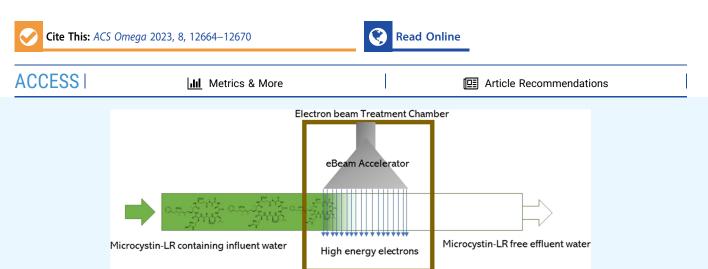




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Applicability of Electron Beam Technology for the Degradation of Microcystin-LR in Surface Waters

Alexandra M. Folcik, Shelby A. Ruggles, and Suresh D. Pillai*



ABSTRACT: Studies were performed to investigate the effects of surface water quality parameters on the degradation of microcystin-LR (MC-LR) using high-energy electron beam (eBeam) technology. Surface water samples were collected across different geographic locations in the United States. Water quality parameters including pH, alkalinity, TDS, and dissolved oxygen were measured in all samples. Degradation of MC-LR in all samples, regardless of parameter concentrations, was above 99%. The effect of natural organic matter (NOM) on MC-LR degradation was also investigated in the presence of fulvic acid. Similarly, the degradation efficiency of MC-LR exceeded 99% for all concentrations of fulvic acid at 5 kGy. This study suggests that surface water quality has a negligible effect on the degradation of MC-LR via eBeam treatment. The results indicate that eBeam technology is a promising technique for the treatment of water contaminated with microcystins.

1. INTRODUCTION

In 2017, the World Health Organization (WHO) estimated over 2.2 billion people throughout the world were without safely managed drinking water services. Drinking water comes primarily from two sources: ground water (aquifers) and surface water (such as streams, rivers, and lakes). In the United States, roughly 60% of drinking water is drawn from surface water sources. There are a variety of manmade and natural pollutants present in surface waters that complicate the availability of safe and affordable drinking water, but nonpoint source pollution from sources such as agricultural or stormwater runoff are leading causes of water pollution within the United States.

Nonpoint source pollution contributes to increased concentrations of nutrients such as nitrogen (N) and phosphorus (P) resulting in eutrophication of waterbodies which has been linked to the promotion of harmful algal blooms (HABs). Freshwater blooms are often associated with photosynthetic prokaryotic organisms (cyanobacteria; cyanoHABs) and are responsible for producing a range of neuro- and hepatotoxic secondary metabolites termed cyanotoxins. Of particular importance to water quality and human health are a common class of cyanotoxins called

microcystins (MCs). MCs are a group of over 200 hepatotoxic variants that have been found in blooms around the world. Microcystin-LR (MC-LR) is the most common and toxic variant of the MCs. During extensive blooms, these toxins may enter drinking water treatment plants, and if not treated effectively, can contaminate finished tap water.

Across the United States, surface waters have differing water chemistries depending on geographic location, amounts and types of pollution, and usage of the waterbody. These chemical characteristics can affect the efficacy of water treatment and therefore underscore the importance of water treatment technologies that are less parameter-specific. Electron beam technology utilizes ionizing radiation generated from commercial electricity and has been shown effective for degradation of both dissolved and suspended pollutants, as well as pathogens,

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Figure 1. Sampling locations of water samples used in this study. An interactive version of this map can be accessed at https://bit.ly/2UeZhAO. Credit: Google Maps.

in water. 8-11 High-energy electron beam (eBeam) irradiation technology presents a viable addition to current water treatment strategies for many emerging contaminants, including MCs. 12 Unlike other advanced oxidative processes (AOPs), eBeam acts as an advanced oxidative and reductive process (AORP) by creating both oxidative and reductive reactive species through the radiolysis of water. This characteristic aids in the broad range of pollutants eBeam is able to target.

