RESEARCH ARTICLE

Cattle manure DOM on adsorption of copper by the cyanobacterium *Aliinostoc* species

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Abstract: Dissolved organic matter (DOM) and Cu(II), originated from livestock manure, often co-exist in livestock effluents. The effects of DOM on adsorption of Cu(II) by adsorbent remain unknown, which may prevent the removal of Cu(II) from livestock effluents using the method of adsorption. In this study, the effects of DOM on adsorption behaviors of Cu(II) by *Aliinostoc* sp. YYLX235, a epiphytic cyanobacterium, were investigated. The results showed that *Aliinostoc* could effectively bind with Cu(II) and remove it from water. Rather than absorption, most of Cu(II) were bound on the cell surface through adsorption. The decay of *Aliinostoc* did not resulted in rapid release of Cu(II) into water. The amount of Cu(II) adsorbed by *Aliinostoc* through ion exchange and complexation was decreased by DOM addition.
Keywords: Dissolved organic matter, copper ion, epiphytic cyanobacterium, competitive adsorption

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Received: September 24, 2021; **Accepted:** October 30, 2021; **Published Online:** December 2, 2021
Citation: Yan, Q., Sun, C., Feng, Y., Miao, K., Wang, S., Shao, J., 2021. Cattle manure DOM on adsorption of copper by the cyanobacterium *Aliinostoc* species. *Applied Environmental Biotechnology*, 6(2): 13-18. http://doi.org/10.26789/AEB.2021.02.002

1 Introduction

Heavy metal contamination has accelerated in recent decades, and poses great risks to public health (Yu et al., 2020.; Chen et al., 2019; Gu, 2018). Cu(II) is essential for many enzymes to main their property structure and function. In order to get high growth rate of animals, Cu(II) is frequently used as feed additive in farming (Zhang et al., 2005; Kumar et al., 2013). High level of Cu(II) addition in feed results in high content of Cu(II) in livestock manure. For example, the content of Cu(II) in livestock manure reached 642.1 mg/Kg (Zhang et al., 2005). Livestock manure without well management would cause Cu(II) contamination in livestock effluents. Although Cu(II) is a biologically essential trace element, too much of Cu(II) is toxic to biota (Cárdenas-Aguiar et al., 2017). Heavy metal ions in waste effluents could be removed by approaches of ion exchange, precipitation and membrane separation. However, these approaches are high cost or high energy consumption. What's more, some of which may cause secondary pollution to environment. Apart from these approaches, adsorption is a low cost, efficient, environmentally-friendly approach for heavy metal removal from waste effluents (Vendruscolo et al., 2017; Kiran and Thanasekaran, 2011; Maini et al., 2019).

The cell wall and exopolysaccharides of bacteria enrich hydroxyl, acetylated amino, carboxyl, and may also contain sulfydryl and/or phosphate (Ferreira et al., 2017; Joo et al., 2010). These groups could bind with Cu(II) through complexation. Cyanobacteria belong to gram-negative bacteria. Compared with other kinds of bacteria, Cyanobacteria have higher content of exopolysaccharides, usually presented as sheath, capsule, and slime. Besides, cyanobacterial exopolysaccharides have higher content of uronic acid relative to other bacteria, making cyanobacteria have higher affinity toward heavy metals than other kinds of bacteria (De Philippis et al., 2011). The adsorption of *Spirulina platensis* (0.2 g/L) toward Cu(II) reached 60 mg/g at an initial Cu(II) concentration of 100 mg/L (Fang et al., 2011). Cyanobacterial bloom-derived biomass (1 g/L) could remove 80% of Cu(II) at an initial Cu(II) concentration of 10 mg/L (Wang et al., 2010). Previous studies about adsorption of heavy metals by cyanobacteria mainly focused on pelagic cyanobacteria, few focused on epiphytic cyanobacteria. Epiphytic cyanobacteria are easy to form biofilm on matrices in waters, which makes them having greater potential in heavy metals removal by adsorption relative to pelagic cyanobacteria.

