 25 and 70% of the world's cyanobacterial blooms are potentially toxic [3]. The major genera producing toxins are *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Lyngbya*, *Microcystis*, *Nostoc* and *Oscillatoria* [9]. In the last decades in Brazil, the presence of cyanobacteria potentially toxic were recorded. It was registered the following: *Cylindrospermopsis*; *Microcystis*; *Planktothrix*; *Aphanizomenon*; *Oscillatoria*; *Anabaena*; *Dolichospermum*; *Raphidiopsis* genera [2;7].

3. CYANOTOXINS

Cyanobacterial toxins, or cyanotoxins, are toxic secondary metabolites and responsible for acute and chronic poisoning of wild/domestic animals and human. Cyanotoxins are divided into hepatotoxins (microcystin, nodularin), cytotoxins (cylindrospermopsin), neurotoxins (anatoxins, saxitoxins, β -methylamino-L-alanine), and dermatotoxins (lipopolysaccharide, lyngbyatoxins, aplysiatoxin) [10;11].

The release of these toxins from the cells into water occurs mainly during cellular senescence, death, and lysis, rather than continuous excretion. It is enhanced by chemical treatments for the eradication of cyanobacteria, especially the use of algicides (copper sulfate or organic herbicides). Worldwide, the hepatotoxins are the most studied group and known toxins. Microcystins (MC) are among the most widespread cyanobacterial toxins detected in freshwaters.

3.1. Microcystins and Nodularins: Hepatotoxins

Hepatotoxin intoxication is the most common and documented worldwide. It has a slower action, causing death between hours and few days, inducing intrahepatic hemorrhage and hypovolemic shock (Carmichael & Schwartz, 1984; Beasley et al., 1989). The genera identified as producers of hepatotoxins are *Microcystis*, *Oscillatoria*, *Aphanizomenon*, *Anabaena*, *Planktothrix*, *Nodularia* and *Nostoc* [11]. It was described two types of hepatotoxins: cyclic heptapeptides and cyclic pentapeptide.

The cyclic heptapeptides are known as microcystins (MCs) and they are found in freshwater cyanobacterial blooms of *Microcystis* genera. Microcystins are named after *Microcystis aeruginosa*, the cyanobacteria in which the toxin was first isolated and described [12]. These MCs are produced by numerous genera of cyanobacteria such as *Oscillatoria*, *Aphanizomenon*, *Anabaena*, *Planktothrix*, and *Anabaenopsis*. For microcystins, the qualitative variations observed in their two L-amino acids were used to designate the different microcystins, for example, MC-LR (leucine-arginine); -MC-RR (arginine-arginine); MC-YA (tyrosine-alanine) [10].

The nodularins (NODs) were first identified in the species *Nodularia spumigena* [12;13]. Nowadays, eight nodular toxins are known, classified according to variations in the degree of methylation, composition, and isomerization of their amino acids [14]. NODs production has been reported only for species *Nodularia spumigena*, *Nodularia sphaerocarpa* and for the genera *Nostoc*.

Globally, the cyanotoxins most frequently found in cyanobacteria flowering freshwater are the cyclic peptide toxins of the microcystin and nodularin families [10]. The most serious known episode associated with human MC exposure occurred in Caruaru, Brazil, where 54 of the 130 hemodialysis patients died after treatment with water accidentally contaminated with MC [14].

3.2. Anatoxins, Saxitoxins, and BMAA: Neurotoxins

Neurotoxins are produced by the genera *Anabaena* [5], *Aphanizomenon* [6], *Oscillatoria*, and *Cylindrospermopsis* [12]. At least five neurotoxins produced from species of these genera are known.

The most studied cyanobacterial neurotoxins are alkaloids compounds, namely, anatoxins and saxitoxins (also known as the paralytic toxins of shellfish-PSPs, mainly produced by marine dinoflagellates [15]. Cyanobacterial alkaloid neurotoxins act on the cholinergic synapses or ion-dependent voltage channels, blocking the skeletal and/or respiratory muscles; in mammals, death can occur in lethal doses due to respiratory failure [15;16].

Anatoxin-a (ATX) was the first cyanobacterial toxin to be chemically and functionally defined and is a secondary amine bicyclic alkaloid [17]. Signs of acute poisoning by ATX in wild and domestic animals include death by respiratory arrest and occurs from a few minutes to a few hours, depending on the dosage. Clinical signs of intoxication show a progression of muscle fasciculation, decreased movement, exaggerated abdominal breathing, cyanosis, and convulsion. This neurotoxic alkaloid is a potent post-synaptic neuromuscular blocker of nicotinic and

cholinergic receptors. This action occurs because the anatoxin-a binds irreversibly to acetylcholine receptors, as it is not degraded by acetylcholinesterase.

Anatoxin-a(S) (ATX-s) is another neurotoxin, later characterized, that shows the same signs of toxicity of the anatoxin-a, added to the intense salivation. This neurotoxin has a mechanism of action similar to anatoxin-a, as it inhibits the action of acetylcholinesterase, preventing the degradation of acetylcholine-gated receptors.

