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Control of *Microcystis Aeruginosa* growth and the Associated Microcystin Cyanotoxin Remediation by Electron Beam Irradiation (EBI)

Shuyu Liu [1,2*], Yueping Zhao[1], Fang Ma[2], Liyan Ma[4], Kevin O'shea[3], Cen Zhao [3], Xiaohui Hu[1], Minghong Wu*[1],

1. School of Environment and Chemical Engineering, Shanghai University, 201800 P.R. China

2. Harbin Institute of Technology ,State Key Laboratory of Urban Water Resource and Environment, Harbin 150090, P.R. China

3. Department of Chemistry and Biochemistry, Florida International University, Miami, FL 33199, United States

4.Key Laboratory of East China Sea and Oceanic Fishery Resources Exploitation, Ministry of Agriculture, Chinese Academy of Fishery Sciences, Shanghai, 200090, China

*** Corresponding Author:** email: liushuyu@shu.edu.cn

Ph: +86-1350-194-7933

Fax: +86-021-66137787

email: mhwu@shu.edu.cn

Ph: +86-21-66137787

Fax: +86-021-66137787

Capsule abstracts

Application of EBI as a promising treatment technology for control of *Microcystis aeruginosa* algae cultures and simultaneous degradation of microcystin (MC-LR, $C_{49}H7_4N_{10}O_{12}$).

Abstract

Microcystin-LR (MC-LR), a problematic potent cyanotoxin, is produced by a variety of cyanobacteria. The presence of MC-LR threaten drinking water is a serious human health and environmental concern. The control of these algae blooms and associated toxins are critical for

ensuring safe drinking water to significant populations. To our best knowledge, this is the first detailed study about application of Electron Beam Irradiation (EBI) for control of Microcystis aeruginosa algae cultures and simultaneous degradation of MC-LR. Effects of EBI dose on MC production and removal efficiency were investigated by measuring intercellular and extracellular MC concentrations. The dramatic decreases of cellular MC concentration and MC in solution were observed under our experimental conditions. Correlation between Chl-a and MC concentrations is eliminated. Inhibition of cell growth and degradation of MC-LR by EBI is highly-efficient during radiolysis.

Keywords

Microcystin; electron beam irradiation; degradation; advanced oxidation

Highlights

EBI treatment of Microcystin in the cell and free in the solution

High dose of EBI leads to high removal percentage of MC in the cell and free in the solution

Correlation between Chl-a and MC concentration was studied under EBI

Abbreviations

EBI--Electron beam irradiation

MC-- Microcystin

HAB--Harmful cyanobacterial blooms

Microcystis aeruginosa--*M. aeruginosa*

Ctrl--Control

Chl-a--Chlorophyll

OD--Optional density

1. Introduction

Cyanobacteria known as blue-green algae commonly exist in drinking water sources and can lead to

harmful algal blooms (HABs). Cyanobacteria can produce a range of potent toxins such as nodularin, cylindrospermopsin and microcysin [1,2], which threaten drinking water sources and human health. Harmful algal blooms (HABs) have become one of the most important environmental problems in recent years due to the increased presence in water bodies. *Microcystis aeruginosa* (*M. aeruginosa*), the most common cyanobacterial blooms, has been reported to predominate 90% of HABs in natural water bodies and produces toxic microcystins (MC) [3]. The cynotoxin microcystins is a potent hepatotoxins with the effects of the inhibition of protein synthesis [4,5]. Microcystin also act as tumor promoters [6] and may induce oxidative DNA damage in human hepatoma cell line HepG2 [7]. The microcystin structure is shown in **Fig.1.**

Due to the significant increases in the occurrences and volume of toxic algae blooms in industrial and potable water, effective treatments are critical to control and eliminate HABs. A number of studies have been reported for the treatment of MC [8,9,10]. The conventional removal methods such as filtration, flotation or coagulation are often not viable for removal of cyanotoxins [11]. Although chemical oxidation treatments such as chlorine and ozone have been studied for removal cyanotoxins, however, by products like trihalomethanes (THMs) (by chlorination) and bromate (by ozonation) are a concern because of the associated negative health consequences [12,13]. Advanced oxidation processes (AOPs) have been widely studied for removal of a variety of pollutants and toxins from the wastewater [11,14,15] and they involve the generation of highly reactive hydroxyl radical to react with pollutants leading to the degradation.

Among advanced oxidation processes, electron beam irradiation (EBI) is a promising technique with the advantages of high removal efficiency and lower temperature required compared with other traditional treatment methods [16]. EBI can produce reactive species such as e_{aq}^-, ·H, ·OH and H_2O_2 by radiolysis of water shown in Eq. 1. These produced reactive species are capable of efficiently degrading the pollutants from the wastewater.

H_2O /\/\/\ → e_{aq}^- (0.27) + ·H (0.06) + ·OH (0.28) + H_2 (0.05) + H_2O_2 (0.07) + H_3O^+ (0.27) (1)

(where the numbers in brackets are the radiation chemical yields of these species (G-values) per 100 eV absorbed energy).