Livestock manure contains high content of DOM. If livestock manure without well management, DOM may be loaded into livestock effluents, and form co-contamination with Cu(II). According to the results of gas chromatography mass spectrometry (GC-MS) and Fourier transform infrared spectroscopy (FTIR) analyses, DOM originated from livestock manure contains hydroxyl, carboxyl, and some other anions (Sun et al., 2021; Lin et al., 2017). These groups could bind with Cu(II) through ion exchange and complexation (Sun et al., 2021), which may interfere the uptake of Cu(II) by cyanobacteria. Although adsorption characteristics of Cu(II) by some cyanobacteria were investigated in previous studies, The effects of DOM originated from livestock manure on the adsorption of Cu(II) by cyanobacteria remain unknown. In order to elucidate this issue, the adsorption behaviors Cu(II) by a epiphytic cyanobacterium with or without livestock manure DOM were investigated in this study.

2 Materials and Methods

2.1 Cyanobacterial strain

An epiphytic cyanobacterium *Aliinostoc* sp. YYLX235 was used in this study. This epiphytic cyanobacterium was isolated from surface of paddy soil. *Aliinostoc* was cultured in BG11 medium (Rippka et al., 1979) at 25±1°C under the illumination of 30 μE/(s•m^2). The light/dark cycle was 12h:12h.

2.2 DOM extraction

Cattle manures were obtained from cattle farm of Hunan Institute of Animal Husbandry & Veterinary (Changsha, China). DOM in the cattle manure was extracted by dH_2O under shaking (150 rpm) at 25°C for 4 h, and then passed through 0.45 μm cellulose acetate membrane. The concentration of DOM in the supernatant was indicated by TOC, which was determined on a vario TOC analyser (Elementar Analysen systeme, Hanau, Germany).

2.3 Adsorption process

The cultures of *Aliinostoc* were harvested at late exponential-phase by centrifuge at 10,000×g for 10 min, and then suspended into deionized water (dH_2O). The suspensions were used as adsorbent in the following adsorption study. Batch adsorption was performed in plastic vessels. Adsorption system contained suspended cells of *Aliinostoc* (0.3 g/L wet weight), aliquot volumes of Cu(II) and DOM stock solutions. In order to study effects of DOM on adsorption of Cu(II) by *Aliinostoc*, DOM concentration in the adsorption system was set to 118.61, 237.23, and 474.45 mg TOC/L, the concentration of Cu(II) was 5 mg/L. Adsorption process was carried out at 25°C under shaking with a speed of 200 rpm. Our pre-study showed that adsorption reached equilibrium within 2 h. Thus, the time for adsorption lasted 2 h. Adsorption mixture received centrifuge at 10,000×g for 8 min. The fraction of Cu(II) in the supernatant was regarded as the part which was not adsorbed by cells of the *Aliinostoc*. This part of Cu(II) was directly determined by atomic absorption spectrophotometer (AAS, Hitachi, Tokyo, JP). In order to study effect of initial Cu(II) concentration on adsorption capacity of *Aliinostoc* toward Cu(II) with or without 118.61 mg /L DOM, initial Cu(II) concentration was set to 5, 10, 20, 40, 80, 160, and 200 mg/L, respectively. The pH of adsorption system was adjusted to 4, 5, 6, and 7 to study effects of pH on adsorption of Cu(II) by *Aliinostoc*.

2.4 Desorption experiments

Batch adsorption process was carried out with 5 mg/L Cu(II), 0.3 g/L biomass of *Aliinostoc* (wet weight), and DOM (118.61 mg TOC/L). After adsorption equilibrium, adsorption mixture received centrifuge at 10,000×g for 8 min. The biomass of *Aliinostoc* with adsorbed Cu(II) was in pellet, which was used for desorption analyses. Two desorbents, 2 mol/L NH4NO3 or 0.1 mol/L EDTA-2Na were used to desorb Cu(II) from cells of *Aliinostoc*, respectively. Desorption process was performed using the method reported by Kuang et al. (2019).