Saxitoxin (STX) is a group of more than 30 natural alkaloids produced by some genera of marine dinoflagellates, but also by freshwater cyanobacteria blooms. They are usually grouped into carbamate (STX, neoSTX, and GTX1-4), sulfamate (GTX Toxins 5-6, C1-4) and decarbamol (dcSTX, dcneoSTX, dcGTX1-4) based on the substituent at position R4 [2].

Finally, the last neurotoxin described is β -N-methylamino-L-alanine (BMAA). It is a non-proteinogenic amino acid produced by diverse cyanobacteria [18] and it has been associated with neurodegenerative disease. As BMAA is not lipophilic, it would not be expected to biomagnify, yet the indirect evidence suggests that it can biomagnify in at least certain circumstances [19].

3.3. Cylindrospermopsin: Cytotoxins

The cylindrospermopsin (CYN) was isolated in 1992 for the first time from the cyanobacteria *Cylindrospermopsis raciborskii* but it is also biosynthesized by other cyanobacteria genera including *Anabaena*, *Aphanizomenon*, and *Raphidopsis*. It is classified as cytotoxin because it can affect both the liver (hepatotoxic) and the nervous system (neurotoxic) [2;11].

4. GUIDANCE VALUES FOR CYANOTOXINS

According to the World Health Organization (WHO), the maximal acceptable MC-LR concentration in drinking water is equal to $1 \mu\text{g}\cdot\text{L}^{-1}$. This guidance value has been adopted by different countries in Europe, South America, and Africa. In Australia, the guidance value for this hepatotoxin in drinking water is equivalent to $1.3 \mu\text{g}\cdot\text{L}^{-1}$, and in Canada, the provisional maximum value for MC-LR has been fixed at $1.5 \mu\text{g}\cdot\text{L}^{-1}$ [11].

Specifically, in Brazil, the guidance values for cyanotoxins is determined by Health Ministry (Regulation 2914/2011), which provides the procedures and responsibilities related to the control and monitoring of water quality for human consumption and standards of potability. Regulation 2914/2011 determines the monthly monitoring at the point of water captation when the number of cyanobacteria cells does not exceed $10.000 \text{ cells mL}^{-1}$. When a value of more than $20.000 \text{ cells mL}^{-1}$ of cyanobacteria occurs, the weekly analysis of cyanotoxins must be performed, mainly for analyzing microcystins and saxitoxins due to their acute and carcinogenic effect.

For cylindrospermopsin, Regulation 2914/2011 recommends this analysis whenever the presence of genera potentially toxin-producing are detected, with the maximum acceptable value of $1.0 \mu\text{g L}^{-1}$. For the presence of anatoxin-a (s), it should be analyzed when the presence of cyanobacteria genera with the potential of producing this cyanotoxin is spotted during the monitoring of the water body. It is worth mentioning that there is no determination of an acceptable maximum limit (AML) regarding anatoxin-a.

In Brazil, the most used technology in water treatment is the conventional one, composed of coagulation, flocculation, decantation or flotation, filtration, disinfection, pH correction, and fluoridation [20]. In natural waters with high algae concentration, the use of flotation guarantees better removal efficiency in relation to the sedimentation. If decantation is adopted, periodic removal of sludge at the bottom of the tank is necessary to reduce the holding time of the retained cells, thus avoiding the release of toxins into the water [20] granular and powdered activated carbon are suitable if addressing extracellular cyanotoxins [21].

5. ADVANCED OXIDATION PROCESSES FOR THE REMOVAL OF CYANOBACTERIA IN WATER

Advanced Oxidation (AOP) processes are characterized by the formation of reactive molecules, such as hydroxyl radicals (HO•). they are known to utilize their high reactivity for the degradation of persistent organic compounds. The hydroxyl radical is a powerful oxidant, of short duration, being highly reactive and non-selective [22]. HO• has electrophilic properties and its reactions with appropriate substrate molecules are kinetically controlled, generally exhibiting second order very high rate constants, which are often close to (or even above) the diffusion-controlled boundary [23]. In addition, the hydroxyl radical is a transient species of ubiquitous nature and is a very important agent in numerous diseases or human disorders and in the aging process [24].

Ionizing radiation generates highly reactive products (electrons, free radicals, ions, excited species) in any system. Among the existing AOPs, Fenton, photo-Fenton, UV / H₂O₂, ozonization, photocatalytic processes, and ionizing radiation stand out, the latter being an efficient method of generating radicals and, therefore, provides oxidation and degradation of organic molecules [25].

4.1. Gamma-ray radiation

Irradiations with Gamma rays are described also as ionizing radiation. The absorbed energy in the material due to irradiation generates instability with atoms and molecules, producing free radicals in cells. The radicals can damage or modify important components of plant cells and affect the morphology, anatomy, biochemistry, and physiology of plants depending on the dose [26].

