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The Journal of Environmental Sciences, an international monthly journal established since 1989 and sponsored by the Research Centre for Eco-Environmental Sciences, Chinese Academy of Sciences. Journal of Environmental Sciences is devoted to publish original, peer-reviewed research papers and reviews on main aspects of environmental sciences, such as environmental chemistry, soil chemistry, atmospheric chemistry, environmental biology, ecotoxicology, geosciences. The aim of the journal is to provide a platform for the latest research advancement.

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Preliminary characterization of bound extracellular polymeric substances (bEPS) of cyanobacteria is crucial to obtain a better understanding of the formation mechanism of cyanobacterial bloom. However, the characterization of bEPS can be affected by extraction methods. Five sets (including the control) of bEPS from *Microcystis* extracted by different methods were characterized using three-dimensional excitation and emission matrix (3DEEM) fluorescence spectroscopy combined chemical spectrophotometry; and the characterization results of bEPS samples were further compared. The agents used for extraction were NaOH, pure water and phosphate buffered saline (PBS) containing cationic exchange resins, and hot water. Extraction methods affected the fluorescence signals and intensities in the bEPS. Five fluorescence peaks were observed in the excitation and emission matrix fluorescence spectra of bEPS samples. Two peaks (peaks T1 and T2) present in all extractions were identified as protein-like fluorophores, two (peaks A and C) as humic-like fluorophores, and one (peak E) as a fulvic-like substance. Among these substances, the humic-like and fulvic-like fluorences were only seen in the bEPS extracted with hot water. Also, NaOH solution extraction could result in strong fluorescence intensities compared to the other extraction methods. It was suggested that NaOH at pH 10.0 was the most appropriate method to extract bEPS from *Microcystis*. In addition, dialysis could affect the yields and characteristics of extracted bEPS during the determination process. These results will help us to explore the issues of cyanobacterial blooms.

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with cell aggregates (Reynolds, 2007). The bEPS as the coating of cyanobacterial cells have various additional functions, such as avoiding ultraviolet radiation, storing nutrients, attaching trace metals, and preventing virus infection (De Philippis et al., 2011). Therefore, the characterization of cyanobacterial bEPS is helpful to acquiring knowledge of the mechanism of cyanobacteria bloom formation and to finding a way of bloom control.

Up to now, the majority of studies have focused on extraction of bEPS from bacteria in sludge (Bourven et al., 2011; Comte et al., 2006; d’Abzac et al., 2010; Frolund et al., 1996; Liu and Fang, 2002) or diatoms in waters (de Brouwer et al., 2002; Takahashi et al., 2009) using physical methods (sonication, water heating, cation exchange resin (CER)) and chemical methods (ethylene diamine tetraacetic acid (EDTA), NaOH and formaldehyde). Among these methods, cation exchange resin (CER) (dowex resin) extraction was recommended by Comte et al. (2006) and Takahashi et al. (2009) as the most efficient method. It was also noted that hot water extraction could disrupt cells (Takahashi et al., 2009). In a recent study, de Brouwer et al. (2002) found that the extraction of bEPS from benthic diatoms with warm distilled water (30°C) was more efficient than that with EDTA (4 mmol/L). For cyanobacteria, many studies have been conducted to compare the effectiveness of extraction methods on quantifying polysaccharides in bEPS. The method of extraction with hot water (80°C for 1 hr) yielded more polysaccharides from the bEPS of Microcystis aeruginosa and Microcystis flos-aquae than those with cold water (4°C for 7 days) (Forni et al., 1997). Alkaline solution (NaOH, pH 10.0) was always used to extract polysaccharides of bEPS from algae (Yang et al., 2008, 2009).

According to previous research, extraction methods could affect the composition and function of bEPS (Bourven et al., 2011; d’Abzac et al., 2010; Frolund et al., 1996). Effective extraction and determination of bEPS is critical in order to understand the physicochemical properties of bEPS (Wingender et al., 1999). However, so far, there are few published universal methods that can be used to extract bEPS from cyanobacteria. Although the comparison of extraction methods has been discussed by Xu et al. (2013b), their research objects were field cyanobacteria. As known, there are a lot of heterotrophic bacteria adhering to the surface of cyanobacterial cells, which could affect the extraction contents or components of bEPS from cyanobacteria. Thus, in this study, the axenic vitro culture of pure cyanobacterium Microcystis was selected as the study object to investigate the effect of extraction methods on the characterization of cyanobacterial bEPS. In addition, samples were dialyzed before measuring the contents of protein and carbohydrate; and a series of dialysis tubings of different pore sizes and molecular weight cutoff were used (Takahashi et al., 2009; Yang and Kong, 2012, 2013; Yang et al., 2008, 2009, 2010). For this purpose, the effect of dialysis on the components and contents of cyanobacterial bEPS was also studied.

