Colloids and Surfaces B: Biointerfaces 128 (2015) 115–118

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Effects of irradiation and pH on fluorescence properties and flocculation of extracellular polymeric substances from the cyanobacterium *Chroococcus minutus*

Wenjuan Song[a], Chenxi Zhao[c], Shuyong Mu[a], Xiangliang Pan[a,*], Daoyong Zhang[a,b,**], Fahad A. Al-Misned[d], M. Golam Mortuza[d,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] *Xinjiang Academy of Environmental Protection Sciences, Urumqi, Xinjiang 830011, China*
[d] *Department of Zoology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia*
[e] *Department of Zoology, Faculty of Life and Earth Science, Rajshahi University, Rajshahi 6205, Bangladesh*

ARTICLE INFO

Article history:
Received 22 August 2014
Received in revised form 3 February 2015
Accepted 9 February 2015
Available online 16 February 2015

Keywords:
Cyanobacteria
EEM fluorescence spectroscopy
Fluorophores
Heavy metals
Nutrient cycles

ABSTRACT

Microbial extracellular polymeric substances (EPS) may flocculate or be decomposed when environmental factors change, which significantly influences nutrient cycling and transport of heavy metals. However, little information is available on the stability of EPS in natural environments. Fluorescence and flocculation properties of EPS from *Chroococcus minutus* under different irradiation and pH conditions were studied. Two aromatic protein-like fluorescence peaks and one tyrosine protein-like peak were identified from the excitation–emission-matrix (EEM) fluorescence spectra of EPS. UVB (ultraviolet B) and solar irradiation increased the fluorescence intensity of all the three peaks while UVC (ultraviolet C) irradiation had little effect. EPS formed unstable flocs after exposure to UV (ultraviolet) irradiation and formed stable flocs under solar irradiation. EPS were prone to flocculation under highly acidic conditions and minimal fluorescence of peaks was observed. The fluorophores in EPS were relatively stable under neutral and alkaline conditions. These findings are helpful for understanding the behavior of EPS in aquatic environments and their role in biogeochemical cycles of the elements.

1. Introduction

Microbial extracellular polymeric substances (EPS) are one of the important types of dissolved organic matter in natural environments and can exert a significant influence on the cycling of nutrient elements such as carbon and nitrogen [1,2]. EPS is mainly composed of polysaccharides, proteins, humic substances and nucleic acids [3,4]. EPS has important cellular functions, including the accumulation of nutrients, formation of a barrier against toxins and in the construction and stability of biofilms [5,6]. EPS can form flocs with various suspended solids such as kaolin clay particles, MgO and Al_2O_3 [7]. The properties and functions of EPS vary with changing temperature, salinity and irradiation [8–10].

Cyanobacteria are important photosynthetic microorganisms in both aquatic and terrestrial environments. They can produce large amounts of EPS, which leads to a better survival capacity than many other phototrophic microorganisms [11]. The protein-like fluorescent substances in EPS from cyanobacteria have significant impact on the speciation and mobility of heavy metals [12–14]. EPS acts as an effective buffering zone between living cells and the ambient environment and can alleviate adverse effects from the ambient environment. Algal EPS can be decomposed by heterotrophic microbial populations [15,16]. Aluwihare and Repeta [15] observed selective microbial degradation and flocculation of EPS from a marine strain of *Thalassiosira weissflogii*. The selective microbial degradation of EPS might enhance the adhesion properties of EPS and promote the formation of transparent exopolymeric particles and aggregates [16]. The decomposition of EPS can

* Corresponding author at: Xinjiang Key Laboratory of Environmental Pollution and Bioremediation, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi 830011, China. Tel.: +86 991 7823156; fax: +86 991 7823156.
** Corresponding author at: State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang 550002, China. Tel.: + Þ86 851 5890483; fax: + Þ86 851 5890483.
E-mail addresses: panxl@ms.xjb.ac.cn (X. Pan), zhang-daoyong@163.com (D. Zhang).

http://dx.doi.org/10.1016/j.colsurfb.2015.02.017
0927-7765/

therefore affect migration of elements such as carbon and nitrogen, as well as the release of heavy metals which have been adsorbed by the EPS.

Elevated UV (ultraviolet) radiation is one of the most detrimental environmental factors for photosynthetic organisms in arid zones [9]. A few studies have shown that EPS protects the cells against the damage from UV irradiation [17,18]. UVB (ultraviolet B) irradiation can decompose humic substances to small molecular weight substances in river water [1]. 96% of freshwater chromophoric dissolved organic matter and 41% of dissolved organic carbon were decomposed under solar irradiation [19].pH is another important environmental factor that might influence the yield, structure and chemical properties of EPS [20,21]. Because of the competition for binding sites between protons and metal cations in natural environments, pH is usually thought to be one of the most important factors which affects adsorption of heavy metal ions to EPS [22,23]. The metal binding sites in EPS from activated sludge increased with increasing pH [24]. Therefore, the stability of EPS under different pH conditions might affect the behavior and fate of metal ions.

Despite the important protective role of EPS toward cellular function, there is little information on the decomposition and stability of EPS under different pH and irradiation conditions. In the present study, the typical cyanobacterium *Chroococcus minutus* was chosen as a model organism to examine the effect of irradiation and pH on the composition, fluorescence and flocculation properties of EPS.