The high efficiency of gamma-ray irradiation to the treatment of odoriferous compounds geosmin and methylisoborneol (MIB) from drinking water was demonstrated. These organic compounds are formed by algal proliferation, specifically cyanobacteria, which confer earthy and moldy taste and odor to the water. The samples of raw water and after treatment of Guarapiranga reservoir (São Paulo - Brazil) were tested with 0.5, 1.0, 2.0, and 3.0 kGy. All the doses were effective in removing these compounds from water [27].

The *Microcystis panniformis* was exposed to three treatments: 4 kGy (6 hours and 27 minutes exposition), equivalent to a dose rate of 0.551 kGy h⁻¹; 5 kGy and 6 kGy. The results showed that this toxic cyanobacterium is resistant to doses lower than 4 kGy. On the other hand, doses higher than 4 kGy are efficient for the control of *M. Panniformis* [28].

Doses from 1 to 9 kGy to evaluate the effects in *Microcystis aeruginosa*. The study demonstrated that the removal efficiency of chlorophyll-a concentration decreased at 5 days after gamma irradiation. It was observed the removal of 98% of the pigment when treated with 9 kGy. The samples submitted to 2 – 5 kGy enhanced the activity of superoxide dismutase (SOD) and peroxidase (POD) in *M. aeruginosa* cells, but the activity of SOD and POD decreased at higher doses (6–9 kGy). The lipid peroxidation in *M. aeruginosa* increased as the radiation dose increased [30].

4.2. Electron beam irradiation (EBI)

Electron beam irradiation (EBI) is widely used in sterilization of medical devices and materials modification, food pasteurization, etc. The exposure of water to EBI produces ionized and excited water molecules and free electrons. These radicals are highly reactive and responsible for breaking the organic compounds in wastewater. In general, the products of EBI are hydrated electron (e_{aq}^-), hydroxyl radical ($HO\bullet$), and hydrogen radical ($H\bullet$) in water and wastewater, according to the equations 1 to 4 [30]:



The electron beam accelerator was applied for irradiations of cyanobacteria at 2.0 kGy up to 5.0 kGy. They obtained efficient removal of *M. aeruginosa*, achieving 91% after 4 kGy decreased the Chl a concentration and photosynthesis rate [31].

The effect of EBI at different doses (1 and 5 kGy) on the production, release, and degradation of microcystin was studied. It was observed that 1kGy presented satisfactory results, as the production and the accumulation of MC in cells. At doses between 2 and 5 kGy, it was possible to inhibit MC production inside *M. aeruginosa* cells. The results demonstrated that ionizing radiation is a viable technique for the remediation of water bodies contaminated with microcystin-based cyanobacteria and their associated toxins [32].

Table 1 shows the chemical compound and cyanobacteria treated with gamma-ray and EBI and the efficiency of each treatment.

Table 1: Comparison between electron-beam and gamma-ray for the inhibition to *M. aeruginosa* and organic compound produced by cyanobacteria according to literature.

	AOP ionizing radiation	Doses (kGy)	Results	Reference
Odoriferous compounds geosmin and methylisoborneol (MIB)		0.5, 1.0, 2.0, and 3.0 kGy.	All the doses were effective in removing these compounds from water.	Duarte et al. (2008)
<i>Microcystis panniformis</i>	Gamma-ray	4.0 to 6.0 kGy *	The doses higher than 4 kGy are efficient for the control of <i>M. panniformis</i> .	Cavalcante-Silva et al. (2010)
<i>Microcystis aeruginosa</i>		1.0 to 9 kGy	The removal efficiency of <i>M. aeruginosa</i> was 98% after 5 days of culture at 9 kGy.	Zheng et al. (2012)
<i>Microcystis aeruginosa</i>		1.0 to 5.0 kGy	The removal efficiency of <i>M. aeruginosa</i> reached 91% when it is subjected to 4 kGy EBI.	Liu et al. (2014)
	Electron beam			
<i>Microcystis aeruginosa</i>		1.0 to 5.0 kGy	The doses 2 to 5 kGy EBI could inhibit the MC production in <i>M. aeruginosa</i> cells.	Liu et al. (2015)

*Dose rate of 0.551 kGy h⁻¹.

3. CONCLUSIONS

The risk of exposure to cyanotoxins for human and animal health occurs due to the increasing demand of water resources for agricultural activities, irrigation, recreation, and public supply. Avoiding aquatic contamination is crucial to controlling the flowering of cyanobacteria.

In order for risk management procedures in this environmental resource to be effective, advances in knowledge are necessary for the adoption of appropriate regulations for the protection of human and animal health. In this way, new technologies for the remediation of water bodies already affected by cyanobacteria are necessary.

Advanced oxidation processes, specifically ionizing radiations, appear to be a promising technology for the destruction of cyanobacterial cells and/or cyanotoxins in drinking water. Further research is needed to further develop these technologies and develop systems suitable for large-scale treatment.

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