Recently, three-dimensional excitation and emission matrix (3DEEM) fluorescence spectroscopy has been applied to characterize extracellular polymeric substances (EPS), and has proved to be an appropriate and effective method (Sheng and Yu, 2006; Zhang et al., 2010). The results of 3DEEM showed that several peaks of the soluble EPS excreted by Microcystis were assigned to protein-like substances, humic-like substances, and fulvic-like substances (Henderson et al., 2008). Qu et al. (2012) also characterized the soluble EPS and bEPS from Microcystis. Nevertheless, there are few papers reporting and assessing the effect of extraction methods on the spectrofluorometric characterization of bEPS from Microcystis by fluorescence excitation and emission matrix (EEM) technique. Accordingly, in this article, we compared four extraction methods of bEPS from cultured cyanobacterium Microcystis. The quantity and quality of bEPS by 3DEEM fluorescence spectroscopy were assessed; and the protein, carbohydrate and phycobilin protein concentrations of extracted bEPS by chemical methods were determined.

### 1. Materials and methods

#### 1.1. Algal cultures

Microcystis wesenbergii as the preponderant algae in cyanobacterial blooms was obtained from the Institute of Hydrobiology, Chinese Academy of Sciences, in Wuhan city, China, and cultured in a BG-11 medium at 25°C under a light–dark (12 hr–12 hr) cycle at 4000 lx. After one month, when they were in stationary growth phase, the cultures (cell density, 1.42 × 10^9 cells/mL) were harvested.

#### 1.2. bEPS extraction processes

Before extraction, in order to separate the supernatant soluble EPS from pellets, an aliquot of 20 mL from cultures was centrifuged at 10,000 × g for 5 min at 4°C. Then the pellets were re-suspended in 20 mL of extracted solution to extract bEPS as per four different procedures. For the control group, the pellets were re-suspended in 20 mL of ultrapure water. Each extraction method was performed in triplicate.

The procedures of four extraction methods were performed as follows: (1) The extraction reagent was ion-exchange resin CER (Amberlite® IR120, Na form, Rohm & Haas Co., Philadelphia, Pennsylvania, USA) in phosphate buffered saline (PBS) (0.1 mol/L, PBS, pH 7.0) (abbreviated CER (PBS)) and was inoculated for 1 hr at 4°C (Frolund et al., 1996; Takahashi et al., 2009). The quantity was 50 g CER per gram biomass (Liu and Fang, 2002), but a 70 g CER per gram biomass was used by Frolund et al. (1996). Owing to differences between extracted materials, a sufficient amount of CER was used to ensure the adequacy of cyanobacterial bEPS extraction. The amount of CER used in this study was ca. 150 g CER (wt weight)/g alga (wt weight). (2) CER in pure water (abbreviated CER (water)) was used (Takahashi et al., 2009); the quantity of CER was used equal to that used in the CER (PBS). (3) Hot pure water extraction for 1 hr after preheating at 80°C was performed (Forni et al., 1997). (4) NaOH solution (pH 10) for 4 hr after preheating at 45°C was processed (Yang et al., 2008, 2009). The control group did not require any treatment (abbreviated NT), so it was centrifuged directly. The centrifugation of bEPS from treated pellets was conducted at 10,000 × g for 5 min at 4°C. The supernatants were filtered through 0.45 μm GF/F glass microfiber filters (Whatman™, Whatman International Ltd. Maidstone, England), and then filtered through 0.2 μm cellulose acetate filters (Sartorius Stedim Biotech GmbH, Goettingen, Germany). The filtrates were used as bEPS samples and the pellets were discarded. In order to test the effect of pore sizes of dialysis membranes on the fluorescence signals of 3DEEM and on the chemical compositions of extracted bEPS, bEPS samples were dialyzed using 3500 or 14,000 Da membranes. Dialysis tubings containing bEPS samples were placed in 500 mL of pure water for 50 hr at 4°C before the determination of 3DEEM characteristics and concentrations of carbohydrate and protein. Four extraction methods and the control group are summarized in Fig. 1.