EBI applications have been reported on the treatment of a variety of pollutants, such as polychlorinated biphenyls, thioanisole, azo dye, and Polycholoro diabenzo-p-dioxin (PCDD) in the wastewater [17,18,19]. However, few applications on the treatment of *M. aeruginosa* have been reported. Herein we report for the first time treatment of toxin producing culture *M. aeruginosa*, focusing on the development of EBI utilizing on controlling MC concentration in the algae cell and free in the solution. We investigated the controlling effect of EBI on MC production by varying the different doses irradiation. Our results demonstrate EBI can be widely applied on the water purification of cyanotoxin based pollutants.

2. Materials and Methods

2.1 Culture of M. aeruginosa

M. aeruginosa specimen (FACHB 905) culture was obtained from the Freshwater Algae Culture collection of the Institute Hydrobiology (Chinese Academy of Sciences, Wuhan, China). It was cultured in autoclaved BG-11 medium at pH around 7.5. Cultures were incubated at 28 ºC in the Light-Emitted Feeding Chamber with an automated light/dark cycle of 14h light/10h dark.

2.2 Electron Beam Irradiation

The electron beam irradiation experiments were conducted at Institute of Radiation Application, Shanghai University using a linear electron accelerator (GJ-II, XianFeng, Shanghai) with 1.0 MeV operating voltage and 1.0 mA beam intensity. Six groups of 100 mLs algal suspension were irradiated in glass petri dishes (90 mm in diameter). The irradiation dose was controlled by setting specific irradiation time to give 0 (control), 1, 2, 3, 4 and 5 kGy dose.

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106 **2.3 Chl-a measurement**

107 The concentration of Chl-a was calculated using ODs at 663, 645, 630 and 750 nm of extracts from
108 5 mLs the culture with a 90 % acetone and10% water solution. The ODs were measured using an
109 UV-Vis spectrophotometer (U-3100, Hitachi). The Chl-a concentration was calculated by the
110 following equation [20]:

111 $$\text{Chl-a (mg L}^{-1}) = [11.64(A_1 - A_4) - 2.16(A_2 - A_4) + 0.10(A_3 - A_4)]v/v_g \quad (2)$$

112 where A_1, A_2, A_3, and A_4 are the absorbance at 663, 645, 630, and 750 nm, respectively, v is the volume of the
113 extract (5 mLs), and vg is the volume of filtered water. The optical densities of algae were monitored at 680
114 nm wavelength.

115

116 **2.4 Microcystin Analysis method**

117 The procedures were summarized in **Fig.2.** For each treatment, 25 mLs of *M. aeruginosa* culture
118 were filtered through a 0.8 μm pore size membrane filter. Then, the sediment was frozen and thawed
119 for 3 times with a little ultra-pure water. The sample was centrifuged for 10 min at 7000 r/min and 4
120 ºC, the supernatant was filtrated through filter membrane of 0.22 μm, the filtrate was subjected to
121 ELISA kit for determination of MC concentration.

122

123 ELISA kit (from Chinese Academy of Sciences) was used to determine MC concentration. The
124 procedure was described as follows: First, add 50 μLs of the standard solutions and 50 μLs of the
125 antibody solution and then cover the wells with parafilm or tape and mix the contents for 30
126 seconds. Afterward, incubate the strips for 90 minutes. And add 100 μLs of the enzyme conjugate
127 solution and mix the contents for 30 seconds. And then add 100 μLs of substrate (color) solution for
128 30 seconds. Finally, 50 μLs of stop solution was added to the wells in the same sequence as for the
129 substrate (color) solution. Read the absorbance at 450 nm using a microplate ELISA photometer
130 (TU-1901, Puxi Company) within 15 minutes after the addition of the stopping solution. All
131 measurements were carried out in triplicate and the data were expressed in the form of mean ±

standard deviation. The calibration curve of MC concentration was shown **in Fig. 3.**

3. Results and discussion

3.1 Changes of associated cellular Microcystin concentration

The *M. aeruginosa* culture can produce MC in the cell during growth process. In order to study the effect of different dose EBI on controlling MC production, we first measured intercellular MC concentration using ELISA. As shown in **Figure 4-a**. MC concentration in Ctrl and 1 kGy treated *M. aeruginosa* cells increased steady during 12 days growth period. MC concentration in 1 kGy treated sample was lower than that in ctrl, which indicates that EBI can inhibit MC production and the *M. aeruginosa* cell growth rate. At last, the MC concentrations after EBI were 60% much lower than control experiment. The fluctuation of intercellular MC concentration was observed under 2-5 kGy EBI irradiation. This is likely because that the decrease of algae cell number can affect philosophy activity of algae cell and further gradually interrupt the MC concentration, leading to the fluctuation of intercellular MC concentration. Further treatment of *M. aeruginosa* cell by EBI resulted in the significant decrease of intercellular MC concentration in the following days. Our results indicate the appropriate dose of EBI can effectively inhibit the intercellular MC production in the *M. aeruginosa* cells.