There have been a few studies in the literature suggesting the applicability of this technology for the degradation of algal toxins. ^{12–15} We have also critically reviewed the literature surrounding the use of ionizing technology for the remediation of waters containing microcystin-LR. ¹⁶ While these studies broadly suggest that eBeam could be suitable for cyanobacteria and cyanotoxin degradation, it needs to be emphasized that the energy of the electrons employed, and the assays used to demonstrate the degradation of the toxin, need be applicable for environmental applications.

The goal of this study was to employ high-energy eBeam treatment on water samples collected from around the United States to understand how water quality affects MC-LR degradation. The underlying hypothesis was that the degradation efficiency of MC-LR by eBeam would be unaffected by pH, alkalinity, total dissolved solids (TDS), and dissolved oxygen and natural organic matter (fulvic acid). Therefore, the goal of this study was to obtain surface water samples with differing chemical characteristics from across the United States, amend these samples with known concentrations of MC-LR, and expose these samples to 5 kGy eBeam dose. The work presented here is part of A.M. Folcik's dissertation research.

2. MATERIALS AND METHODS

2.1. Sampling. Samples were solicited from 22 locations throughout the United States as representative samples for the various geographic regions in the lower 48 states and Hawaii

(Figure 1). Surface water sources that supply drinking water in each location were determined using the US EPA's Drinking Water Mapping Application to Protect Source Waters (DWMAPS).¹⁷ Volunteers were provided with three 60 mL low-density polyethylene bottles (Thermo Fisher Scientific, Waltham, MA), a water sampling protocol, and were asked to take all samples in triplicates. Samples were then mailed back to College Station, TX, where they were stored for further analysis.

2.2. Water Chemistry. The pH, alkalinity, TDS, and dissolved oxygen were measured in samples to understand the diversity of the water chemistries from the different locations. pH was measured with a Manual 430 pH Meter (Corning, Corning, NY) and calibrated with reference standards pH 4.00 \pm 0.01 and 10.00 \pm 0.01 (VWR International, Radnor, PA). A HI775-Alkalinity handheld colorimeter (Hanna Instruments, Woonsocket, RI) was used to measure alkalinity levels. The instrument range was 0-500 mg/L CaCO₃. TDS was measured using a Traceable Conductivity/TDS Pocket Tester with Calibration Meter (Cole-Parmer, Chicago, IL). The instrument range was 0-1999 mg/L. The TDS factor was set to 0.66, the temperature normalization value was set to 25 °C, and the temperature compensation coefficient was set to 2.0% as recommended for this instrument when measuring freshwater. A Model 830 Dissolved Oxygen Meter (Orion, Beverly, MA) was calibrated and used to measure the dissolved oxygen within the water samples. The probe was inserted into the samples and gently stirred until the readings were stable.

2.3. Electron Beam Treatment. Electron beam irradiation dosing was performed at Texas A&M University's National Center for Electron Beam Research in College Station, TX. A high-energy (10 MeV), 15 kW pulsed S-band linear accelerator was used (dose rate 3 kGy/s). L-α-Alanine dosimeters and EPR-based spectroscopy using the Bruker escan reader (Billerica, MA) were used to confirm dose received. Initial dosing experiments were conducted to determine the dose used for spiked surface water samples.

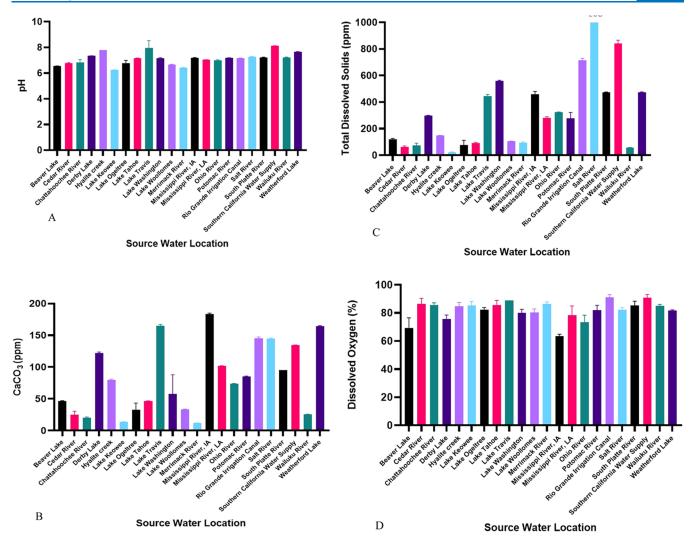


Figure 2. (A) pH of surface water samples collected from around the United States. (B) Alkalinity of surface water samples collected from around the United States. (C) Total dissolved solids in surface water samples collected from around the United States. (D) Dissolved oxygen in surface water samples collected from around the United States.