2.5 Long term incubation experiment

In order to study the adsorption (including absorption) of Cu(II) by *Aliinostoc* and release of Cu(II) during decay of algal cells, a long term incubation experiment was carried out. Adsorption process was performed using the method described above (Subsection 2.3). The adsorption mixture contained 1 mg/L of Cu(II), 0.3 g/L biomass of *Aliinostoc* (wet weight), with or without 118.61 mg TOC/L DOM, and pH was set to 7.0. After 2 h of adsorption under shaking, the adsorption mixtures were then transferred to an incubator. The temperature of incubator was 25±1°C. The illumination strategy was the same to the description in the subsection 2.1. The incubated adsorption mixtures were sampled on the 2^{nd} h, 24^{th} h (Day 1), and Days 8, 17, and 29, and then centrifuged at 10,000×g for 8 min. The Cu(II) in the supernatant was regarded as the part which was not been adsorbed or absorbed. The concentration of Cu(II) in the supernatant was determined used the method described in subsection 2.3. Due to lack of nutrients in the adsorption mixture, cells of *Aliinostoc* would gradually die. The alive biomass of *Aliinostoc* was indicated by chlorophyll *a* (Chl *a*). Cells of *Aliinostoc* in the adsorption mixture were obtained through centrifuge at 10,000×g for 8 min. The content of Chl *a* in cells of *Aliinostoc* were extracted using 80% acetone and determine spectrophotometrically at 663 and 645 nm (Shao et al., 2015).

2.6 Statistical analyses

The differences were analyzed by one-way ANOVA (LSD). Difference was regarded as significant at $p < 0.05$.

3 Results

3.1 Effects of DOM concentration on adsorption of Cu(II) by *Aliinostoc*

As shown in Figure 1, the DOM, originated from cattle manure, had negative effects on adsorption of *Aliinostoc* toward Cu(II). The adsorption of Cu(II) by *Aliinostoc* was 48.2%, 27.7%, and 0.2% of the control at DOM addition level of 118.61, 237.23, and 474.45 mg/L, respectively. The results of statistical analyses showed that these differences were all statistically significant.

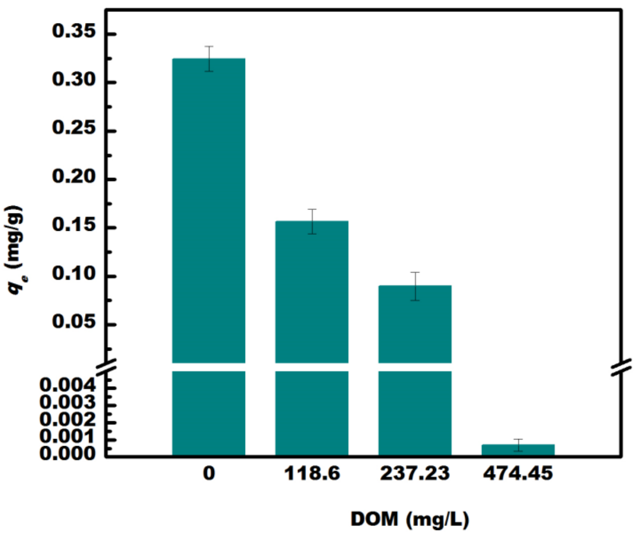

Figure 1. Effects of DOM originated from cattle manure on adsorption of Cu(II) by *Aliinostoc* sp. YYLX235.

Table 1. Adsorption isothermal parameters of Cu(II) by *Aliinostoc* with or without DOM

	Freundlich model			Langmuir model		
	K_F	n	R^2	q_{max} (mg/g)	b	R^2
Aliinostoc	0.759	3.688	0.776	3.003	0.116	0.944
Aliinostoc + DOM	0.323	2.407	0.776	2.987	0.038	0.894

3.2 Effects of different initial Cu(II) concentration and adsorption isotherm

As shown in Figure 2, the adsorption of Cu(II) by *Aliinostoc* rapidly increased along with increase of initial Cu(II) concentration, and it reached plateau at the initial Cu(II) concentration of 80 mg/L. Adsorption data were fitted by Langmuir model and Freundlich model to study adsorption isotherm of Cu(II) by *Aliinostoc* with or without DOM. As shown in Table 1, The regression coefficient (R^2) for Langmuir model was obviously higher than that for Freundlich model. Although DOM addition slightly decreased R2 for