3.2 Changes of free Microcystin concentration in the solution

The *M. aeruginosa* cells under EBI irradiation may lead to the MC releasing into the solutions. The free MC contents in the solution were also investigated to evaluate the effect of EBI treatment on MC production. **Figure 4-b** illustrates the free MC concentration in solution as a function of EBI exposure days. The MC concentration in 1 kGy group was similar with Ctrl group after 12 days EBI irradiation. The 2-4 kGy EBI exposure led to similar decrease of free MC concentration in the solution with removal percentage of 76 ± 1 %. High dose of EBI can produce more reactive oxygen species (ROS) such as hydroxyl radical (·OH) in the solution [21]. As a result, 5 kGy EBI exposure can significantly remove free MC up to 97 %. Our results suggest ROS produced in high dose of

EBI irradiation are critical for removal and degradation of free MC concentration in the *M. aeruginosa* solution.

3.3 Changes in Total MC concentration

The total MC concentrations were measured to fully evaluate the effect of EBI irradiation on control of MC production. As shown in **Figure 5**, MC concentration increased by 69.8% and 37.2% under Ctrl and 1 kGy EBI irradiation, respectively, after 12 days growth. MC concentration significantly decreased under 2-5 kGy EBI exposure with the removal percentages of 60.8%, 59.6%, 60.2% and 72.1% in 2, 3, 4 and 5 kGy groups, respectively. This also demonstrated that 5 kGy EBI irradiation can exhibit the best performance on the removal of total MC concentration under our experimental conditions (pH is about 7, room temperature is 24 ºC and atmospheric pressure) **(Figure 6)**.

3.4 Correlation between MC concentration and algae cell growth

Figure 7 (a and b) was MC concentration as a function of Chl-a during the algae growth after irradiation. We calculated an important coefficient for MC production capability and cell growth under different dose of EBI **in Table.1**. In Ctrl group, the good correlation ($R_a^2 = 0.983$) between associated MC concentration and Chl-a concentration indicates that cellular MC production increased with algae cell growth. R_b^2 showed MC in the solution increased with algae growing, indicating that MC was produced and released during growing process. When the algae was irradiated by EBI irradiation, both slopes decreased indicating that irradiation inhibited algae cell growth and MC production. As a result, MC concentration in the cell did not keep increasing, leading to MC concentration released into solution decreased simultaneously. The slope (in **Fig. 7b**) between free MC concentration in solution and Chl-a concentration decreased with EBI dose increasing, which showed that the free MC in the solution did not keep increasing with the cell growth.

184 The cell substance of algae, including protein, carbohydrate and lipid often have two functions, one
185 was to combine the nutrient for growth, and the other was to resist circumstance intimidation. When
186 the electron beam irradiated the solution, it made a kind of extreme condition for *M. aeruginosa*. As
187 a result, parts of algae cells would die and the survival cells would produce more carbohydrate or
188 protein to resist environment change, which also contributed to MC production decreasing. The
189 corresponding MC part released into solution decreased as well. Upon EBI treatment a variety of
190 ROS are produced which can result in cell damage and ultimately death rupture. Cells that are not
191 killed can recover and couture produce MC. Those cell that die and release MC into the solution.
192

193 ## 4. Conclusions

194 We systematically investigated effect of different dose (1~5 kGy) of EBI on cellular MC production,
195 releasing and degradation. The changes of cellular MC and the free MC content in solution
196 changing process were tested individually. We found that 1kGy EBI could control MC production
197 and accumulation. 2~5 kGy EBI could inhibit the MC production in *M. aeruginosa* cells and also be
198 considered as a good range for removal of MC from contaminated water bodies. An enhancement in
199 both cellular MC and solution MC removal were observed with EBI dose increasing. EBI destroys
200 the correlation between intercellular and exocellular Chl-a and MC concentrations. The results
201 demonstrate MC production and release are reduced following EBI and the MC concentration in the
202 solution can be reduced as a function of radiation dose though various algae growth stages. These
203 results can provided a understanding of the removal and degradation of cellular MC and free MC in
204 solution under the EBI, which can improve the viability of EBI technologies for the remediation of
205 contaminated water with microcystin based cyanobacteria and their associated toxins. Ongoing
206 studies are underway to further develop the fundamental understanding and better assess EBI as a
207 potential water treatment for cyanotoxins.
208

