WATER RESEARCH 69 (2015) 51–58

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/watres

Extracellular polymeric substances buffer against the biocidal effect of H_2O_2 on the bloom-forming cyanobacterium *Microcystis aeruginosa*

Lei Gao [a,d], *Xiangliang Pan* [a,*], *Daoyong Zhang* [a,b,**], *Shuyong Mu* [a], *Duu-Jong Lee* [c], *Umut Halik* [e]

[a] *Xinjiang Key Laboratory of Environmental Pollution and Bioremediation, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi 830011, China*

[b] *State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang 550002, China*

[c] *Department of Chemical Engineering, National Taiwan University, Taipei 10617, Taiwan*

[d] *Graduate University of Chinese Academy of Sciences, Beijing, 100049, China*

[e] *College of Resource and Environment Sciences, Xinjiang Key Laboratory of Oasis Ecology, Xinjiang University, Urumqi 830046, China*

ARTICLE INFO

Article history:
Received 28 July 2014
Received in revised form
23 October 2014
Accepted 28 October 2014
Available online 13 November 2014

Keywords:
Biocide
Bloom
Exopolysaccharide
Exopolymer
Antioxidant
Photosynthesis

ABSTRACT

H_2O_2 is an emerging biocide for bloom-forming cyanobacteria. It is important to investigate the H_2O_2 scavenging ability of extracellular polymeric substances (EPS) of cyanobacteria because EPS with strong antioxidant activity may "waste" considerable amounts of H_2O_2 before it kills the cells. In this study, the buffering capacity against H_2O_2 of EPS from the bloom-forming cyanobacterium *Microcystis aeruginosa* was investigated. IC_{50} values for the ability of EPS and vitamin C (VC) to scavenge 50% of the initial H_2O_2 concentration were 0.097 and 0.28 mg mL^{-1}, respectively, indicating the higher H_2O_2 scavenging activity of EPS than VC. Both proteins and polysaccharides are significantly decomposed by H_2O_2 and the polysaccharides were more readily decomposed than proteins. H_2O_2 consumed by the EPS accounted for 50% of the total amount of H_2O_2 consumed by the cells. Cell growth and photosynthesis were reduced more for EPS-free cells than EPS coated cells when the cells were treated with 0.1 or 0.2 mg mL^{-1} H_2O_2, and the maximum photochemical efficiency Fv/Fm of EPS coated cells recovered to higher values than EPS-free cells. Concentrations of H_2O_2 above 0.3 mg mL^{-1} completely inhibited photosynthesis and no recovery was observed for both EPS-free and EPS coated cells. This shows that EPS has some buffering capacity against the killing effect of H_2O_2 on cyanobacterial cells. Such a strong H_2O_2 scavenging ability of EPS is not favorable for killing bloom-forming cyanobacteria. The high H_2O_2 scavenging capacity means considerable amounts of H_2O_2 have to be used to break through the EPS barrier before H_2O_2 exerts any killing effects on the cells. It is therefore necessary to determine the H_2O_2 scavenging capacity of the EPS of various bloom-forming cyanobacteria so that the cost-effective amount of H_2O_2 needed to be used for killing the cyanobacteria can be estimated.

* *Corresponding author*. Tel./fax: +86 991 7885446.
** *Corresponding author*. Tel./fax: +86 851 5890483.
E-mail addresses: xlpan@ms.xjb.ac.cn (X. Pan), zhang-daoyong@163.com (D. Zhang).

http://dx.doi.org/10.1016/j.watres.2014.10.060
0043-1354/

1. Introduction

The cyanobacterial blooms of freshwater lakes are a great ecological and human health risk due to the production of toxins such as microcystins and anatoxins (El-Shehawy et al., 2012; Horst et al., 2014). Exposure to cyanobacterial toxins may cause acute or chronic toxicity to neurosystems and many organs including the liver (Sivonen and Jones, 1999). Oxidation of the cyanobacteria cells by oxidants is one of the common ways to inhibit growth of harmful cyanobacteria (Matthijs et al., 2012; Rajasekhar et al., 2012). In comparison with O_3 and ClO_2^-, H_2O_2 is an emerging and inexpensive but effective biocide (Xu et al., 2007; Matthijs et al., 2012; Fan et al., 2013). A number of studies show that H_2O_2 can effectively kill various bloom-forming cyanobacteria (Drábková et al., 2007; Barrington and Ghadouani, 2008; Matthijs et al., 2012; Qian et al., 2010, 2012). The physiological processes inhibited by H_2O_2 include photosynthesis, antioxidant systems, synthesis of pigments and circadian rhythms (Hoeger et al., 2002; Barrington and Ghadouani, 2008; Qian et al., 2010, 2012).

Cyanobacteria are able to produce extracellular polymeric substances (EPS) which are mainly composed of polysaccharides and proteins (Wingender et al., 1999; Pan et al., 2012; Zhang et al., 2013; Pivokonsky et al., 2014). Some of the EPS are loosely attached to the cell surface but other parts can be tightly bound to the cell wall (Pan et al., 2010a, 2010b). The EPS physically serves as a barrier between the cell and the ambient environment (Pan, 2010). Owing to their strong ability to bind toxic pollutants, water holding capacity and antioxidant activity, they play a key role in buffering against adverse effects of various environmental stressors (Shepherd and Beilby, 1999; Pan, 2010). EPS isolated from bacteria *Pseudomonas* PF-6 (Ye et al., 2012) and *Bacillus megaterium* RB-05 (Chowdhury et al., 2011) have a stronger antioxidation ability to hydroxyl free radicals and the superoxide anion compared to the typical antioxidant, vitamin C (VC). The antioxidant activity, in the sense of physiological health of bacteria, makes EPS an indispensable protective buffering zone against damage from ROS (reactive oxygen species). On the other hand, from the perspective of killing toxic bloom-forming cyanobacteria, such strong ROS scavenging capacity of cyanobacteria implies that a high proportion of H_2O_2 would be consumed by the EPS before it can exert killing activity on the cell. In other words, the massive EPS around the cells can significantly reduce the killing effect of H_2O_2. Therefore, it is of importance to know how much H_2O_2 would be consumed when H_2O_2 is applied to kill harmful cyanobacteria. However, no information on this is currently available.