#### 1.3. Chemical analysis of bEPS (phycobilin proteins, carbohydrate and protein concentrations)

For all bEPS extracts, the assay of chemical compositions was carried out by colorimetric methods as follows:
1.3.1. Phycobilin proteins

Determination of phycobilin proteins is a good way to test for cell lysis (Schluchter and Glazer, 1999). So in order to determine which extraction procedure caused less cell lysis, we investigated the release of intracellular phycobilin protein. The measurement was conducted on a Shimadzu UV-2401PC UV–vis spectrophotometer; and absorbances at the wavelength of 620 nm (OD{\text{620}}), 650 nm (OD{\text{650}}), and 565 nm (OD{\text{565}}) were recorded to calculate the concentrations of three pigments within the phycobilin family, allophycocyanin (APC, mg/mL), phycocyanin (PC, mg/mL) and phycoerythrin (PE, mg/mL) based on the equations described below by Abelson and Simon (1988).

\[
\text{PC} = \frac{\text{OD}_{620} - 0.7 \times \text{OD}_{650}}{7.38} \\
\text{PE} = \frac{\text{OD}_{565} - 2.8 \times \text{PC} - 1.34 \times \text{(AP)}}{1.27} \\
\text{APC} = \frac{\text{OD}_{650} - 0.19 \times \text{OD}_{620}}{5.65}
\]

1.3.2. Carbohydrate

The extracts were dialyzed for at least 48 hr (about 50 hr in this study) against MilliQ water using 3500 and 14,000 Da membranes at 4°C. The carbohydrate contents were determined by the method of Dubois et al. (1956) with glucose as the standard. Briefly, 1 mL of 5% phenol and 5 mL of sulfuric acid (AR, analytical reagent) were added to 1 mL of the dialyzed extract. The mixture was vortexed and analyzed after 30 min using spectrophotometry at the wavelength of 488 nm and glucose as the standard for the calibration curve.

1.3.3. Protein

Aliquots of extracts (0.05 mL) were dialyzed against MilliQ water at 3500 or 14,000 Da at 4°C for about 50 hr and were then taken for protein analysis. The total protein concentrations were tested using Coomassie Blue, which is a reagent kit purchased from Nanjing Jiancheng Bioengineering Institute of Jiangsu Province, Nanjing city, China.

**Fig. 1 – Flow chart of bound extracellular polymeric substances extraction from cultured Microcystis.**
1.3.4. 3DEEM fluorescence spectroscopy

The 3DEEM fluorescence of bEPS samples before and after dialysis with 3500 or 14,000 Da molecular weight cut-off (MWCO) membranes was characterized on a Hitachi F-7000 fluorescence spectrometer (Hitachi High-Technologies, Tokyo, Japan). The 3DEEM spectra were collected with subsequent scanning emission spectra from 250 to 550 nm at 1 nm increments, by varying the excitation wavelength from 200 to 450 nm at 5 nm increments, and with a speed of 2400 nm/min. The blank spectrum was recorded with only extraction solution.

1.4. Statistics

Analysis of variance was applied to the concentrations of carbohydrate before and after dialysis as well as to the intensities of protein-like peaks (peaks T1 and T2) to check the significance of difference between the data. The independent-sample t-test was also employed to compare the contents of carbohydrate and protein between using two types of dialysis membranes. Statistical analysis was performed using Origin 8.0 software (OriginLab Corporation Northampton, USA) and SPSS 17.0 software (Statistical Program for Social Sciences, Chicago, USA).

2. Results

2.1. Results of chemical determination (concentrations of carbohydrate, protein, and phycobilin proteins)

The comparison of the contents of carbohydrate before and after dialysis with 3500 or 14,000 Da did not show a significant difference among the extraction methods (Fig. 2) (p > 0.05). However, significant differences were displayed for the contents of protein extracted using five methods no matter whether the dialysis was used or not (p < 0.05). Before dialysis, the protein contents extracted by NaOH solution were relatively higher than those extracted by other methods. The contents of protein extracted with hot water were the highest after dialyzing with 3500 Da. However, compared to the contents of protein dialyzed with 14,000 Da, CER (water) method could extract more protein.

There were significant differences in carbohydrate and protein contents between samples dialyzed with molecular weight cut-offs of 3500 and 14,000 Da. For all extraction methods, the concentrations of carbohydrate dialyzed with 14,000 Da were significantly higher than those with 3500 Da (p < 0.01). This trend is similar to that in protein (p < 0.05) (Fig. 2). As shown in Fig. 3, the phycoerythrin contents of phycobiliproteins were significantly higher than the contents of allophycocyanin and phycocyanin (p < 0.01). There were no significant differences among the concentrations of allophycocyanin, phycocyanin and allophycocyanin extracted by four methods and NT.