209 ## Acknowledgements

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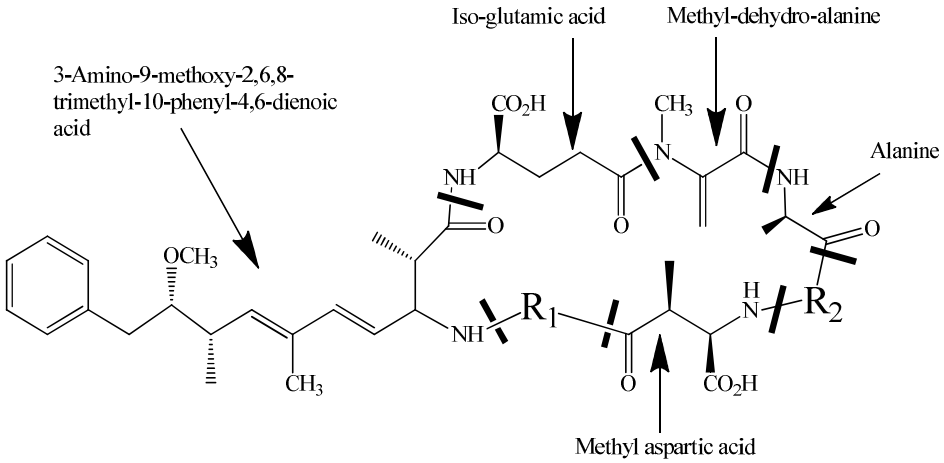
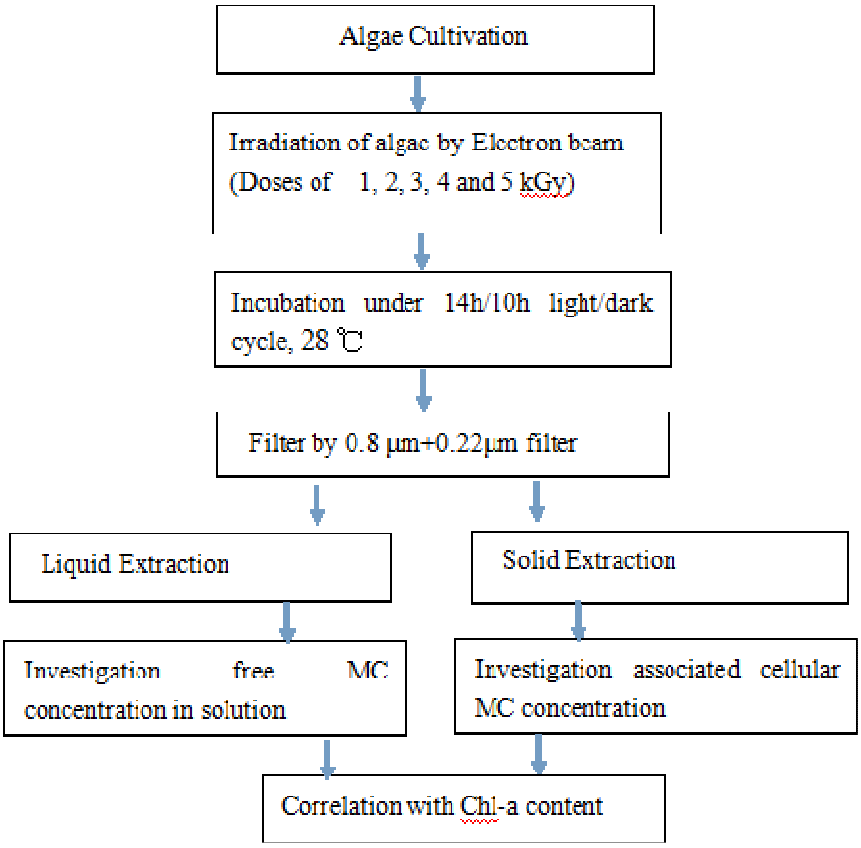


Figure 1. Molecular structure of microcystin



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224 Figure 2. Procedure of Effect of EBI on MC production correlating with algae growth

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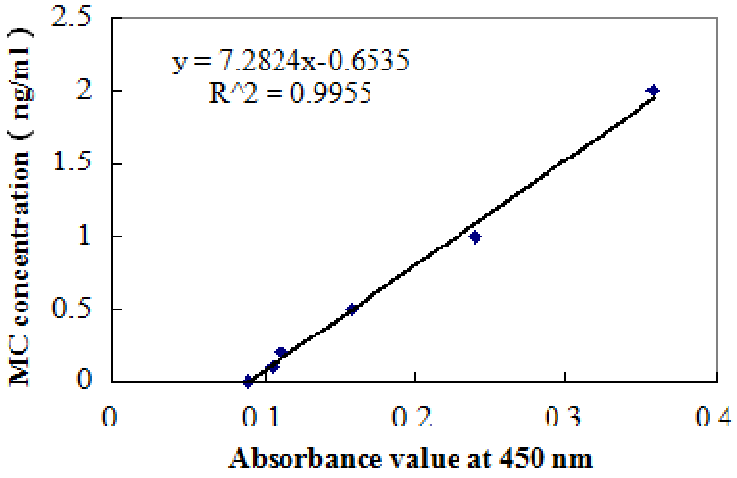

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228 Figure 3. Calibration curve of MC concentration

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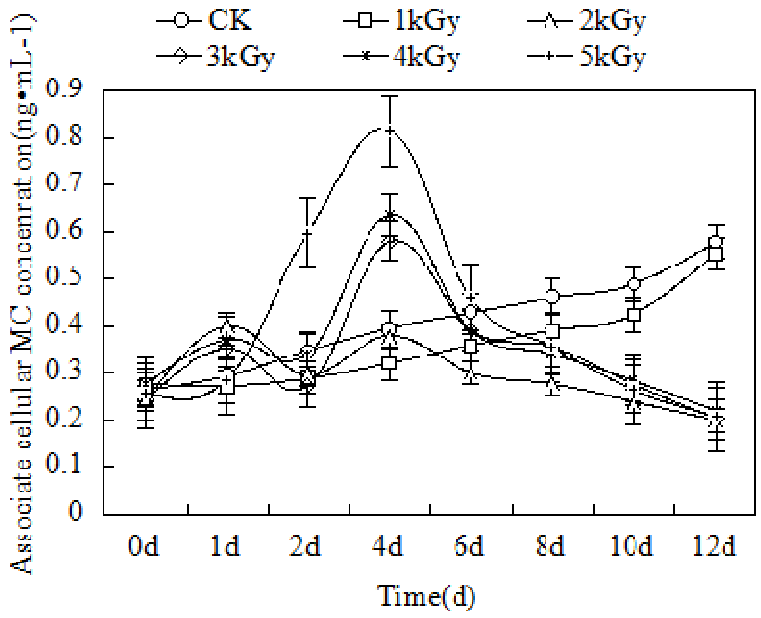