In the present study, the influence of EPS on the toxic effects of H_2O_2 on photosynthesis of *Microcystis aeruginosa*, a common bloom-forming cyanobacterium, was studied. The H_2O_2 scavenging ability of EPS from *M. aeruginosa* was quantitatively examined using VC as a reference.

2. Materials and methods

2.1. Cultivation of cyanobacterium

The cyanobacterium *M. aeruginosa* (FACH#905) was purchased from the Freshwater Algae Culture Collection of the Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China), and cultured in BG-11 medium (Stanier et al., 1971) at 30 °C under 50 μmol photons m^{-2} s^{-1} PAR with a 12:12 h light–dark cycle. Cyanobacterial cells in the exponential growth phase were used for extraction of EPS and physiological tests.

2.2. Effects of EPS on the killing activity of H_2O_2 on M. aeruginosa

The effect of H_2O_2 on the photosynthesis of *M. aeruginosa* was comparatively assessed in the absence and presence of EPS. In order to remove or extract EPS from the cells, the cell suspension was irradiated with 200 w ultrasound (HU20500B, Shanghai) for 20 min and then centrifuged at 14,000 r min^{-1} for 20 min (Pan et al., 2010a). The supernatant was collected as the raw EPS solution and the residual cells were considered as EPS free cells (herein defined as −EPS cells. The −EPS cells were checked by the Alcian blue 8GX staining method. 0.05% Alcian blue solution was prepared by dissolving Alcian blue 8GX of analytical grade in 3% acetic acid solution at pH 2–3. After 30 min of staining with Alcian blue solution, EPS on surfaces of cells was checked with a light microscope. If the cells were not stained with Alcian blue, this indicated that the EPS was successfully removed from the cells (Shepherd and Beilby, 1999; Böckelmann et al., 2003; Kallmeyer et al., 2008). The Alcian blue 8GX staining results showed that most EPS on the surface of the *M. aeruginosa* cells was successfully removed by the ultrasound-centrifugation extraction (Fig. S1).

In addition, in order to assess the effect of EPS extraction on the physiological status of cells, the maximum photochemical efficiency of photosystem II (Fv/Fm), which is a reliable indicator for photosynthesis of cyanobacteria, of cells before and after extraction of EPS was monitored using a dual-modulation fluorometer (FL-3500, PSI, Brno, CZ) (Pan et al., 2008, 2009). The cells were dark-adapted for 5 min before chlorophyll fluorescence testing (Pan et al., 2008). It was found that Fv/Fm was kept at relatively constant values of about 0.34 after EPS extraction, a little lower than the original value (0.35), indicating that the physiological status of the cells was not adversely affected by EPS extraction (Table S1).

The original cyanobacterial cells without EPS extraction, i.e., eps-coated cells (herein defined as +EPS cells) and −EPS cells were separately incubated in BG-11 medium containing various concentrations (0, 0.4, 0.8, 1.2 and 1.6 mg L^{-1}) of H_2O_2. The optical density at 668 nm (OD_{668}), chlorophyll *a* content and Fv/Fm of the cells untreated and treated with various H_2O_2 concentrations were measured at different time intervals. The Chl *a* content was determined by the colorimetric method (Arnon, 1949). Three mL of cell suspension was centrifuged at 8000 rpm for 5 min. The residual cells were extracted with 3 mL of 80% acetone at 4 °C in the dark for 24 h and then centrifuged at 8000 rpm for 5 min. The absorbance at 663 nm and 645 nm of the acetone extract was recorded with 80% acetone being used as the reference blank using a spectrophotometer (Unico 2000, Shanghai, China). Chl *a* content was estimated using the following formula: Chl *a* (mg L^{-1}) = $12.7 \times A_{663} - 2.69 \times A_{645}$ (Arnon, 1949).

2.3. Extraction and characterization of EPS

The raw EPS solution was filtered through a 0.45 μm membrane filter and then repeatedly purified with a dialysis membrane (3500 Da cut-off) in distilled water every 4 h for 24 h at 4 °C. Total organic carbon (TOC) of EPS solution was measured by wet combustion using a TOC analyzer (TOC-4100, Shimadzu, Japan). Content of polysaccharides was measured by the phenol-sulfuric acid method, using glucose as the standard (Dubois et al., 1956). The content of proteins was measured by the Bradford method, with bovine serum albumin as the standard (Bradford, 1976). The dry weight of the cells was measured after the cells were dried at 50 °C in a vacuum oven to a constant weight.

A fluorescence spectrophotometer (F-7000, Hitachi, Japan) was used to record the excitation-emission matrix (EEM) fluorescence spectra of the EPS solution (Zhang et al., 2010). The EEM spectra were collected with scanning emission spectra from 250 to 550 nm every 2 nm by varying the excitation wavelength from 200 to 400 nm at 5 nm increments. The EEM spectrum of Milli-Q water was subtracted from the EEM spectra of EPS samples. The EEM spectral images were generated using the software SigmaPlot 10.0 (Systat, US) (Pan, 2010).

