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Biosorption of high-concentration Cu (II) by periphytic biofilms and the development of a fiber periphyton bioreactor (FPBR)

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HIGHLIGHTS

- Periphyton could effectively entrap high-concentration Cu-hydrate ($\leq$20 mg L^{-1}).
- Adsorption is the main Cu removal pathway from water by species-rich periphyton.
- EPS overproduction and porous structure of periphyton extended binding sites for Cu.
- Cu adsorption onto periphyton fits well with the pseudo-second-order kinetic model.

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ABSTRACT

In this study, a kind of microbial aggregates: periphytic biofilms were used for Cu removal and immobilized onto fiber for developing a novel bioreactor. Results show that periphyton can effectively entrap Cu at initial concentrations of 2–20 mg L^{-1} due to the overproduction of EPS and porous structure of periphyton, and biosorption was the primary mechanism of Cu removal. Cu (mainly $Cu_3(OH)_4^{2+}$, $Cu_2(OH)_2^{2+}$ and Cu^{2+}) adsorption onto periphytic biofilms followed the pseudo-second order kinetic model. The biosorption process fitted the Freundlich, Langmuir and Dubinin-Radushkevich Isotherm models well and was thermodynamically spontaneous. The fiber substrate used in the periphyton bioreactor greatly increased the Cation Exchange Capacity (CEC) of the system. This study indicates that immobilization of periphytic biofilms onto fiber for novel bioreactor development is a feasible way of entrapping high-concentration Cu from wastewater.

1. Introduction

Many heavy metals are essential elements for plant and animal growth but can be toxic at high levels (Yang et al., 2016). Exponentially increasing human activities in the last half century have led to corresponding increases in heavy metal discharge, especially into natural waterways, with high threats to ecological safety. *In-situ* bioremediation techniques for the treatment of contaminated material have been widely accepted in treating natural water ecosystems, including heavy metal polluted waters (Liu et al., 2016). Many techniques have been developed to remove heavy metals from natural waterways and wastewater, such as chemical precipitation, ion exchange, adsorption, membrane filtration, coagulation and flocculation, flotation and biological assimilation (Singha and Das, 2013). Among these techniques, adsorption and membrane filtration are the most effective measures (He and Chen, 2014; Singha and Das, 2013). However, the large-scale use of membrane filtration is limited due to its high cost and low permeation flux.

Biosorption is the capacity of the biomass (e.g. yeast, nut shells, macrophytes and many others) to bind heavy metals from water and is a feasible and economical approach to remove heavy metals from water (Areco et al., 2013). It has been widely reported that microorganisms including microalgae, bacteria, and fungi have high heavy metal (e.g., Cu, Pb, Zn, Cr and Cd) adsorption capacities onto their cell surface (Alam et al., 2015; Gadd, 2009; Salehizadeh and Shojaosadati, 2003). Many microorganisms can secrete extracellular polymer substances (EPS) such as proteins, polysaccharides and lipids, which can adsorb metal ions via interactions between the ions and the negative charges of EPS acidic functional groups (Salehizadeh and Shojaosadati, 2003; Sheng et al., 2010).

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Additionally, microorganism cell walls can adsorb heavy metals through ion exchange, surface complexation, Van der Waals' force and electrostatic interaction (Abdolali et al., 2015; Areco et al., 2013). Some bacteria, such as *Aeromonas caviae*, *Bacillus firmus* and *Corynebacterium glutamicum* have high heavy metal adsorption capacities up to 400–600 mg g^{-1} (Choi and Yun, 2004; Loukidou et al., 2004; Salehizadeh and Shojaosadati, 2003). However, high-concentration heavy metals could be toxic to the individual microorganisms, such as impairment of chloroplast, inhibition of photosynthetic activity, cell division and growth, displacing essential ions, and interacting with enzymes (Andosch et al., 2015; Serra et al., 2009).