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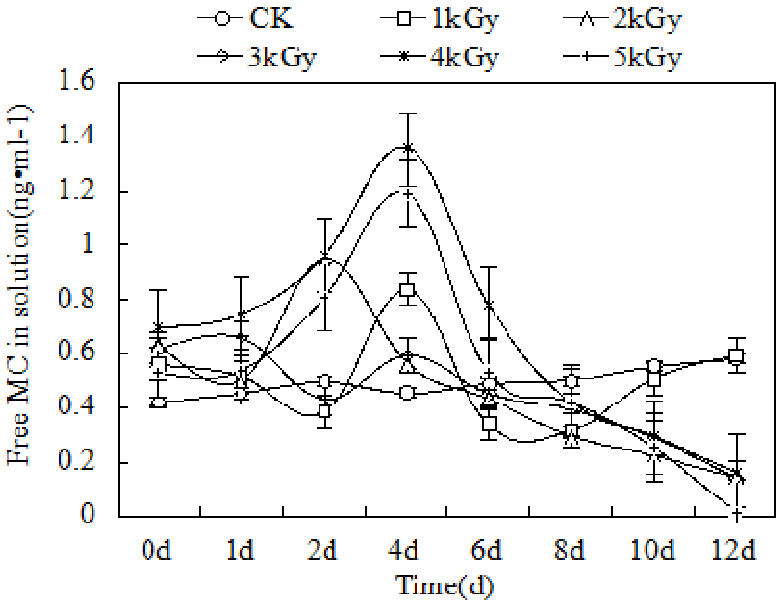
231 a.



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233 b.

234 Figure 4. a. is the associate cellular MC concentration changing after different irradiation doses and b is the

235 changing of free MC concentration in solution after irradiation. Individual cultures were grown under identified

236 conditions.

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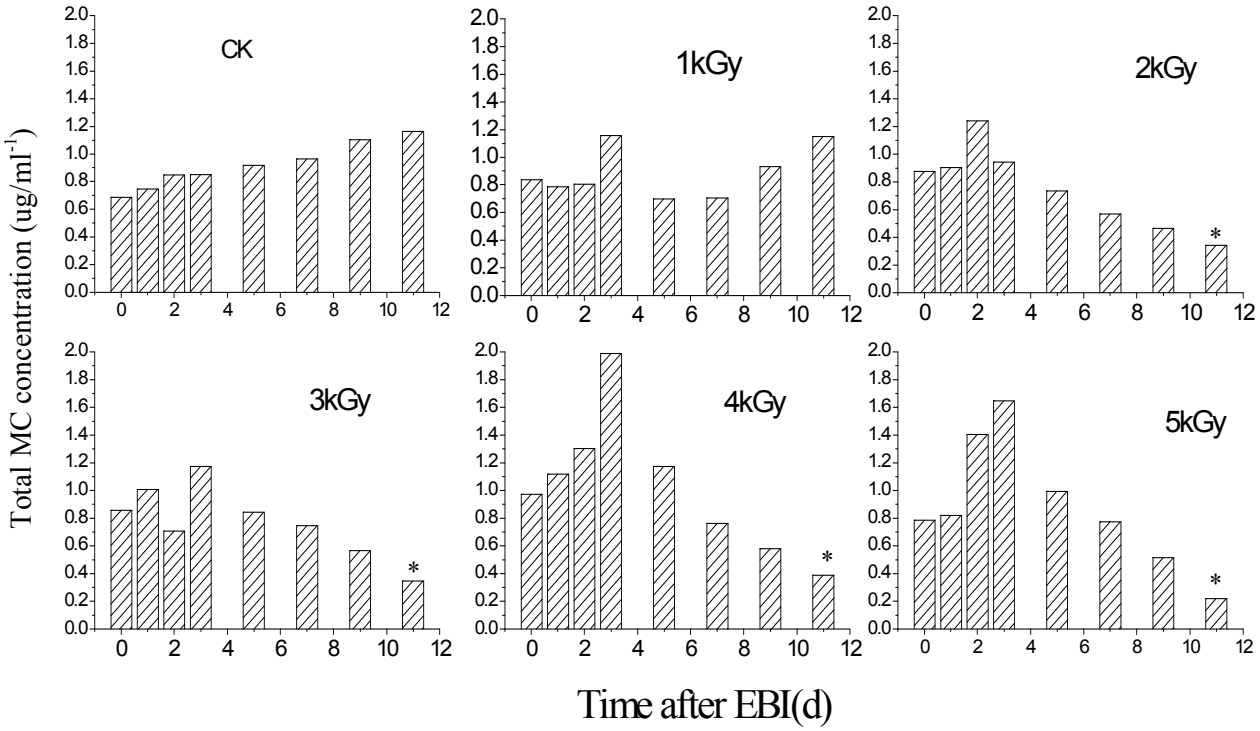

Figure 5. Total MC concentration changing of different treatment(x –ax means the culture time after irradiation).