2.4. H_2O_2 scavenging ability of EPS

The H_2O_2 scavenging capacity of EPS was examined with the typical strong reductant ascorbic acid (VC) as the reference. VC solution was also prepared by dissolving analytical grade VC in deionized water. H_2O_2 solution was added into the EPS and VC solution. The mixed solution volume was 50 mL. The H_2O_2 concentration in EPS or VC solutions was determined by a colorimetric method after 3 h of reaction (Graf and Penniston, 1980). The H_2O_2 scavenging ability of EPS or VC (mg mg^{-1}) was expressed the ratio of the amount of consumed H_2O_2 to the amount of EPS or VC.

The H_2O_2 scavenging kinetics of EPS, VC and cells with EPS were also examined. Two 50 mL bottles of EPS coated cell suspension with same cell density were prepared. One bottle of cell suspension was used to extract EPS by centrifugation. The extracted EPS solution was dialyzed for 24 h at 4 °C and diluted to 50 mL with a concentration of EPS of 0.2 mg mL^{-1}. The other bottle of cell suspension was directly used for testing of H_2O_2 scavenging kinetics. H_2O_2 solution was added to the EPS solution (0.2 mg mL^{-1}), VC solution (0.2 mg mL^{-1}) and the original EPS-coated cell suspension to a final H_2O_2 concentration of 0.4 mg mL^{-1}. Residual H_2O_2 concentrations in EPS solution, VC solution and the cell suspension was determined at different time intervals.

2.5. Statistics

All the experiments were replicated and the mean values were used.

3. Results

3.1. Extraction and characteristics of EPS

There was one typical protein-like peak at Ex = 280 nm/Em = 330 nm in the EEM fluorescence spectra of EPS (Fig. 1a).

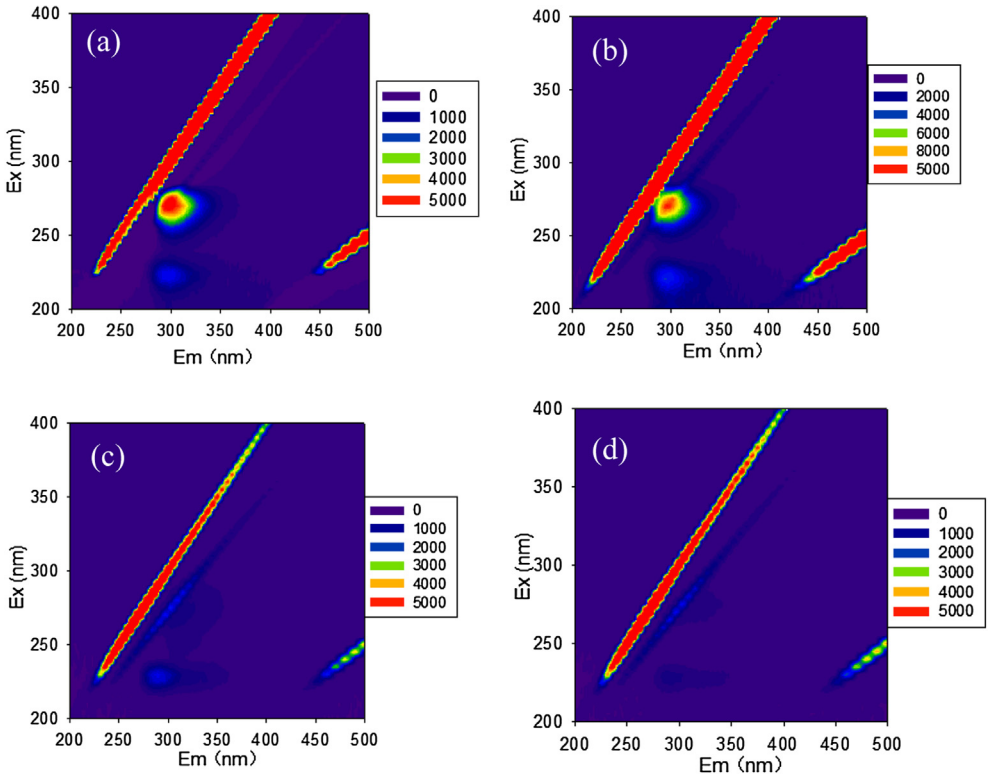

Fig. 1 – Excitation emission matrix to fluorescence spectra photos of EPS oxidized by H_2O_2. (a) 39 mg L^{-1} EPS, (b) 39 mg L^{-1} EPS + 0.03 mg mL^{-1} H_2O_2, (c) 39 mg L^{-1} EPS + 0.05 mg mL^{-1} H_2O_2, (d) 39 mg L^{-1} EPS + 0.2 mg mL^{-1} H_2O_2.

About 40 mg L^{-1} TOC of EPS was extracted. The content of polysaccharides and proteins was 32.6 and 11.3 mg g^{-1} EPS, respectively.

Extraction of EPS had little effect on the physiological performance of the cells. The chlorophyll fluorescence test showed that the maximum photochemical efficiency (Fv/Fm) of photosystem II for both the untreated cells and the cells after extraction of EPS was about 0.35.