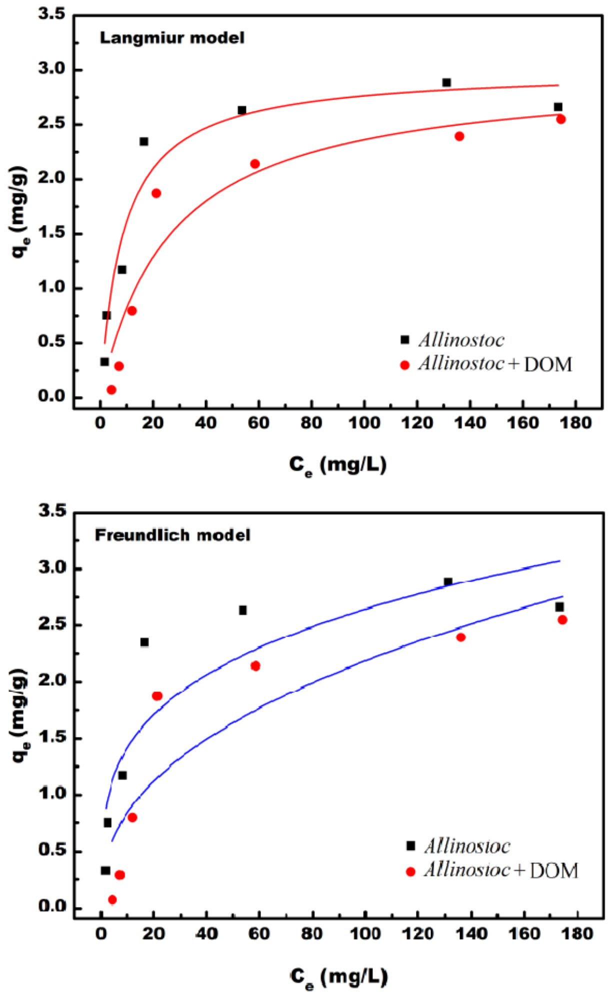

Figure 2. Adsorption isotherms of Cu(II) by *Aliinostoc* sp. YYLX235 with or without DOM. Data were fitted by Langmuir model and Freundlich model.

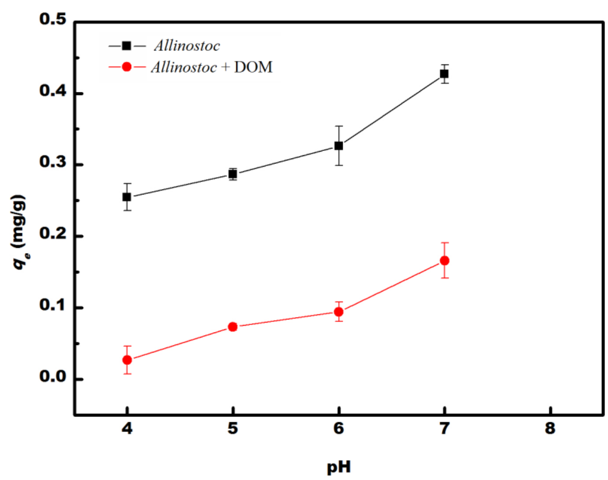

Figure 3. Effect of pH on adsorption characteristics of Cu(II) by *Aliinostoc* sp. YYLX235 with or without DOM.

Langmuir model, it is still more suitable than Freundlich model to explained the adsorption of Cu(II) by *Aliinostoc* + DOM.

3.3 Effects of pH on adsorption

The effects of pH on adsorption of Cu(II) by *Aliinostoc* or *Aliinostoc* + DOM are shown in Figure 3. The adsorption of Cu(II) by *Aliinostoc* rapidly increased along with increase of pH from 4.0 to 7.0. Similar phenomenon was observed in the treatment of *Aliinostoc* + DOM. At a same pH, the adsorption of Cu(II) by *Aliinostoc* was significantly higher than that by *Aliinostoc* + DOM.

3.4 Desorption characteristics

After adsorbed by *Aliinostoc* with or without DOM, the desorption characteristics of Cu(II) from *Aliinostoc* were investigated. In the treatment of without DOM addition, the desorption rates for NH_4NO_3 and EDTA-2Na were 57.3% and 70.1%, respectively (Figure 4). For the treatment of *Aliinostoc* + DOM, NH_4NO_3 released 40.0% of total adsorbed Cu(II), and EDTA-2Na released 44.1% of total adsorbed Cu(II).