This was completed using 3 mg/L MC-LR spiked in deionized water. The target doses were 1, 2, and 5 kGy, and doses received were 1.27, 2.04, and 5.05 kGy. These doses were chosen based on our previous research. A 5 kGy dose resulted in a 98% reduction from nontreated samples and was therefore used as the dose for remaining experiments. The experimental samples were exposed to a target dose of 5 kGy in 2 mL glass screw-thread vials (VWR International, Radnor, PA). Preliminary dose-mapping studies were performed on vials used for irradiation to confirm dose uniformity ratio.

2.4. Water Samples Treatment and Quantification. For surface water samples, 1 mL aliquots were spiked with 3 mg/L microcystin-LR (purity \geq 95%, Cayman Chemical, Ann Arbor, MI). Samples were irradiated at a target dose of 5 kGy, and the actual dose received was 5.11 \pm 0.079 kGy. Quantification of MC-LR after irradiation was determined biologically using the EPA-preferred ADDA-specific ELISA kit (Eurofins Abraxis, Inc., Warminster, PA). The ELISA kit standard curve ranged from 0.15 to 5.0 μ g/L. Plates were read on a Synergy H1 Hybrid Multi-Mode Microplate Reader (Biotek, Winooski, VT) using Gen5 Microplate Reader and Imager software.

2.5. Effect of Fulvic Acid. The effect of NOM was additionally investigated using FA (98.3% purity, AdipoGen Life Sciences, San Diego, CA). Samples were prepared to contain 0, 50, or $100~\mu g/L$ fulvic acid and 2 mg/L MC-LR. Samples were irradiated at a target dose of 5 kGy, and the actual dose received was 5.11 ± 0.079 kGy. Similarly, MC-LR in samples was quantified using an ADDA-specific ELISA kit (Eurofins Abraxis, Inc., Warminster, PA).

2.6. Data Analysis. The data were statistically analyzed and visualized using commercially available GraphPad Prism software, version 9.1.2 (GraphPad Software, San Diego, CA). Shapiro—Wilk tests and qq plots were used to verify the normality of data. According to these results, a two-way ANOVA was used followed by Šidák's multiple comparison test. The tests were performed with a significance of 95% (p < 0.05).

3. RESULTS AND DISCUSSION

3.1. Chemistry of Surface Water Samples. Surface water quality in the United States varies both temporally and spatially depending upon water volume, sediment composition, biodiversity, and other stressors and pollution. ^{19,20} In this study, samples were solicited from 22 locations across the

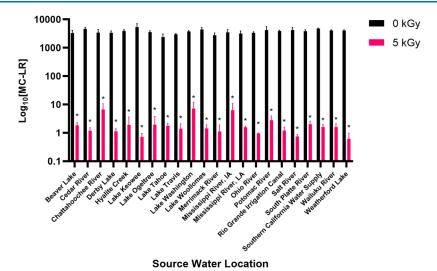


Figure 3. MC-LR degradation in surface water samples following eBeam treatment at 5.11 ± 0.079 kGy ($p \le 0.0001$; error bars represent standard deviation).

Table 1. Water Samples with the Highest Remaining MC-LR Following 5.11 ± 0.079 kGy eBeam Treatment

source water location	pН	alkalinity (mg/L of CaCO ₃)	TDS (mg/L)	dissolved oxygen (%)	MC-LR remaining (%)
Chattahoochee River, GA	6.81 ± 0.25	20.33 ± 1.15	75.33 ± 15.04	85.67 ± 1.53	0.19
Lake Washington, FL	7.15 ± 0.04	57.66 ± 30.24	558.67 ± 4.73	80 ± 2.65	0.19
Mississippi River, IA	7.20 ± 0.02	183.33 ± 1.53	459 ± 21.70	63.33 ± 1.53	0.18

lower 48 states and Hawaii to represent geographic differences in surface water composition (Figure 1; https://bit.ly/2UeZhAO). Samples were then analyzed for pH, alkalinity, TDS, and dissolved oxygen (Figure 2).