3.2. Decomposition of EPS by H_2O_2

Table 1 shows the TOC and content of polysaccharides and proteins of EPS after 3 h of treatment with various concentrations of H_2O_2. EPS was clearly decomposed during its removal of H_2O_2. The TOC and polysaccharide and protein content decreased with increasing H_2O_2 concentration. Polysaccharides were more susceptible to decomposition of H_2O_2 than proteins. When EPS was treated with 0.2 mg mL^{-1} H_2O_2 almost all of the polysaccharides were decomposed. The excitation-emission matrix fluorescence spectra showed that 0.05 and 0.2 mg mL^{-1} H_2O_2 quenched most of the fluorescence of the protein-like substances in EPS (Fig. 1 and Table 1), which confirmed that proteins in EPS were also drastically degraded by H_2O_2.

3.3. H_2O_2 scavenging ability and kinetics of EPS

The H_2O_2 scavenging ability of EPS was assessed with VC as the reference. *M. aeruginosa* EPS showed a higher H_2O_2 removal percentage and scavenging ability than VC (Fig. 2a and b). All of the H_2O_2 was removed by 0.2 mg mL^{-1} EPS while only 35.8% of H_2O_2 was removed by 0.2 mg mL^{-1} VC. More than 0.6 mg mL^{-1} VC was required to remove all the H_2O_2. Concentrations of EPS and VC required to scavenge 50% of the initial H_2O_2 were 0.097 and 0.28 mg mL^{-1}.

It was also found that EPS removed H_2O_2 faster than VC (Fig. 3). 36% and 25% of H_2O_2 was removed by EPS and VC at 10 min, respectively and after this time no significant further H_2O_2 removal was observed. Cells with EPS consumed much more H_2O_2 but at a slower rate than EPS and VC. About 75% of H_2O_2 was scavenged by cells with EPS at 60 min and thereafter H_2O_2 concentrations changed little. The difference between H_2O_2 consumed by EPS and cells with EPS was about 50%. This means that about half of the supplied H_2O_2 reacts with EPS when H_2O_2 is used to kill the cyanobacteria.

The H_2O_2 scavenging kinetics of EPS and VC followed a first order exponential decay model ($R^2 = 0.983–0.998$).

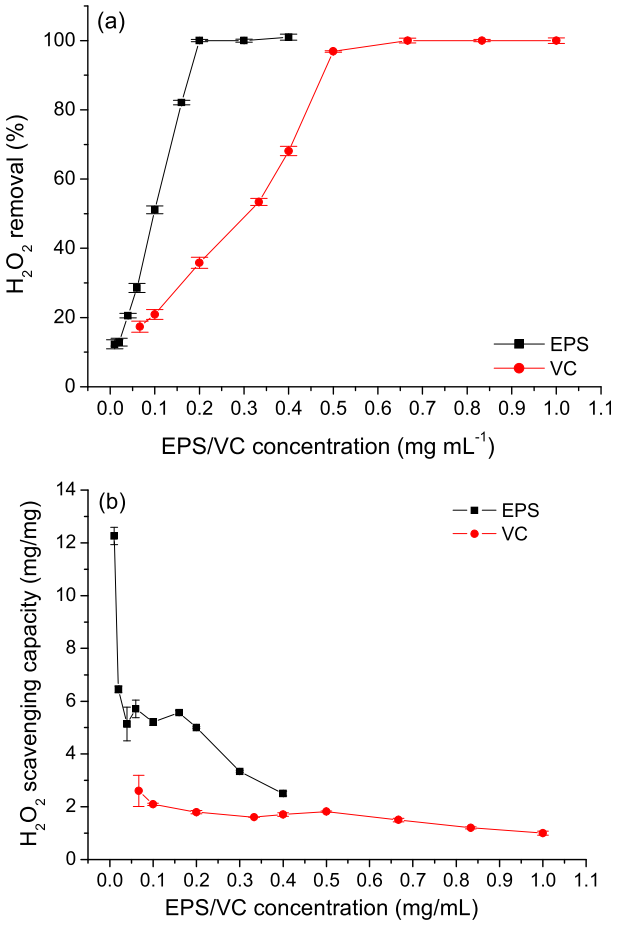

Fig. 2 – Oxidation ability of EPS and VC. (a) the initial concentration of H_2O_2 was 0.4 mg mL^{-1}, (b) the initial concentration of H_2O_2 was 0.4 mg mL^{-1}. The starting concentration of EPS was 0.01 mg mL^{-1}.

$$C_t = C_0 e^{-k(t-t_0)} \quad (1)$$

where C_0 and C_t are the H_2O_2 concentration at time t_0 and t, and k is the rate constant. The values of k of H_2O_2 removal kinetics for cells with EPS, EPS and VC were 0.056, 0.277 and 0.225, respectively. This indicates that EPS scavenged H_2O_2 a little faster than VC. But the H_2O_2 scavenging capacity of EPS was 11% more than that of VC. Cells with EPS scavenged more H_2O_2 than EPS alone and VC but at a much slower rate. The higher H_2O_2 scavenging capacity of cells with EPS is attributed to the additional reaction of H_2O_2 with cell membrane and intracellular mass.

Table 1 – Content of major components of EPS treated with various concentrations of H_2O_2. The data are presented as mean ± S.E.($n = 2$).