Periphytic biofilms, a kind of microbial aggregates composing of microalgae, bacteria, epiphytes and detritus, are common in aquatic ecosystems attaching to the submerged surfaces (Liu et al., 2016; Shangguan et al., 2015). The species-rich periphytic biofilms are capable of adsorbing various substances including phosphorus, organic matter and heavy metals from waters via the secretion of EPS by green algae, cyanobacteria, diatoms and bacteria (Lu et al., 2014a; Yan et al., 2017). The high porosity and numerous void spaces on the exterior surface of periphytic biofilms can facilitate the transport of heavy metals into the interior of periphytic biofilms to be adsorbed or absorbed (Donar et al., 2004; Lu et al., 2014a; Wu et al., 2010). Additionally, because of its complex community structure, periphytic biofilms has a high tolerance and capacity to adapt to high-concentration heavy metal conditions and can maintain sustainable metabolic activities (Mejias Carpio et al., 2014; Valls and De Lorenzo, 2002).

Although many microorganisms and their communities have potentials to adsorb heavy metals from wastewater, few of them have been successfully applied in the *in-situ* removal of heavy metals from polluted water ecosystems (Gadd, 2009; He and Chen, 2014). Among the various factors, immobilization and separation of the biomaterial from water bodies are the critical step for a feasible *in-situ* heavy metal removal technique (Gadd, 2009). Accordingly, selection of suitable substrates and immobilization techniques should be put in priority. Because of the functional groups at the surface of photoautotrophs (e.g., microalgae), the periphytic community usually carries negative surface charges (Ozkan and Berberoglu, 2013). Therefore, periphytic biofilms could be immobilized on the substrate with positively charged ion exchange sites via the electrochemical combination of positive and negative charges.

With this background, the primary objectives of this study were to: (i) to investigate the Cu removal capacity of periphytic biofilms and identify whether adsorption is the primary mechanism of Cu removal by periphytic biofilms; (ii) if removed through adsorption, to describe the Cu adsorption process with kinetic, isotherm and mathematical models; and (iii) to design a new-type bioreactor: fiber periphyton bioreactor (FPBR), and evaluate the potential feasibility of fiber in immobilizing periphytic biofilms for large-scale applications.

2. Materials and methods

2.1. Preparation of microbial aggregates

To obtain natural and species-rich microbial aggregates, fiber substrate (Fiber Carries, FCs, length 0.2 m, width 0.1 m, Millipore®) was suspended in Xuanwu Lake in Nanjing, China. Periphytic biofilms accumulated on the FCs and after two weeks, they were moved into glass tanks (length 0.5 m, width 0.35 m, height 0.24 m) with artificial wastewater (based on the chemical compositions of water in Xuanwu Lake). The composition of the artificial wastewater was as follows: pH 7.22 ± 0.03, TN 1.26 ± 0.02 mg L^{-1}, NO_3^--N 0.73 ± 0.02 mg L^{-1}, NH_4^+-N 0.53 ± 0.01 mg L^{-1}, TP 0.12 ± 0.01 mg L^{-1}, TDP 0.035 ± 0.001 mg L^{-1}, $C_6H_{12}O_6$ 169 mg L^{-1}. The glass tanks were kept in a greenhouse at 20–25 °C for 25 days until the brown and microporous microbial communities formed on the FCs with a thickness of 5–8 mm. Thereafter, the increase in thickness of periphytic biofilms with incubation time was negligible and the microbial community were regarded as stable and scraped from the fiber substrate for characteristic analyses and the following experiments.

2.2. Cu removal and adsorption experiments

To prevent interference from other substances in real wastewater and simultaneously provide essential nutrients for the growth of the microbial community, artificial wastewater with various Cu concentrations was used in this study ($C_6H_{12}O_6$ 169 mg L^{-1}, NaCl 63 mg L^{-1}, $(NH_4)_2SO_4$ 63 mg L^{-1}, KH_2PO_4 44 mg L^{-1}, $NaHCO_3$ 94 mg L^{-1}, $MgSO_4{\cdot}7H_2O$ 94 mg L^{-1}, $CaCl_2{\cdot}2H_2O$ 31 mg L^{-1}, $FeSO_4{\cdot}7H_2O$ 3.25 mg L^{-1}, $CuSO_4{\cdot}5H_2O$ 7.86–78.6 mg L^{-1}, pH 7.20).

To investigate the Cu removal process, 5.0 g of fresh periphytic biofilms (moisture 85 ± 5%) was added to 500 ml beakers filled with 250 ml of simulated wastewater with Cu concentrations of 2, 5, 10 or 20 mg L^{-1}. This was done in triplicate for each Cu concentration. To investigate whether adsorption was the main mechanism of Cu removal, NaN_3 was used to inhibit the microbial activity of periphytic biofilms. Specifically, 0.5 g L^{-1} of NaN_3 was added to three additional 500 ml beakers with 250 ml of simulated wastewater and a Cu concentration of 5.0 mg L^{-1}. Cu concentration of the wastewater was determined after 2, 6, 12, 24, 36, 48, 60, 84 and 108 h. All simulated microcosms (beakers) were kept at 25 °C with a light intensity of 2500 lux under a 14/10 h light/dark cycle in an incubator.