Generally, the pH range in surface water systems is 6.5-8.5 as corroborated by the water samples obtained in this study. PH remained relatively consistent among sample locations with Lake Keowee, SC, having the lowest pH (6.24 ± 0.026) and the Southern California Water Supply having the highest pH (8.12 ± 0.02) (Figure 2A). The pH can affect a variety of chemical and biological processes in water by altering the solubility, transport, and bioavailability of many chemicals and pollutants. Low pH has been associated with nearby mining operations, industrial effluents, and agricultural runoff while high pH has been associated with alkaline geology and soils, oil and gas brines, and limestone gravel roads in the waterbody vicinity. 22

Alkalinity is related to the acid-neutralizing capacity of a liquid due to the presence of chemical species such as bicarbonate, carbonates, and hydroxides. Alkalinity measurements in sample locations had much more variability ranging from 11.67 to 183.33 mg/L CaCO3 in the Merrimack River, NH, and the Mississippi River, IA, respectively (Figure 2B). Alkalinity is most often determined from the rocks and sediments surrounding the body of water. Alkalinity normally ranges from 20 to 200 mg/L CaCO3 but has the potential to exceed 400 mg/L CaCO3 in areas with high amounts of urban runoff or limestone application.

Total dissolved solids represent the sum of all organic and inorganic substances dissolved in water. Measurable sample TDS values ranged from 21.33 to 840.67 in samples from Lake Keowee, SC, and the Southern California Water Supply, respectively (Figure 2C). However, one sample location, Salt River, AZ, was above our limit of detection (LOD). TDS is not often considered a health hazard, but instead the US EPA

includes TDS as a voluntary guideline for water quality. ²⁴ TDS is often indicative of ionic strength within a waterbody and amounts of TDS influence mineral content in water. ²⁵ High TDS content is more likely in ground water than surface water, but high TDS content may cause toxicity to aquatic organisms due to mineral shifts as well as corrosion of plumbing fixtures. Concentrations greater than 500 mg/L are not recommended for drinking water. ²⁶

Finally, dissolved oxygen is a measure of the oxygen gas incorporated in the water. Dissolved oxygen in samples ranged from 63.33% in the Mississippi River, IA, samples, to 91% in the Rio Grande Irrigation Canal, TX, samples (Figure 2D). Oxygen normally enters the water through direct atmospheric absorption or through the production of oxygen via aquatic plants.²⁷ Low dissolved oxygen is far more likely than excessive dissolved oxygen and can result from algal blooms, high temperature, and ammonia content. Healthy dissolved oxygen levels typically range from 80 to 120% (6.5–8 mg/L), and values of less than 2 mg/L indicate hypoxic zones.²⁸

Overall, pH, alkalinity, TDS, and dissolved oxygen values measured in samples were within expected ranges for each parameter, respectively. High alkalinity was observed in more urban locations such as Lake Travis, TX, Weatherford Lake, TX, the Mississippi River, and Salt River, AZ. However less urban locations that still had high alkalinity measurements included Hyalite Creek, MT, and Derby Lake, VT. This may be indicative of sediment composition lending to high alkalinity or in the case of Derby Lake, VT, the presence of dairy farming nearby (Figure 2B). Similar locations also contained high amounts of TDS. The Southern California Water Supply and Salt River, AZ, samples contained the greatest amount of TDS at 840.68 ± 24.7 and >LOD, respectively. This may be due to runoff and leaching from natural deposits in both locations as most of Southern