	0 mg mL^{-1} H_2O_2	0.03 mg mL^{-1} H_2O_2	0.05 mg mL^{-1} H_2O_2	0.2 mg mL^{-1} H_2O_2
TOC (mg L^{-1})	39.94 ± 0.0316	16.55 ± 0.03432	16.42 ± 0.0134	15.13 ± 0.06213
Polysaccharide (mg g^{-1})	32.58 ± 0.06472	23.25 ± 0.05603	12.65 ± 0.11206	0.19 ± 0.22412
Protein (mg g^{-1})	11.31 ± 0.05667	8.75 ± 0.01211	5.25 ± 0.08021	2.73 ± 0.10651
Fluorescence intensity (I_n)	6971 ± 6.22718	4823 ± 2.0185	520.1 ± 0.82125	508.6 ± 0.61734

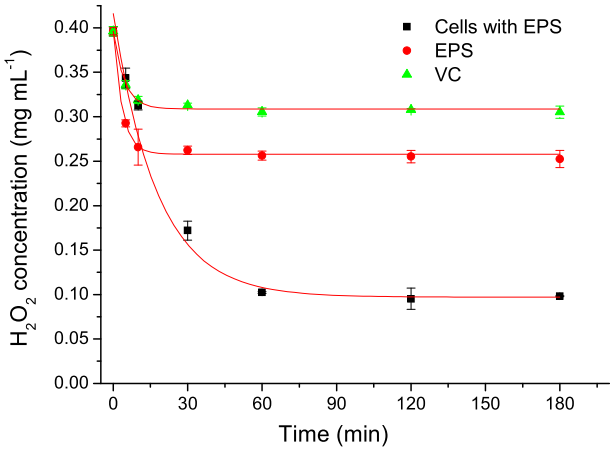

Fig. 3 – Oxidation rate of EPS and VC by H_2O_2 (the initial concentration of H_2O_2 was 0.4 mg mL^{-1}, EPS and VC was 0.2 mg mL^{-1}).

3.4. *Effects of H_2O_2 on cell growth and photosynthesis in the absence/presence of EPS*

Cell growth was inhibited by 0.4 mg L^{-1} or higher concentrations of H_2O_2 (Table 2). However, cell growth and photosynthesis were reduced more for EPS-free cells than for EPS coated cells, when the cells were treated with 0.4 or 0.8 mg L^{-1} H_2O_2 (Fig. 4). At such concentrations of H_2O_2, Fv/Fm recovered significantly within 6 h, and Fv/Fm of EPS coated cells recovered to higher values than EPS-free cells. 1.2 mg L^{-1} and higher concentrations of H_2O_2 treatment rapidly reduced Fv/Fm to zero for both types of cells, and no subsequent recovery of Fv/Fm was observed (Fig. 4), indicating that such concentrations of H_2O_2 can effectively kill the cells regardless of the EPS. This result shows that EPS has some buffering capacity against the killing effect of H_2O_2 on cyanobacteria cells.

4. Discussion

This study shows that EPS from the cyanobacterium *M. aeruginosa* has a strong H_2O_2 scavenging ability and plays a crucial role in protecting cells from the oxidative toxicity of H_2O_2. However, from the perspective of killing toxic cyanobacteria, the high H_2O_2 scavenging capacity of EPS means considerable amounts of H_2O_2 have to be consumed by the EPS before it can exert toxicity.

EPS efficiently scavenged H_2O_2 at the cost of decomposition of its components in a dose dependent manner (Fig. 2). This implies that both proteins and polysaccharides have strong antioxidant activity. In the present study, the IC50 for EPS to scavenge 50% of initial H_2O_2 was 0.097 mg mL^{-1}, which is much lower than that of VC, indicating a higher antioxidant activity of EPS than VC. Furthermore, it was found that EPS scavenged H_2O_2 faster than VC (Fig. 3). A number of studies showed that EPS, especially the exopolysaccharides, of various microbes have a strong scavenging ability toward various reactive oxygen species including superoxide and hydroxyl radicals.

Table 2 – The maximum efficiency of PSII photochemistry (Fv/Fm), the optical density at 668 nm (OD_{668}) and Chl *a* content (mg L^{-1}) of cyanobacteria cells with EPS (+EPS) and without EPS (-EPS) treated with various concentrations of H_2O_2 for different times and fluorescence peak positions. The data presented here are the mean values of three independent measurements at $p < 0.005$.

	H_2O_2 treatment time	0.1 mg mL^{-1} H_2O_2 (+EPS)	0.1 mg mL^{-1} H_2O_2 (−EPS)	0.2 mg mL^{-1} H_2O_2 (−EPS)	0.2 mg mL^{-1} H_2O_2 (−EPS)	0.3 mg mL^{-1} H_2O_2 (+EPS)	0.3 mg mL^{-1} H_2O_2 (−EPS)	0.4 mg mL^{-1} H_2O_2 (+EPS)	0.4 mg mL^{-1} H_2O_2 (−EPS)
Fv/Fm	0 min	0.375	0.370	0.366	0.340	0.366	0.357	0.405	0.363
	180 min	0.266	0.230	0.145	0.129	0.036	0.053	0.019	0.044
	1 d	0.338	0.285	0.309	0.215	0.000	0.001	0.000	0.000
OD_{668}	0 min	1.991	1.991	1.991	1.991	–*	–*	–*	–*
	2 d	1.721	1.609	1.721	1.533	–*	–*	–*	–*
Chl *a* content	0 min	2.125	2.105	2.125	2.105	–*	–*	–*	–*
	2 d	1.37	1.346	1.093	0.98	–*	–*	–*	–*

* The optical density and Chl *a* content were not measured since the cells were actually killed by H_2O_2.