2. Materials and methods

2.1. Organism and culture conditions

The *C. minutus* was isolated from a soil sample. Cyanobacterial cells were grown in BG-11 medium [25] at 30 °C under 55 μmol photons m^{-2} s^{-1} PAR (photosynthetically active radiation) with a 12 h/12 h light/dark cycle. After 1 week of incubation, the cells were harvested to extract the EPS.

2.2. EPS extraction

EPS was extracted using a cation exchange resin (001 × 7, Shanghai) [26]. The culture was centrifuged at 5500 rpm for 10 min at 4 °C and then washed twice with Milli-Q water. The supernatant was discarded and the cyanobacterial cells were re-suspended in Milli-Q water. The cell suspension was mixed with some resin and shaken at 120 rpm and 30 °C for 8 h. The mixture was then centrifuged at 16,000 rpm for 10 min at 4 °C. The supernatant, i.e., the raw EPS solution, was filtered through a 0.22 μm membrane and purified using a dialysis membrane (3500 Dalton) at 4 °C for 24 h.

2.3. Determination of the biochemical composition of EPS

The total organic carbon (TOC) content of EPS solution was measured with a TOC analyzer (TOC-4100, Shimadzu, Japan). The polysaccharide content was measured by the phenolsulfuric acid method [27]. Protein content was determined by the Bradford-method [28]. Turbidity of the EPS solution was measured using a light-scattering photoelectric turbidity meter (HACH-2100P, Shanghai). 15 mL of EPS sample was put in a clean sample cell, which was capped with a thin film of silicone oil over the entire surface. The turbidity of the sample was determined using the automatic range for the equipment.

2.4. Irradiation treatment

EPS solutions in Petri dishes were irradiated with UVB light, UVC (ultraviolet C) light and simulated solar light with a background illumination of 55 μmol photons m^{-2} s^{-1} PAR supplied by white fluorescent light. UVB irradiation was supplied by an ultraviolet irradiation lamp (EB-160C/FE, USA) with a peak emission at 312 nm. UVC irradiation was generated by an ultraviolet irradiation lamp (SW-CJ-1FD, Shanghai) with a peak emission at 253.7 nm. Simulated solar light was produced by an iodine–tungsten lamp. The irradiation intensity of UVB, UVC and simulated solar light on the surface of EPS was adjusted to 70 μW cm^{-2}. All lamps were turned on 30 min prior to irradiation tests to allow for stable output of the irradiation. The irradiation time for each treatment was 120 min.

2.5. EEM (Excitation–emission-matrix) fluorescence spectroscopy

EEM spectra of EPS solutions were recorded with a fluorescence spectrophotometer (F-7000, Hitachi, Japan) equipped with one 1.0 cm quartz cell and a thermostatic bath [14]. EEM spectra were collected from 200 to 550 nm of emission wavelength every 2 nm and from 200 to 400 nm of excitation wavelength every 5 nm. The width of the excitation/emission slit and the scanning speed were set to 5.0 nm and 1200 nm min^{-1}, respectively. The spectrum of Milli-Q water was subtracted from the fluorescence spectra of EPS. The three-dimensional EEM spectra were plotted using the software Sigmaplot 10.0 (Systat, US).

3. Results and discussion

3.1. The yield and biochemical composition of EPS

The EPS solution was pH 5.8. The yield of EPS was 60.7 mg g^{-1} dry weight cells. The TOC content was 2.0 mg L^{-1}. There were much more proteins (0.69 mg L^{-1}) present than polysaccharides (0.11 mg L^{-1}) in the EPS.

3.2. The fluorescence property of EPS

The EEM fluorescence spectra of EPS are shown in Fig. 1. Two aromatic protein-like fluorescence peaks (peak A at Ex/Em = 230/302 nm and peak B at Ex/Em = 235/352 nm) and one tyrosine protein-like peak (peak C at Ex/Em = 265/372 nm) were identified from the EEM spectra of EPS [14,18,29]. This result indicates that protein-like substances were the dominant fluorescent

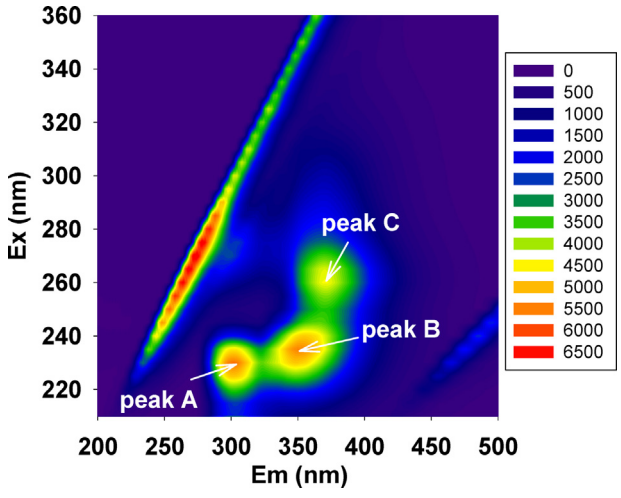

Fig. 1. The EEM fluorescence spectrum of EPS from *C. minutus*.