Table 2. Water Samples with the Lowest Remaining MC-LR Following 5.11 ± 0.079 kGy eBeam Treatment

source water location	pН	alkalinity (mg/L of $CaCO_3$)	TDS (mg/L)	dissolved oxygen (%)	MC-LR remaining (%)
Lake Keowee, SC	6.24 ± 0.03	13.67 ± 0.58	21.33 ± 4.04	85.33 ± 2.89	0.01
Lake Woollomes, CA	6.67 ± 0.02	33.33 ± 0.58	105.67 ± 1.53	80.33 ± 2.52	0.02
Salt River, AZ	7.25 ± 0.04	144.67 ± 1.15	>LOQ?	82.33 ± 1.53	0.02

California's water supply is imported from the Colorado River. 29,30

3.2. Effect of Water Quality on MC-LR Degradation. MC-LR in all 22 source water samples were significantly reduced with removal efficiencies ranging from 99.81 to 99.98% (Figure 3). Locations with the highest remaining MC-LR following eBeam treatment were the Chattahoochee River, GA $(6.63 \pm 3.98 \ \mu\text{g/L})$ from $3423.05 \pm 1036.12 \ \mu\text{g/L}$), Lake Washington, FL $(7.28 \pm 4.78 \ \mu\text{g/L})$ from $3747.16 \pm 321.97 \ \mu\text{g/L}$), and the Mississippi River, IA $(6.31 \pm 4.57 \ \mu\text{g/L})$ from $3454.74 \pm 921.93 \ \mu\text{g/L}$). Despite this, the three locations did not show similarities in their alkalinity, TDS, or dissolved oxygen content (Table 1).

Locations with the greatest MC-LR degradation following eBeam treatment were Lake Keowee, SC (0.73 \pm 0.23 μ g/L from 5335.54 \pm 1774.63 μ g/L), Lake Woollomes, CA (1.45 \pm 0.48 μ g/L from 4036.70 \pm 226.63 μ g/L), and Salt River, AZ (0.75 \pm 0.13 μ g/L from 4185.93 \pm 1031.87 μ g/L). Similarly, no trends were observed between any measured parameters and MC-LR breakdown (Table 2).

pH, alkalinity, TDS, and dissolved oxygen were chosen as measured parameters due to their noted ability in the literature to affect MC-LR breakdown in various oxidative treatment strategies.^{31–33} All water quality parameters, especially alkalinity and TDS, ranged widely in collected surface water samples. Regardless of each sample's water chemistry, degradation efficiency in all samples exceeded 99%, and there were no trends observed between water parameters and degradation. For example, the Salt River, AZ, samples and the Mississippi River, IA, samples both contained high measured alkalinity (144.67 \pm 1.15 mg/L of CaCO₃ and 183.33 \pm 1.53 mg/L of CaCO₃, respectively). Despite this similarity, the Salt River, AZ, samples showed 99.9% degradation efficiency with 5 kGy-treated samples containing 0.75 \pm 0.13 μ g/L MC-LR, and the Mississippi River, IA, samples showed 99.8% degradation efficiency with 5 kGy-treated samples containing 6.31 \pm 4.57 μ g/L MC-LR. Nevertheless, these differences of MC-LR degradation efficiency are overall negligible. These results suggest that eBeam treatment of MC-LR in surface water is not water-quality-dependent and further underscores the utility of eBeam technology over other chemical treatments.

3.3. Fulvic Acid Effects on MC-LR Degradation. NOM has been shown to have negative impacts on MC-LR degradation for a variety of AOPs. 31,32,34,35 As with the parameters previously discussed, NOM can influence the concentration of radicals and their contributions to MC-LR degradation. NOM consists mainly of fulvic acid and other humic substances and may act as a scavenger for hydroxyl radicals depending on solution pH. In this experiment, fulvic acid was utilized to study the effects of NOM on eBeam degradation of MC-LR (Figure 4). Samples containing both 50 and 100 μ g/L FA showed an overall decrease in MC-LR degradation at 5 kGy. However, degradation of spiked MC-LR was still significant at all concentrations of FA employed with degradation efficiencies of 99.99, 99.96, and 99.92% for 0, 50, and 100 μ g/L, respectively.