2.2. Results of 3DEEM

The number of main peaks in a 3DEEM fluorescence spectrum ranged from 2 to 5 (T1, T2, A, C, and E), depending on the extraction methods and dialysis protocols used (Table 1, and Fig. 4). The major peak T1 was at the excitation/emission wavelength (Ex/Em) of 270–275/278–356 nm, while peak T2 was at Ex/Em of 210–230/310–344 nm. These peaks were considered as protein-like peaks, which were assigned to the fluorescence of tryptophan (protein-like substances) (Coble, 1996; Henderson et al., 2008). The A and C peaks were located at Ex/Em of 330–355/451–463 nm and 275/451–465 nm, respectively. Similar peak-locations were assigned to humic-like substances (Coble, 1996). Peak E located at Ex/Em of 240/448–463 nm in this study was considered to represent the fulvic-like substance according to Wang et al. (2009).

While there were five main fluorescence peaks of bEPS extracted from Microcystis by the hot water method, there were only two main fluorescence peaks (T1 and T2) in the EEM spectra for the other three extraction methods (Table 1). Peaks T1 and T2 were present in all extractions. Dialysis could remove A, C and E peaks under some protocols (Table 1), and could affect the fluorescence intensities of peak T1 and T2 (Figs. 5 and 6).

By comparing the intensity of peak T1 (a protein-like substance) without dialysis process, the two strongest intensities were shown in the bEPS extracted by hot water and NaOH methods. These intensities were significantly higher than those extracted by the other three extraction methods (including NT) (p < 0.05). There was no significant difference in peak T1 intensity extracted with hot water and NaOH solution. The peak intensity of the bEPS by CER (water) extraction was significantly higher than that by CER (PBS) and NT methods. There were no significant differences among peak intensities extracted by CER (PBS) and NT methods.

Similarly, for peak T2 before dialysis, a protein-like substance, the strongest intensity was shown in bEPS by NaOH extraction. The intensity of peak T2 was significantly higher than that by CER (water) extraction and CER (PBS) extraction, but it was not significantly different from that by hot water.
Fig. 3 – The bound extracellular polymeric substances yields of three phycobilin proteins (phycocyanin, allophycocyanin, and phycoerythrin), extracted by different methods. Data are means with standard errors.

3. Discussion

Previous research had revealed that bEPS played important roles in the formation of colony or aggregation of cyanobacteria (Reynolds, 2007; Yang et al., 2008, 2009). Besides, as the coating of cells, the characterization of bEPS must affect the behavior and fate of cyanobacterial blooms. Therefore, it is essential to know in depth the characteristics of bEPS to better control the nuisance blooms. In this article, four common extraction methods and the control group were employed to assess the characteristics of bEPS, and to screen for the optimum method to extract bEPS of Microcystis for further research.

3.1. Characteristics of bEPS

In this article, the protein and carbohydrate contents of bEPS from lab-cultivated M. wesenbergii were determined. As shown in Fig. 2, the ratios of protein to carbohydrate ranged from 0 to 11.8, with a mean value of 3.7 for all of the extraction methods including all the dialysis processes, indicating that the bEPS of M. wesenbergii was mainly comprised of protein, and the wide range of values suggested that the ratios could be affected by dialysis processes and extraction methods. Qu et al. (2012) found a mean value of 6.61 in the bEPS from M. aeruginosa. However, as reported by Xu et al. (2013b), the contents of polysaccharides of bEPS from cultivated M. aeruginosa in the standard medium were found to be higher than those of protein. The objects of their studies were both M. aeruginosa, but they got the completely opposite conclusions. This may be caused by different extraction methods or different culture phases for the M. aeruginosa (Pivokonsky et al., 2006; Qu et al., 2012; Xu et al., 2013b).

In general, two main peaks (peak T1 and T2) belonging to the protein-like substances were identified in the extracted bEPS of M. wesenbergii. Similar results were also obtained by Xu et al. (2013a) and Qu et al. (2012). Although the studied species (M. wesenbergii in this study vs M. aeruginosa in their studies) were different, the major bloom-forming cyanobacterial species Microcystis showed similar 3DEEM fluorescence spectra for bEPS. Additionally, as the two peaks were always observed in extracted bEPS, the intensities of these peaks or the ratios may give us a deep understanding of the characteristics of bEPS, especially the features of protein in bEPS.