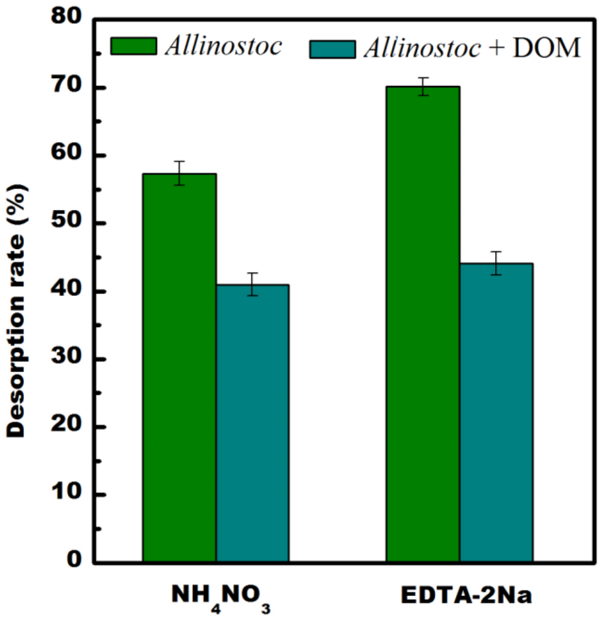

Figure 4. Desorption rate of Cu(II) from *Aliinostoc* and *Aliinostoc* + DOM by different desorbents.

3.5 Cu(II) uptake and release during long term of incubation

For the treatment of without DOM, with extending of incubation time, the concentration of free Cu(II) in adsorption mixture on the 24^{th} h (Day 1) was slightly lower than that on the 2^{nd} h, and it maintained 0.17-0.19 mg/L from Day 1 to Day 19, and then slightly increased to 0.22 mg/L on Day 29. Unlike the treatment of without DOM, the concentration of free Cu(II) in the treatment of *Aliinostoc* + DOM continually decreased with the extension of incubation. The concentration of Chl *a* in the treatment of without DOM addition rapidly decreased during incubation, from 1.55 mg/L on Day 1 to 0.09 mg/L on Day 29. For the treatment of *Aliinostoc* + DOM, its Chl *a* concentration dropped from 1.47 on Day 1 to 0.90 mg/L on Day 8, and followed by slight decrease from Day 8 to Day 17, and then rapidly decreased to 0.57 mg/L on Day 29.

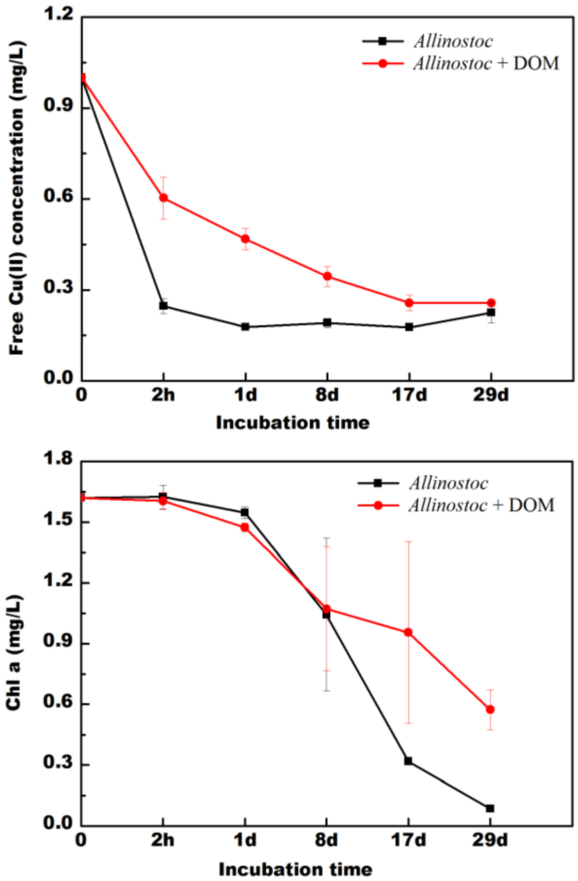

Figure 5. Variations of free Cu(II) concentration and algal cell viability in the adsorption mixture during long term of incubation.

4 Discussion

Cyanobacteria show high efficiency in removal of heavy metal ions from water by adsorption (Kiran and Thanasekaran, 2011; Sen et al., 2017). In this study, *Aliinostoc* (1 mg/L) removed 82.2% of Cu(II) from water within 24 h at an initial Cu(II) concentration of 1 mg/L, which showed potential in Cu(II) removal from water. Either DOM or cyanobacterial cell surface enrich hydroxyl, amino, carboxyl, and some other anions (Fang et al., 2011; Sun et al., 2021). These functional groups can bind with heavy metals through ion exchange and complexation (Sun et al., 2021). The adsorption of Cu(II) by *Aliinostoc* was decreased by

DOM addition, suggesting that DOM may compete with *Aliinostoc* for adsorption of Cu(II). Thus, decrease of DOM concentration is helpful for removal of Cu(II) from livestock effluents by adsorption using epiphytic cyanobacteria.