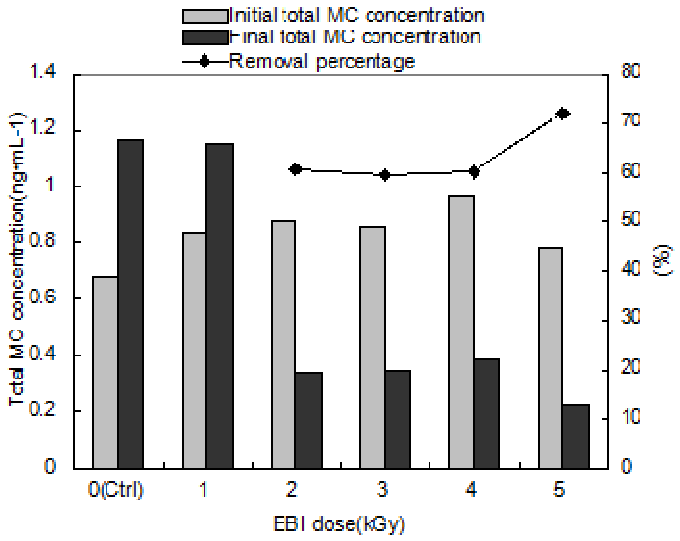

Figure 6. Variation of total MC concentration (including both cellular MC and MC in solution) and its removal percentage by different irradiation dose. The MC removal ratio was calculated by (Final total MC concentration --initial total MC concentration)/initial total MC concentration×100%

257 Table .1 Dependency between MC concentration and Chl-a concentration

Parameters	Correlation between associate cellular MC concentration and Chl-a content		Correlation between free MC concentration in solution and Chl-a content	
	Slopes	R_a^2	Slopes	R_b^2
Ctrl	Y_c=0.0499x+0.0053	0.983	Y_c=0.0239x+0.3028	0.8728
2 kGy	Y_2=0.0434x+0.1032	0.5477	Y_2'=0.1953x-0.2579	0.8028
4 kGy	Y_4=0.0341x+0.2605	0.2223	Y_4'=0.2266x+0.0341	0.5599
5 kGy	Y_5=0.0755x+0.1224	0.1177	Y_5'=0.01910x-0.1784	0.4973