3.2. Cell lysis and release of intracellular phycobilin proteins

To evaluate whether extraction methods resulted in cell lysis, the contents of intracellular phycobilin proteins (phycocyanin, allophycocyanin, and phycoerythrin) were measured. This kind of evaluation had been used by the authors and proved feasible (Schlu却不ting and Glazer, 1999; Yang et al., 2011). Since the cells were in stationary phase, there was cell death in the culture medium. In fact, during the whole culture period, there was a balance between the number of dead or dying cells and new daughter cells. If the growth rate exceeded the death rate, the cyanobacterial populations would undergo net growth. When the growth rate and death rate are equal, then stationary phase was attained. Therefore, many types of intracellular phycobilin proteins (phycocyanin, allophycocyanin, phycoerythrin) were also observed in the control. The amounts of intracellular phycobilin proteins in extracted bEPS were not significantly different among the extraction methods, but the amounts of phycobilin extracted with hot water were higher than those with the other extraction methods (excluding CER (PBS)). This suggested that the use of hot water (80°C) was likely to lead to cell lysis. According to the evaluation of 3DEEM spectra, peak A located Ex/Em = 330–355/451–463 nm in our study was similar to the peak of intracellular algae organic matter (IOM) from Microcystis that occurred at Ex/Em of 365/460 (Li et al., 2012). As shown in Fig. 4, peak A was present only in bEPS by hot water.

<table>
<thead>
<tr>
<th>Extraition method</th>
<th>Before dialysis</th>
<th>Dialysis with 3500 Da</th>
<th>Dialysis with 14,000 Da</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT (not treated)</td>
<td>T1, T2</td>
<td>T1, T2</td>
<td>T1, T2</td>
</tr>
<tr>
<td>CER (PBS)</td>
<td>T1, T2</td>
<td>T1, T2</td>
<td>T1, T2</td>
</tr>
<tr>
<td>CER (water)</td>
<td>T1, T2</td>
<td>T1, T2, A, C, E</td>
<td>T1, T2, A, C</td>
</tr>
<tr>
<td>Hot water</td>
<td>T1, T2</td>
<td>T1, T2</td>
<td>T1, T2</td>
</tr>
<tr>
<td>NaOH</td>
<td>T1, T2</td>
<td>T1, T2</td>
<td>T1, T2</td>
</tr>
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</table>

T1 and T2 peaks were seen in all 3DEEM fluorescence spectra. The presence of additional A, C, and E peaks depended on the extraction method and dialysis protocol used.

3DEEM: three-dimensional excitation and emission matrix.
Fig. 4 – The three-dimensional excitation and emission matrix fluorescence spectra of bound extracellular polymeric substances extracted by different methods. The main peaks are identified as T1, T2, A, C and E.

Fig. 5 – Maximum fluorescence intensities of T1 (a) and T2 (b) protein-like substances of Microcystis bound extracellular polymeric substances under different extraction methods, and three dialysis protocols. Data are means with standard deviations.

Fig. 6 – Maximum fluorescence intensities of humic-like (peaks A and C) and fulvic-like (peak E) substances of Microcystis bound extracellular polymeric substances using hot water extraction. Zero intensity was seen in peak A (14,000 Da) and peak E (3500 and 14,000 Da). Data are means with standard deviations.
methods directly. For all extraction methods, the intensity of peak T₃ was higher than that of peak T₂ (Fig. 5) through comparing the average intensities of peak T₁ and peak T₃ before dialysis. This indicated that this kind of protein-like substance was abundantly present in bEPS. For peak T₁, the intensity extracted by NaOH method was 2.6 times more than that extracted by NT, and 2.7 and 1.8 times more than those by CER methods (two methods: CER (PBS) and CER (water)). For peak T₂, NaOH solution could extract the highest intensity. It produced the peak T₂ with its intensity 1.3 times more than that by hot water extraction, 1.3 times of that by NT, and 2.2 and 2.9 times of those by CER methods (two methods).

In general, extraction using a NaOH solution at pH 10.0 resulted in the strongest fluorescence intensity at peak T₂, and strong intensity at peak T₁ among the methods tested. Although hot water could result in strong peaks of extracted bEPS, it may be easier to provoke cell lysis as mentioned above. Thus, we suggested that NaOH (pH 10) solution was a more suitable method to extract the bEPS of Microcystis.