Adsorption thermodynamic experiments were carried out to investigate adsorption kinetics and isotherms. Specifically, 8 g of wet periphytic biofilms (moisture 85 ± 5%) scraped from the FCs was added to individual 250 ml Erlenmeyer flasks with 100 ml of simulated wastewater (same composition as above) at Cu concentrations of 2, 5, 10 or 20 mg L^{-1}. This was done in triplicate for each Cu concentration. The microbial activity of the microbial community in all the flasks was inhibited by adding 0.5 g L^{-1} NaN_3. Cu concentration was determined after 2, 6, 12, 24, 36 and 48 h. This experiment was conducted in an incubator with a light intensity of 2500 lux under a 14/10 h light/dark cycle at 25 °C.

2.3. Development of the fiber periphyton bioreactor (FPBR)

FCs (length 0.2 m, width 0.1 m) were immersed in 500 ml beakers filled with 250 ml artificial wastewater (as described above) with a density of 0.3 m^2 FCs per cubic meter of artificial wastewater. Then, 2.0 g of fresh periphytic biofilms was suspended in the beaker in contact with the FCs and cultured in a greenhouse at 20–25 °C for 25 days. The periphytic biofilms with FCs were then taken out to measure the cation exchange capacity (CEC) and evaluate the Cu adsorption/exchange capacity of the FPBR.

2.4. Sample analysis

The morphology of the fresh periphytic biofilms was characterized at 400 × magnification under an Optical Microscope (H600L, Nikon, Japan) and a Scanning Electron Microscope (Quanta 200, FEI, USA). To estimate the periphytic dry weight (DW), moisture was removed by oven-drying wet samples at 80 °C for 72 h. The microbial diversity and metabolic activity of the periphytic biofilms with and without the addition of NaN_3 were investigated using the Biolog™ ECO Microplate (Hayward, CA, USA) following Shangguan et al. (2015). Specifically, 50 ml of water with washed

fresh periphytic biofilms was used for each Biolog and 150 μl was added to each well of every ECO Microplate. The plates were incubated at 25 °C and average well color development (AWCD) was evaluated using a Biolog Microplate Reader at 590 nm every 24 h for five days.

To investigate the extracellular polymeric substances (EPS) of periphytic biofilms after exposed to Cu at different concentrations, periphytic biomass samples were collected on day 0, 1, 3 and 5 by taking 5 ml cultures after homogeneous mixing. EPS was extracted from each 5 ml suspended periphytic culture using the formaldehyde/NaOH method (Yan et al., 2017). After extraction, the EPS pellet was dried and thereafter polysaccharide was measured by anthrone-H_2SO_4 colorimetry (Dische, 1962) and protein was measured following the Bicinchoninic Acid (BCA) method (Smith et al., 1985), and the polysaccharide and protein contents were expressed as mg/L culture.

To investigate the microbial community composition, periphytic biofilms samples were collected from the 500 ml beakers at the beginning and the end of the Cu removal experiments. The periphytic biofilms microbial diversity level was analyzed via MiSeq sequencing as described by Shangguan et al. (2015). These were done by Shanghai Majorbio (http://www.majorbio.com/). After sequencing, the PE reads of the raw sequence data were separated and overlapped to assemble the final sequences with a minimum overlap of 10 bp for each two PE reads. The PE reads with more than two mismatches within the 10 bp length were discarded during the overlap step. The sequence trim was processed using Trimmomatic. Operational taxonomic units (OTUs) were then determined at a similarity of 97% using Usearch. The taxonomy assignment of tags was performed on OTUs using a RDP classifier and the taxonomic structure compositions of the microbial community were expressed as relative abundances.

pH of the artificial wastewater was measured every 12 h using a pH/mV meter (Mettler Toledo). To measure Cu concentration, 3 ml of wastewater was collected by syringe from each beaker, then filtered through a 0.25 μm micro-membrane. Cu concentration was determined following an inductively coupled plasma atomic emission spectrometry method (Optima 8000, Perkin Elmer, USA). The percentage of Cu-hydrates in the total Cu (II) at varying wastewater pH was obtained using Visual MINTEQ 3.1 software (http://hem.bredband.net/b108693/index.html).