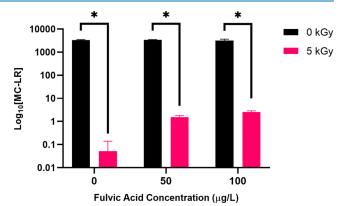


Figure 4. MC-LR degradation in deionized water supplemented with 0, 50, or 100 μ g/L FA following eBeam treatment at 5.11 \pm 0.079 kGy ($p \le 0.0001$; error bars represent standard deviation).

NOM is composed of an array of moieties with various charges including carboxylic and phenolic functional groups. Therefore, solution pH can influence the overall charges of NOM. Increasing pH of solution results in an overall negative charge on phenolic type groups in both FA and humic acids (HA), while at lower pH, this negative charge is associated with carboxylic groups. Further studies should be completed to determine differences in potential NOM radical scavenging as a function of pH to understand the full effects of NOM concentration of MC-LR degradation by eBeam technology.

4. CONCLUSIONS

We investigated several water parameters that could possibly influence eBeam degradation of MC-LR in surface water by obtaining samples gathered around the United States. All samples were analyzed for pH, alkalinity, TDS, and dissolved oxygen. Although some samples showed greater rates of degradation than others, average degradation efficiency at 5 kGy across water samples was above 99%. No trends were observed in any measured parameters as influencers of MC-LR degradation. We also investigated the influence of FA on MC-LR breakdown. MC-LR degradation efficiency was decreased in samples containing FA and was concentration-dependent. However, degradation at all tested concentrations was still significant ($p \leq 0.0001$).

We have previously shown that even at doses as low as 400 Gy, 0.5 mg/L MC-LR was degraded to below a detection limit of 1 ng/L. This falls well below the US EPA's Health Advisory (HA) for microcystin at 300 ng/L. The information from this batch study suggesting that 5 kGy is sufficient to degrade MC-LR is valuable. This information can be used to design a flow-through system or Under Beam Conveyance (UBC) of water (using an eBeam treatment platform) that controls the flow rate so that every water molecule is subjected to a uniform 5 kGy dose. These results highlight the value of eBeam technology as an additional tool in the "toolbox of technologies" that water quality managers can utilize in managing algal toxin-contaminated drinking water resources.

Technological advances are also needed to make this technology suitable for field use, for example, installing the technology on floating barges, or integrating it into current drinking water treatment trains. Economic analyses and optimization studies are also warranted. However, these results help provide an understanding of the potential interactions of water quality parameters with the degradation of MC-LR using eBeam.

AUTHOR INFORMATION

Corresponding Author

Suresh D. Pillai — Interdisciplinary Faculty of Toxicology, Texas A&M University, College Station, Texas 77843, United States; Department of Food Science and Technology, Texas A&M University, College Station, Texas 77843, United States; National Center for Electron Beam Research, an IAEA Collaborating Centre for Electron Beam Technology, College Station, Texas 77845, United States; orcid.org/0000-0002-9766-8765; Email: suresh.pillai@ag.tamu.edu

Authors

Alexandra M. Folcik — Interdisciplinary Faculty of Toxicology, Texas A&M University, College Station, Texas 77843, United States; Exponent, Maynard, Massachusetts 01754, United States

Shelby A. Ruggles – Department of Food Science and Technology, Texas A&M University, College Station, Texas 77843, United States

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.2c07448

Author Contributions

This manuscript was written through contributions of all authors. A.M.F. completed irradiation and MC-LR analysis. S.A.R. completed water quality parameter measurements. A.M.F. and S.D.P. contributed to writing, editing, and revision of the manuscript. All authors have given approval to the final version of the manuscript.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

MCs, microcystins; MC-LR, microcystin-LR; eBeam, electron beam; NOM, natural organic matter; WHO, World Health Organization; HABs, harmful algal blooms; cyanoHABs, cyanobacterial harmful algal blooms; AOP, advanced oxidation

process; AORP, advanced oxidation reduction process; TDS, total dissolved solids; FA, fulvic acid; DWMAPS, drinking water mapping application to protect source waters; EPA, Environmental Protection Agency; LOD, limit of detection

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