3.4. Effects of dialysis

The dialysis processes were always done before the determination of the contents of carbohydrate and protein, but different dialysis tubings were used. We chose the common dialysis tubings with weight cut-off 3500 and 14,000 Da in this study. As shown in Fig. 2, we compared the contents of carbohydrate and protein using different membranes. Usually dialysis leads to dilution of samples inside the dialysis tubing. Dialysis involves molecules passing through the semi-permeable membrane in both directions and each substance reaches its own equilibrium independently of the other substances; therefore, sample dilution can occur. If a substance’s concentration is high in the outside of the membrane and its size is small enough to pass through the membrane pores, a net movement from the dialysis buffer into the sample would occur. Water is such a small molecule that it is capable of passing through the pores of virtually all dialysis membranes. When dialyzing a high solute concentration against a dilute dialysis buffer, there will thus be a net movement of water (and possibly salts) into the dialysis unit through the membrane. Similarly to the data presented by Pan et al. (2010), the concentrations of protein and carbohydrate of extracted EPS decreased after dialysis. However, interestingly, the concentrations of carbohydrate and protein increased after dialysis with a big cutoff. The carbohydrate and protein contents dialyzed with the 14,000 Da weight cut-off membrane were significantly higher than those dialyzed with the 3500 Da membrane. In the experimental process, it was noted that the volume of dialysis tubings decreased. This suggested that extracted polymers may stick to the dialysis membrane, resulting in single-pass of small molecules from the hypertonic samples into dialysis buffer (pure water). Another possible explanation is that some small-molecule chemicals influencing the determination of protein and carbohydrate contents were removed during dialysis and thus the contents of protein and carbohydrate might have increased. The exact reasons await future study.

Dialysis could also affect both the quantities and the characteristics of the fluorescence spectra of bEPS (Table 1, Figs. 5 and 6), which is consistent with the report of Pan et al. (2010). The fluorescence intensities at peak E in the bEPS of Microcystis were eliminated after dialyzing with a 3500 Da membrane. The intensities of peak A decreased significantly or disappeared after using 14,000 Da molecular cutoff dialysis membranes. As shown in Fig. 6, after dialysis (3500 Da), the fluorescence intensities at peak A and peak C in the bEPS sample by hot water extraction decreased by 67.3% and 64.2%, respectively. This indicates that most of the humic-like substances were of less than 3500 Da molecular weight. The fluorescence spectra of peak E were not tested in hot water extraction with 3500 Da cut-off weight dialysis, thus the fulvic-like substances were also less than 3500 Da molecular weight.

For peak T₃, by comparing the intensities among the extracted bEPS without dialysis, with 3500 Da dialysis and 14,000 Da dialysis, the fluorescence intensities of peak T₃ were shown in a decreasing order as follows: dialysis with 3500 Da > dialysis with 14,000 Da > without dialysis. For peak T₃, the observed order was: dialysis with 14,000 Da > dialysis with 3500 Da > without dialysis. As shown in the analyzed results above, the amounts of protein-like substances without dialysis were the lowest, and using various dialysis membranes could result in different results.

As reported by Sheng and Yu (2006), the fluorescence intensities increased with pH in the range of 3–10, but they were not affected by ion intensities (Sheng and Yu, 2006). Dialysis diluted the pH value, e.g., dialysis decreased the pH value of the alkaline solution (NaOH extraction method); this should have lowered the intensities of peaks. However, in this study, most of the peak intensities increased after dialysis. Because some metals could quench the fluorescence such as Al³⁺ (Ruan et al., 2013), dialysis could remove these metals in the extracted solution and lead to reactive fluorescence. This would explain the results that the concentrations of peaks after dialysis were higher than those before dialysis. Thus, the use of different dialyses should be paid an attention to during the preparation of EPS samples, as shown by this study.

4. Conclusions

As bEPS strongly impacts the behavior and fate of cyanobacteria, their quantification and characteristics are essential to explore the mechanism of cyanobacterial bloom outbreaks. Since extraction methods can lead to different results, this study provides a comparison of different extraction methods of bEPS from Microcystis. Our results have shown that extraction with hot water can lyse cells. The NaOH solution (pH 10) method compared to the other extraction methods extracted strong fluorescence intensities in bEPS. Therefore, we consider NaOH solution extraction as the most suitable method among the four extraction methods. In addition, we should pay more attention to the dialysis processes, which can significantly affect the efficiency of extraction methods. Furthermore, care is needed in the preparation of bEPS.

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Aims and scope

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