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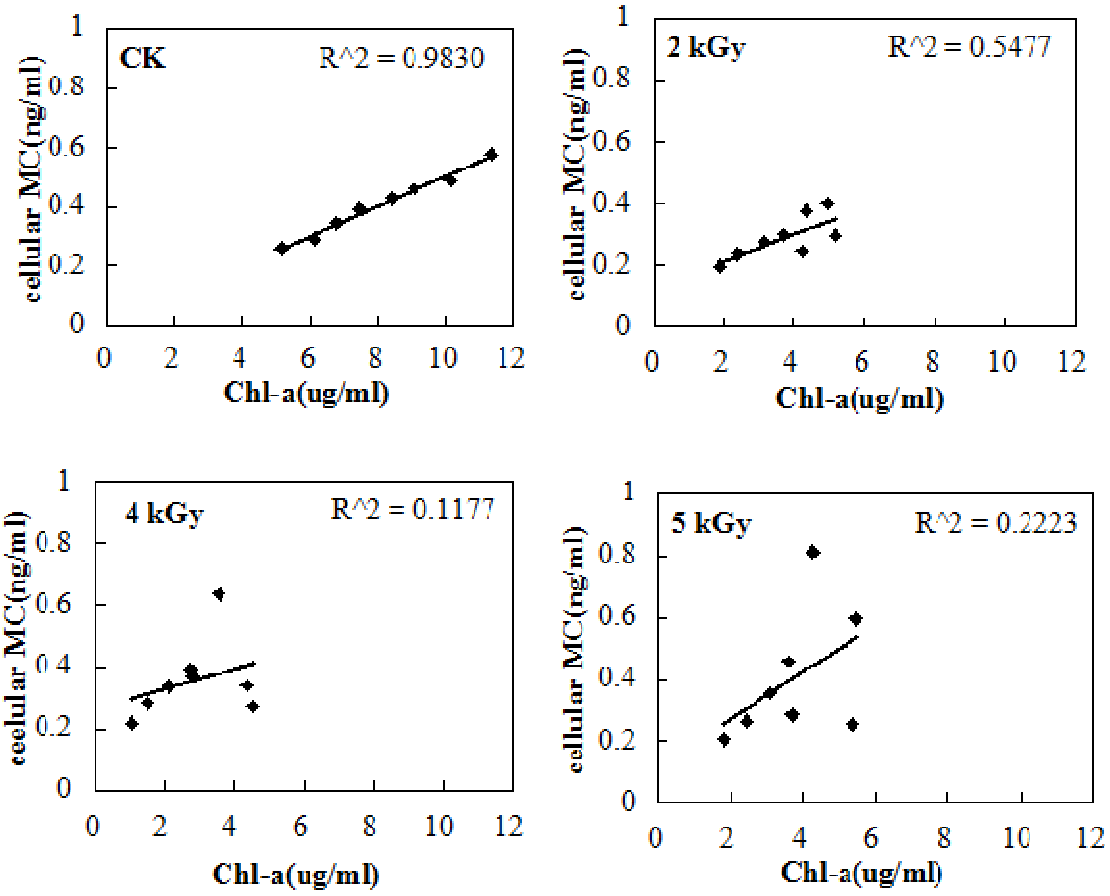
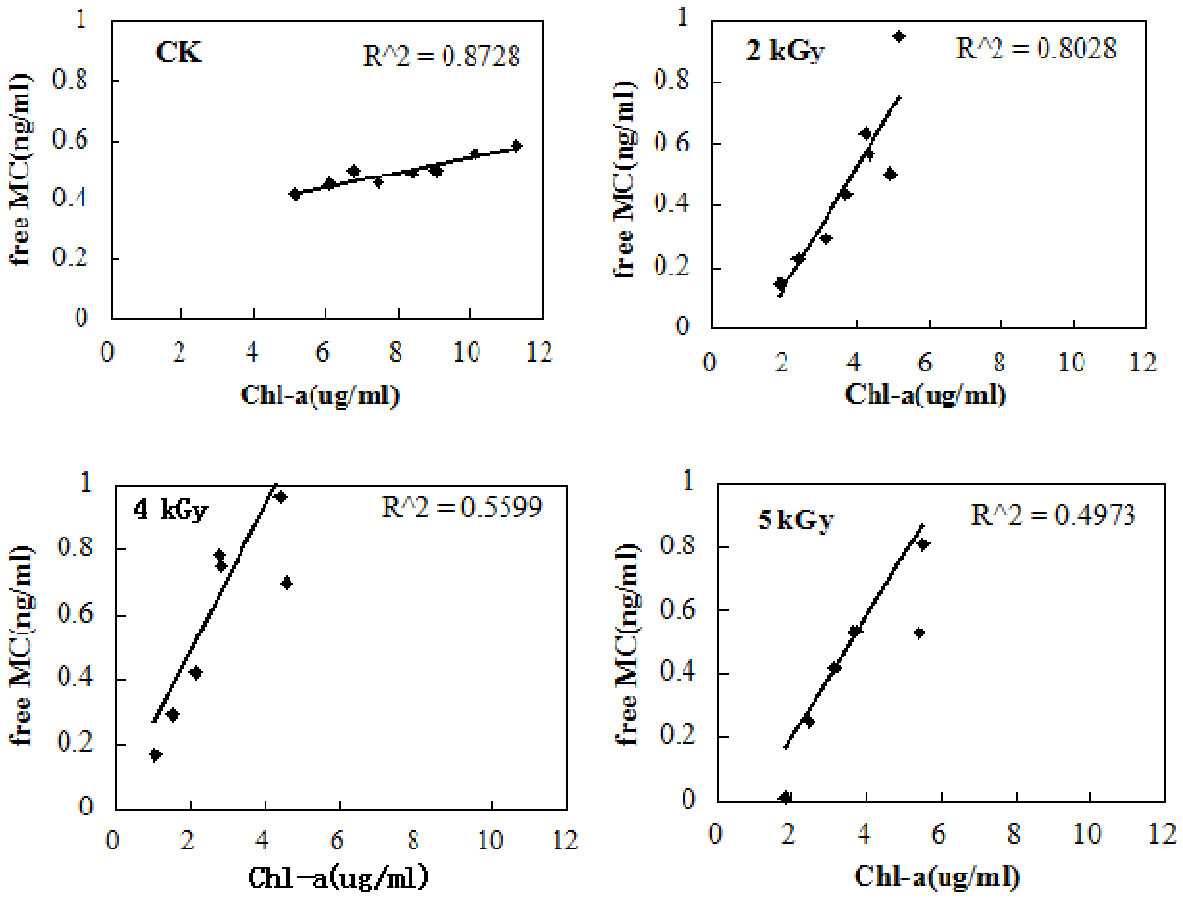


a.



b.

Figure 7. Correlation fit of Chl-a content with the MC concentration, a.is the relationship between Chl-a content and associate cellular MC concentration . b is the relationship between Chl-a content and free MC content in solution

References

1. G. Zanchett, E.C. Oliveira-Filho, Cyanobacteria and cyanotoxins: from impacts on aquatic ecosystems and human health to anticarcinogenic effects, *Toxins* 5 (2013) 1896-1917, 1822 pp.

2. A.A. de la Cruz, A. Hiskia, T. Kaloudis, N. Chernoff, D. Hill, M.G. Antoniou, X. He, K. Loftin, K. O'Shea, C. Zhao, M. Pelaez, C. Han, T.J. Lynch, D.D. Dionysiou, A review on cylindrospermopsin: the global occurrence, detection, toxicity and degradation of a potent cyanotoxin, *Environ. Sci.: Processes Impacts* 15 (2013) 1979-2003.

3. Majsterek, I.; Sicinska, P.; Tarczynska, M.; Zalewski, M.; Walter, Z., Toxicity of microcystin from cyanobacteria growing in a source of drinking water. *Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol.* 139C (2004) 175-179.

4. L. Voloshko, J. Kopecky, T. Safronova, A. Pljusch, N. Titova, P. Hrouzek, V. Drabkova, Toxins and other bioactive compounds produced by cyanobacteria in Lake Ladoga, *Estonian J. Ecol.* 57 (2008) 100-110.