Cation exchange capacity (CEC) was used to evaluate the Cu adsorption/exchange capacity of the FPBR by comparisons with periphytic biofilms attached to soil and polythene, as soil is the common solid substrate supporting periphytic biofilms under natural conditions and polythene is the conventional carrier of periphytic biofilms. CEC was determined according to the method proposed by Chamuah and Dey (1982). Briefly, 20 ml KCl solution (0.1 mol L^{-1}) was added into a flask with 5 g fresh periphytic biofilms scraped from fiber, soil or polythene, and centrifuged for 10 min. After centrifugation, the supernatant was collected for the determination of KCl and the deposit was used for further extractions. A total of 25 ml NH_4Cl solution (1.0 mol L^{-1}) was added to the flask with the deposit and centrifuged for 10 min. After centrifugation, the supernatant was collected for the measurement of NH_4^+ using a Flow Analyzer (Auto Analyzer 3, Seal, Germany). The CEC values were expressed as cmol kg^{-1} dry periphytic biofilms.

2.5. Data analysis

Each experiment was conducted in triplicate and the results were expressed as mean ± 1 S.D. A *t*-test was used to compare the microbial activity of periphytic biofilms with and without NaN_3 treatment using SPSS Version 22.0. α was set at 0.05. One-way ANOVA was used to test the difference in EPS production of periphytic biofilms at different initial Cu concentrations and *p* was set at 0.05.

The amount of Cu adsorbed onto periphytic biofilms at time *t* and the equilibrium time was obtained by means of Eqs. (1) and (2) (Areco et al., 2013).

$$q_t = \frac{(C_0 - C_t)V}{m} \quad (1)$$

$$q_e = \frac{(C_0 - C_e)V}{m} \quad (2)$$

where q_t is the amount of Cu adsorbed onto periphytic biofilms at time *t* (mg g^{-1}), q_e is the amount of Cu adsorbed onto periphytic biofilms at equilibrium (mg g^{-1}), *t* is contact time (h), C_0 is the initial Cu concentration (mg L^{-1}), C_t is the Cu concentration at time *t* (mg L^{-1}), C_e is the Cu concentration at equilibrium (mg L^{-1}), V is the volume of solutions (L) and m is the dry weight of periphytic biofilms (g).

Pseudo-second-order adsorption models were used to investigate the adsorption mechanism (Lu et al., 2014a). The intra-particle diffusion model (Eq. (3)) was used to identify the adsorption mechanism (Özcan et al., 2005).

$$q_t = k_{id}t^{0.5} + C \quad (3)$$

where k_{id} is the rate constant of intra-particle diffusion (mg g^{-1} $h^{-0.5}$) and C is the intra-particle diffusion model constant.

Langmuir and Freundlich equations were applied to the isotherm data. Based on further analysis of the Langmuir equation, the dimensionless parameter of the equilibrium or adsorption intensity (R_L) can be expressed by Eq. (4). R_L is one of the most reliable indicators of the adsorption intensity of a surface. There are four possibilities of the R_L value: i) irreversible adsorption, $R_L = 0$; ii) favorable adsorption, $0 < R_L < 1$; iii) linear adsorption, $R_L = 1$; iv) unfavorable adsorption, $R_L > 1$ (Ho et al., 2002).

$$R_L = \frac{1}{1 + K_L C_0} \quad (4)$$

where R_L is the adsorption intensity and K_L is the Langmuir adsorption constant (L mg^{-1}).

The Dubinin-Radushkevich (D-R) isotherm model was used to determine the adsorption type (physical or chemical). The linear form of the D-R model is expressed by the following Eq. (5) (Singha and Das, 2013).

$$\ln q_e = \ln q_m - \beta\varepsilon^2 \quad (5)$$

where ε is the Polanyi potential, and $\varepsilon = RT\ln\left(1 + \frac{1}{C_e}\right)$. R is the ideal gas constant (8.314 J mol^{-1} K^{-1}) and T is the thermodynamic temperature (K).