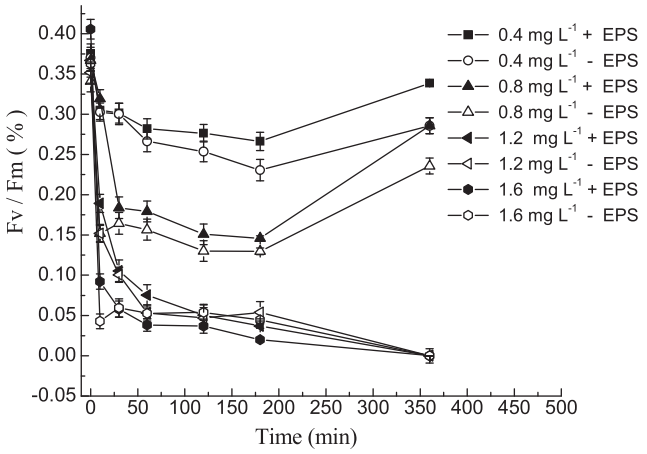

Fig. 4 – The maximum photochemical efficiency (Fv/Fm) of photosystem II of *M. aeruginosa* cells treated with various concentrations of H_2O_2 in the absence/presence of EPS. +EPS: cells with EPS, and –EPS: cells without EPS.

Chowdhury et al. (2011) reported that EPS from a river sediment bacterium *B. megaterium* RB-05 is a superior superoxide radical scavenger than quercetin. The IC50 (half maximal inhibition concentration) of the EPS was 24.14 μg mL^{-1} while IC50 for the reference of quercetin was 39.01 μg mL^{-1}. EPS from *Pantoea agglomerans* quenched hydroxyl radicals, superoxide radicals with an IC50 of 0.07 and 0.15 mg mL^{-1}, respectively (Wang et al., 2007). Concentrations for *Lactobacillus planterum* LP6 EPS quenching 50% of the initial radical for DPPH•, OH• and $O_2^{\bullet-}$ radicals were 1.38, 3.43 and 0.11 mg mL^{-1}, respectively (Li et al., 2013). In the present study, both proteins and polysaccharides were significantly decomposed by H_2O_2 and the polysaccharides were more readily decomposed than proteins (Table 1). However, Huang et al. (2013) reported that EPS of the fungus *Cordyceps sinensis* showed a significant dependence on the protein content, being negligible for polysaccharides. The antioxidant activity of EPS is related to the kind of ROS. For example, *Lactobacillus helveticus* MB2-1EPS at 4 mg mL^{-1} from scavenged 80.24% of hydroxyl radicals, being less than the scavenging percentage of VC (97.72%). However, EPS showed a stronger superoxide scavenging ability (71.82%) than VC (56.35%) (Li et al., 2014).

In the present study, the cyanobacterium cell EPS consumed about 50% of H_2O_2 when H_2O_2 was used to kill the cyanobacteria (Fig. 3). On one hand, this shows that the strong H_2O_2 scavenging activity of EPS plays an important role in buffering against the toxicity of H_2O_2. The photosynthetic activity (Fv/Fm) of EPS coated cells recovered significantly to higher values than that of the EPS-free cells (Fig. 4). The ROS scavenging ability of EPS significantly ameliorates various environmental stresses. The protective effect of EPS from the bacterium *P. agglomerans* against UV radiation was most likely to be due to the ability to scavenge free radicals (Wang et al., 2007). The capsular EPS of *Pseudomonas aeruginosa* mitigated toxicity from nanosize titanium dioxide (TiO_2) and this was also attributed to the important role of EPS as a barrier to cell membrane oxidation from ROS (Christopher et al., 2012). However, the protective barrier of bacterial EPS to chemicals might be due to interaction of its hydrophobic fractions with toxins (Henriques and Love, 2007). On the other hand, the strong H_2O_2 reactivity with EPS reduces the efficiency of H_2O_2 for controlling cyanobacteria. The high H_2O_2 scavenging capacity means considerable amounts of H_2O_2 have to be used to break through the EPS barrier before H_2O_2 can exert killing effects on the cells. In this sense, it is necessary to determine the H_2O_2 scavenging capacity of EPS of various bloom-forming cyanobacteria so that we can estimate the cost-effective amount of H_2O_2 needed to kill the cyanobacteria.

More and more studies show that H_2O_2 is an efficient biocide for bloom-forming cyanobacteria or algae (Barrington et al., 2013). Recently, successful field application of H_2O_2 to suppress cyanobacterial and marine algal blooms has been reported (Matthijs et al., 2012; Wang et al., 2012; Burson et al., 2014). Sometimes repeated application is required to avoid the development of renewed dominance of cyanobacteria (Barrington et al., 2013). Extensive and repeated application of H_2O_2 to large lakes for suppression of blooms is expensive and it is necessary to take measures to reduce the cost. It is feasible to estimate the minimum dose of H_2O_2 required for killing harmful cells based on the H_2O_2 scavenging ability of EPS. The ideal dose of H_2O_2 should be just enough to cross the EPS barrier and then kill the cell without disintegration of the whole cell, which would consume much more H_2O_2 than that is needed only for killing the cell. If H_2O_2 is not applied enough, growth of cyanobacteria is inhibited temporarily and the inhibited dominant population can recover soon. In this case, H_2O_2 has to be repeatedly applied and the cost increases significantly. Therefore, it is necessary to estimate the amount of H_2O_2 consumed by EPS and the amount to kill the cell. It is also important to judge the cyanobacteial or algal cells are irreversibly killed or not by monitoring the physiological indicators, such as photosynthesis parameters, during H_2O_2 application in the field. Furthermore, because different species of cyanobacteria may have different yield of EPS with different H_2O_2 scavenging capacity, understanding the differences in H_2O_2 scavenging capacity of EPS between various populations of bloom forming cyanobacteria or algae is useful for minimizing the cost. The well-known fact that cyanobacterial or algal blooms are usually dominated by one or a few species makes it feasible to design different lowest but effective doses of H_2O_2 for controlling different blooms. The future work should be focused on constructing a database that at least includes information on H_2O_2 consumption of EPS of various bloom-forming species and the lowest but effective killing dose of H_2O_2. Such a database is helpful for one to immediately determine a cost-effective dose of H_2O_2 according to the dominant bloom-forming species when one bloom event occurs.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (U1120302, 21177127 and 41203088).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2014.10.060.