5. J. Mankiewicz, M. Tarczynska, Z. Walter, M. Zalewski, Natural toxins from cyanobacteria, *Acta Biol. Crac. Ser. Bot.* 45 (2003) 9-20.

6. R. Nishiwaki-Matsushima, T. Ohta, S. Nishiwaki, M. Suganuma, K. Kohyama, T. Ishikawa, W.W. Carmichael, H. Fujiki, Liver tumor promotion by the cyanobacterial cyclic peptide toxin microcystin-LR, *J. Cancer Res. Clin. Oncol.* 118 (1992) 420-424.

7. B. Zegura, B. Sedmak, M. Filipic, Microcystin-LR induces oxidative DNA damage in human hepatoma cell line HepG2, *Toxicon* 41 (2002) 41-48.

8. A.M. de Freitas, C. Sirtori, C.A. Lenz, P.G.P. Zamora, Microcystin-LR degradation by solar photo-Fenton, UV-A/photo-Fenton and UV-C/H_2O_2: a comparative study, *Photochem. Photobiol. Sci.* 12 (2013) 696-702.

9. L. Li, C. Shao, T.-F. Lin, J. Shen, S. Yu, R. Shang, D. Yin, K. Zhang, N. Gao, Kinetics of Cell Inactivation, Toxin Release, and Degradation during Permanganation of Microcystis aeruginosa, *Environ. Sci. Technol.* 48 (2014) 2885-2892.

10. J. Andersen, C. Han, K. O'Shea, D.D. Dionysiou, Revealing the degradation intermediates and pathways of visible light-induced NF-TiO_2 photocatalysis of microcystin-LR, *Appl. Catal., B* 154-155 (2014) 259-266.

11. C. Zhao, M. Pelaez, D.D. Dionysiou, S.C. Pillai, J.A. Byrne, K.E. O'Shea, UV and visible light activated TiO_2 photocatalysis of 6-hydroxymethyluracil, a model compound for the potent cyanotoxin cylindrospermopsin, *Catal. Today* 224 (2014) 70-76.

12. R.I. Daly, L. Ho, J.D. Brookes, Effect of Chlorination on Microcystis aeruginosa Cell Integrity and Subsequent Microcystin Release and Degradation, *Environ. Sci. Technol.* 41 (2007) 4447-4453.

13. E. Rodriguez, G.D. Onstad, T.P.J. Kull, J.S. Metcalf, J.L. Acero, G.U. von, Oxidative elimination of cyanotoxins: Comparison of ozone, chlorine, chlorine dioxide and permanganate, *Water Res.* 41 (2007) 3381-3393.

14. V.K. Sharma, T.M. Triantis, M.G. Antoniou, X. He, M. Pelaez, C. Han, W. Song, K.E. O'Shea, A.A. de la Cruz, T. Kaloudis, A. Hiskia, D.D. Dionysiou, Destruction of microcystins by conventional and advanced oxidation processes: *A review, Sep. Purif. Technol.* 91 (2012) 3-17.

15. C. Zhao, L.E. Arroyo-Mora, A.P. DeCaprio, V.K. Sharma, D.D. Dionysiou, K.E. O'Shea, Reductive and oxidative degradation of iopamidol, iodinated X-ray contrast media, by Fe(III)-oxalate under UV and visible light treatment, *Water Res.* 67 (2014) 144-153.

16. C. Guignot, N. Betz, B. Legendre, A. Le Moel, N. Yagoubi, Degradation of segmented poly(ether urethane) Tecoflex induced by electron beam irradiation: Characterization and evaluation, *Nucl. Instrum. Methods Phys. Res., Sect. B* 185 (2001) 100-107.

17. T. Tobien, W.J. Cooper, M.G. Nickelsen, E. Pernas, K.E. O'Shea, K.-D. Asmus, Odor Control in Wastewater Treatment: The Removal of Thioanisole from Water-A Model Case Study by Pulse Radiolysis and Electron Beam Treatment, *Environ. Sci. Technol.* 34 (2000) 1286-1291.

18. G. Mark, H.-P. Schuchmann, M.N. Schuchmann, L. Prager, C. von Sonntag, Electron-Beam Treatment of Aromatic Hydrocarbons that can be Air-Stripped from Contaminated Groundwater. 1. Model Studies in Aqueous Solution, *Environ. Sci. Technol.* 37 (2003) 372-378.

19. K. Hirota, T. Hakoda, M. Taguchi, M. Takigami, H. Kim, T. Kojima, Application of Electron Beam for the Reduction of PCDD/F Emission from Municipal Solid Waste Incinerators, *Environ. Sci. Technol.* 37 (2003) 3164-3170.

20. X.-L. Jin, Q. Xia, X.-Y. Wang, J.-J. Yue, D.-B. Wei, Inactivation of Microcystis aeruginosa with Contact Glow Discharge Electrolysis, *Plasma Chem. Plasma Process.* 31 (2011) 697-705.

21. S. Liu, Y. Zhao, W. Jiang, M. Wu, F. Ma, Inactivation of Microcystis aeruginosa by Electron Beam Irradiation, *Water, Air, & Soil Pollution* 225 (2014) 1-6.