Langmuir model explains monolayer adsorption, while Freundlich model explains multilayer adsorption (Cadogan et al., 2015; Lu et al., 2016). The results presented in Figure 2 and Table 1 showed that the adsorption of Cu(II) by *Aliinostoc* belonged to monolayer adsorption. DOM addition did not change the adsorption isotherm of Cu(II) by *Aliinostoc*. This result suggested that the surface property of *Aliinostoc* was not modified by DOM addition. pH can affect protonation of adsorbent. It often had significant effects on adsorption capacity. The adsorption of Cu(II) by *S. platensis* increased with the increase of pH from 4 to 7 (Fang et al., 2011). However, This phenomenon only observed at pH ranged from 1 to 4 when using cyanobacterial bloom-derived biomass (mainly as *Microcystis*) as adsorbent; at pH ranged from 4 to 7, the adsorption of Cu(II) by cyanobacterial biomass decreased with increase of pH (Wang et al., 2010). Our results support the report form Fang et al. (2011) but inconsistent with that from Wang et al. (2010). Different structure of extracellular polymeric substances of these cyanobacteria may response for these inconsistencies.

The desorbent NH_4NO_3 desorbs the parts of Cu(II) bound by physical adsorption and ion exchange; EDTA-2Na desorbs the fractions of Cu(II) bound by physical adsorption, ion exchange, and complexation (Kuang et al., 2019). The results of desorption in this study showed that the main binding force of *Aliinostoc* toward Cu(II) was ion exchange, and then followed by complexation. These results are consistent with the reports from Wang et al. (2010) and Fang et al. (2011). DOM addition decreased the fraction of Cu(II) bound on the surface of cells of *Aliinostoc* by ion exchange and complexation.

The uptake of Cu(II) by microorganism included adsorption and transportation of Cu(II) from outside to inside of cell (absorption) (Letnik et al., 2017). The uptake of Cu(II) by *Aliinostoc* on the 24^{th} h was only slightly higher than that on the 2^{nd} h, suggesting that most of Cu(II) were bound on the surface of *Aliinostoc*. The results of Chl *a* determination indicated that cells of *Aliinostoc* gradually died during incubation. However, the free Cu(II) in the adsorption mixture was stable during incubation in the treatment of without external DOM, suggesting that the part of Cu(II) adsorbed by *Aliinostoc* still bound with cellular debris after cell death. Although cyanobacteria are autotrophs, they are evidenced capable of degrading organic matters, such as pesticides, dyes, and oil (Subramanian et al., 1994; El-Sheekh et al., 2009; Akoijam et al., 2015). Although cells of *Aliinostoc* were gradually died during long term of incubation, the uptake of Cu(II) by *Aliinostoc* was gradually increased during incubation in the treatment of *Aliinostoc* + DOM, while this phenomenon was not observed in the treatment of without DOM addition. One possible reason for this phenomenon is that *Aliinostoc* could degrade cattle manure DOM, and eliminate negative effects of DOM on Cu(II) adsorption.

5 Conclusion

Livestock manure without well management often results in co-contamination DOM and Cu(II) livestock effluents. Adsorption is a low cost and environmentally-friendly approach for Cu(II) removal from livestock effluents. The effects of DOM on adsorption of Cu(II) by *Aliinostoc* sp. YYLX235 were investigated in this study. *Aliinostoc* could effectively bind with Cu(II) through ion exchange and complexation. Cu(II) mainly bound on the surface of cells of *Aliinostoc*, and it still bound with cellular debris after cell death. Thus, the decay of *Aliinostoc* did not result in rapid release of Cu(II) into water. DOM addition did not change adsorption isotherm of Cu(II) by *Aliinostoc*, but it decreased adsorption capacity of *Aliinostoc* toward Cu(II).

Funding Sources

This research was supported by Natural Science Foundation of Hunan Province (No. 2020JJ4371) and Undergraduate Student Innovation Project of Hunan Provincial Education Department (No. s202010537065).

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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