REFERENCES

Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts. Polyphenol oxidase in Betavulgaris. Plant Physiol. 24 (1), 1–15.

Barrington, D.J., Ghadouani, A., 2008. Application of hydrogen peroxide for the removal of toxic cyanobacteria and other phytoplankton from wastewater. Environ. Sci. Technol. 42, 8916–8921.

Barrington, D.J., Reichwaldt, E.S., Ghadouani, A., 2013. The use of hydrogen peroxide to remove cyanobacteria and microcystins from waste stabilization ponds and hypereutrophic systems. Ecol. Eng. 50, 86–94.

Böckelmann, U., Szewzyk, U., Grohmann, E., 2003. A new enzymatic method for the detachment of particle associated soil bacteria. J. Microbiol. Methods 55 (1), 201–211.

Bradford, M., 1976. A rapid and sensitive method for the quantization of microgram quantities of protein utilizing the principle of protein-dye binding. J. Anal. Biochem. 72 (4), 248–254.

Burson, A., Matthijs, H.C.P., de Bruijne, W., Talens, R., Hoogenboom, R., Gerssen, A., Visser, P.M., Stomp, M., Steur, K., van Scheppingen, Y., Huisman, J., 2014. Termination of a toxic *Alexandrium* bloom with hydrogen peroxide. Harmful Algae 31, 125–135.

Chowdhury, S.R., Basak, R.K., Sen, R., Adhikari, B., 2011. Optimization, dynamics, and enhanced production of a free radical scavenging extracellular polysaccharide (EPS) from hydrodynamic sediment attached *Bacillus megaterium* RB-05. Carbohydr. Polym. 86 (3), 1327–1335.

Christopher, M., Hessler, M.Y., Wu, Z.X., Hyeok, C., Seo, Y., 2012. The influence of capsular extracellular polymeric substances on the interaction between TiO_2 nanoparticles and planktonic bacteria. Water Res. 46 (15), 4687–4696.

Drábková, M., Matthijs, H.C.P., Admiraal, W., Maršálek, B., 2007. Selective effects of H_2O_2 on cyanobacterial photosynthesis. Photosynthetica 45 (3), 363–369.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. J. Anal. Chem. 28 (3), 350–356.

El-Shehawy, R., Gorokhova, E., Fernández-Piñas, F., del Campo, F.F., 2012. Global warming and hepatotoxin production by cyanobacteria: what can we learn from experiments? Water Res. 46 (5), 1420–1429.

Fan, J.J., Ho, L., Hobson, P., Brookes, J., 2013. Evaluating the effectiveness of copper sulphate, chlorine, potassium permanganate, hydrogen peroxide and ozone on cyanobacterial cell integrity. Water Res. 47 (14), 5153–5164.

Graf, E., Penniston, J.T., 1980. Method for determination of hydrogen peroxide, with its application illustrated by glucose assay. Clin.Chem. 26 (5), 658–660.

Henriques, I.D.S., Love, N.G., 2007. The role of extracellular polymeric substances in the toxicity response of activated sludge bacteria to chemical toxins. Water Res. 41 (18), 4177–4185.

Hoeger, S.J., Dietrich, D.R., Hitzfeld, B.C., 2002. Effect of ozonation on the removal of cyanobacterial toxins during drinking water treatment. Environ. Health Perspect. 110 (11), 11–27.

Horst, G.P., Sarnelle, O., White, J.D., Hamilton, S.K., Kaul, R.B., Bressie, J.D., 2014. Nitrogen availability increases the toxin quota of a harmful cyanobacterium, *Microcystis aeruginosa*. Water Res. 54, 188–198.

Huang, Q.L., Siu, K.C., Wang, W.Q., Cheung, Y.C., Wu, J.Y., 2013. Fractionation, characterization and antioxidant activity of exopolysaccharides from fermentation broth of a *Cordyceps sinensis* fungus. Process Biochem. 48 (2), 380–386.

Kallmeyer, J., Smith, D.C., Spivack, A.J., D'Hondt, S., 2008. New cell extraction procedure applied to deep subsurface sediments. Limnol. Oceanogr. Methods 8, 236–245.

Li, J.Y., Jin, M.M., Meng, J., Gao, S.M., Lu, R.R., 2013. Exopolysaccharide from *Lactobacillus planterum* LP6: antioxidation and the effect on oxidative stress. Carbohydr. Polym. 98 (1), 1147–1152.

Li, W., Ji, J., Chen, X.H., Jiang, M., Rui, X., Dong, M.S., 2014. Structural elucidation and antioxidant activities of exopolysaccharides from *Lactobacillus helveticus* MB2-1. Carbohydr. Polym. 102, 351–359.

Matthijs, H.C.P., Visser, P.M., Reeze, B., Meeuse, J., Slot, P.C., Wijn, G., Talens, R., Huisman, J., 2012. Selective suppression of harmful cyanobacteria in an entire lake with hydrogen peroxide. Water Res. 46 (5), 1460–1472.

Pan, X.L., 2010. Microbial extracellular polymeric substances: the ignored but crucial bio-interface affect in mobility of heavy metals in environment (editorial). Res. J. Biotechnol. 5 (3), 3–4.

Pan, X.L., Deng, C.N., Zhang, D.Y., Wang, J.L., Mu, G.J., 2008. Toxic effects of amoxicillin on the photosystem II of *Synechocystis* sp. characterized by a variety of in vivo chlorophyll fluorescence tests. Aquat. Toxicol. 89, 207–213.

Pan, X.L., Zhang, D.Y., Chen, X., Mu, G.J., Li, L.H., Bao, A.M., 2009. Effects of levofloxacin hydrochlordie on photosystem II activity and heterogeneity of *Synechocystis* sp. Chemosphere 77, 413–418.

Pan, X.L., Liu, J., Zhang, D.Y., Chen, X., Li, L.H., Song, W.J., Yang, J.Y., 2010a. A comparison of five extraction methods for extracellular polymeric substances(EPS) from biofilm by using three-dimensional excitation-emission matrix (3DEEM) fluorescence spectroscopy. Water SA 36 (1), 111–116.

Pan, X.L., Liu, J., Zhang, D.Y., Song, W.J., Wu, F.C., 2010b. Binding of dicamba to soluble and bound extracellular polymeric substances (EPS) from aerobic activated sludge: a fluorescence quenching study. J. Colloid Interface Sci. 345 (2), 442–447.

Pan, X.L., Liu, J., Song, W.J., Zhang, D.Y., 2012. Biosorption of Cu(II) to Extracellular Polymeric Substances (EPS) from *Synechoeystis* sp.: a fluorescence quenching study. Front. Environ. Sci. Eng. 6, 493–497.

Pivokonsky, M., Safarikova, J., Baresova, M., Pivokonsak, L., Kopeka, I., 2014. A comparison of the character of algal extracellular versus cellular organic matter produced by cyanobacterium, diatom and green alga. Water Res. 51, 37–46.

Qian, H.F., Yu, S.Q., Sun, Z.Q., Xie, X.C., Liu, W.P., Fu, Z.W., 2010. Effects of copper sulfate, hydrogen peroxide and N-phenyl-2-naphthylamine on oxidative stress and the expression of genes involved photosynthesis and microcyst in disposition in *Microcystis aeruginosa*. Aquat. Toxicol. 99 (3), 405–412.

Qian, H.F., Hu, B.F., Yu, S.Q., Pan, X.J., Wu, T., 2012. The effects of hydrogen peroxide on the circadian rhythms of *Microcystis aeruginosa*. PLOS ONE 7 (3), e33347.

Rajasekhar, P., Fan, L.H., Nguyen, T., Roddick, F.A., 2012. A review of the use of sonication to control cyanobacterial blooms. Water Res. 46 (14), 4319–4329.

Shepherd, V., Beilby, M., 1999. The effect of an extracellular mucilage on the response to osmotic shock in the charophyte alga *Lamprothamnium papulosum*. J. Memb. Biol. 170 (3), 229–242.

Sivonen, K., Jones, G., 1999. Cyanobacterial toxins. In: Chorus, I., Bartram, J. (Eds.), Toxic Cyanobacteria in Water. WHO, E&FN Spon, London, pp. 41–111.

Stanier, R.Y., Kunisawa, R., Mandel, M., Cohen-Bazire, G., 1971. Purification and properties of unicellularblue-greenalgae(order Chroococcales). Bacteriol. Rev. 35 (2), 171–205.

Wang, H.Y., Jiang, X.L., Mu, H.J., Liang, X.T., Guan, H.S., 2007. Structure and protective effect of exopolysaccharide from *P. agglomerans* strain KFS-9 against UV radiation. Microbiol. Res. 162 (2), 124–129.

Wang, Z., Lia, D., Qina, H., Li, Y., 2012. An integrated method for removal of harmful cyanobacterial blooms in eutrophic lakes. Environ. Pollut. 160 (1), 34–41.

Wingender, J., Neu, T.R., Flemming, H.C., 1999. Microbial Extracellular Polymeric Substances: Characterization, Structure, and Function. Springer Verlag.

Xu, Y., Yang, J., Ou, M., Wang, Y., Jia, J., 2007. Study of *Microcystis aeruginosa* inhibition by electrochemical method. Biochem. Eng. J. 36 (3), 215–220.

Ye, S., Liu, F., Wang, J., Wang, H., Zhang, M., 2012. Antioxidant activities of an exopolysaccharide isolated and purified from marine *Pseudomonas* PF-6. Carbohydr. Polym. 87 (1), 764–770.

Zhang, D.Y., Pan, X.L., Mostofa, K.M.G., Chen, X., Wu, F.C., Mu, G.J., Song, W.J., Liu, J., 2010. Complexation between Hg(II) and biofilm extracellular polymeric substances: an application of fluorescence spectroscopy. J. Hazard. Mater. 175 (1–3), 359–365.

Zhang, D.Y., Lee, D.J., Pan, X.L., 2013. Desorption of Hg(II) and Sb(V) on extracellular polymeric substances: effects of pH, EDTA, Ca(II) and temperature shocks. Bioresour. Technol. 128, 711–715.