Finally, thermodynamic parameters including adsorption energy (E) and Gibbs free energy (ΔG°) were evaluated to determine the feasibility of the biosorption process (Chang et al., 2016; Singha and Das, 2013).

3. Results and discussion

3.1. Periphytic biofilms characteristics

After 25 days cultivation, dense and microporous periphytic biofilms formed on the FCs. Under the optical microscope, it was observed under microscope that the periphytic biofilms was highly diverse with filamentous algae and cyanobacteria being the most common taxonomic groups. At the end of the experiment, diatoms, bacteria and protozoa were observed under the scanning electron microscope.

Bacteria are sensitive to environmental changes and adapt quickly to environmental stress such as high-concentration Cu pollution (Mejias Carpio et al., 2014; Valls and De Lorenzo, 2002). Accordingly, the microbial community compositions at the end of the experiment were compared with those at the beginning. The microbial community changed drastically after exposure to the high-concentration Cu wastewater (Fig. 1A and B). For instance, at the beginning of the experiment cyanobacteria were the dominant species (50.1%) followed by *Gammaproteobacteria* (20.0%), *Betaproteobacteria* (10.4%) and *Cytophagia* (9.3%, Fig. 1A). After exposure to Cu, the relative abundances of *Gammaproteobacteria* and *Bacteroidia* greatly increased (48.8% and 27.8%, respectively), while cyanobacteria and *Cytophagia* decreased to 6.2% and 1.7%, respectively (Fig. 1B).

3.2. Cu removal by periphytic biofilms

Periphytic biofilms were effective in removing Cu from wastewater at various concentrations. Regardless of the initial Cu concentration (2, 5, 10 or 20 mg L^{-1}), Cu removal efficiency during the first 24 h was the greatest, ranging from 63% to 73% (Fig. 2A). Considering this high removal rate, it was suspected that Cu removal by periphytic biofilms during the first 24 h was dominated by processes other than assimilation.

To determine whether adsorption was involved, NaN_3 was used to impede microbial activity by restraining microbial respiration and inhibiting assimilation at an initial Cu concentration of 5 mg L^{-1} (Saisho et al., 2001). AWCD values (representing microbial activities) in the NaN_3 treatment were nearly zero during the incubation time, while that of the control group increased up to 0.5 (Fig. 2B). These results indicated that microbial respiration in periphytic biofilms was completely inhibited by NaN_3 and Cu assimilation by periphytic biofilms was trivial. Moreover, the Cu removal efficiency of the periphytic biofilms with the addition of NaN_3 showed no significant difference from that without NaN_3 during the first 48 h ($\alpha > 0.05$, Fig. 2A).

pH is known to greatly affect the speciation of Cu-hydrates in the aqueous phase, for instance, a high pH can cause $Cu(OH)_2$ precipitation (Janyasuthiwong et al., 2015). In this study however, the pH decreased from 7.2 to 6.0 during the Cu removal experiment (Fig. 2C). This pH decrease must be due to (i) the increasing release of CO_2 from heterotrophic bacteria respiration, and (ii) decreasing CO_2 consumption by the photoautotrophs as indicated by their decreasing relative abundance (Fig. 1C and D). At this pH, $Cu_3(OH)_4^{2+}$ was the dominant Cu-hydrates species, followed by $Cu_2(OH)_2^{2+}$ and Cu^{2+} (Fig. 2D). There was no $Cu(OH)_2$ precipitation, which indicates that $Cu(OH)_2$ precipitation can be ignored in Cu removal by periphytic biofilms. In summary, these results validated the hypothesis that adsorption by periphytic biofilms was the dominant mechanism of Cu removal in the first 24 h.

It is generally accepted that EPS plays a critical role in heavy metal adsorption by living cells, such as algae, bacteria, fungi and yeast (Gadd, 2009; Sheng et al., 2010; Yan et al., 2017). In this study, compared to the control, significant overproduction ($p < 0.05$) of polysaccharide (0.4–2.5 mg L^{-1}) and protein (0.4–3.4 mg L^{-1}) in EPS of periphytic biofilms was observed (Fig. 2E and F) when exposed to Cu at concentrations of 2–20 mg L^{-1}, and the EPS overproduction showed an increasing tendency following the increase of initial Cu concentration. Adsorption of heavy metals by EPS is non-metabolic and can be caused by interactions between metal ions and negative charges of acidic functional groups of EPS (Gadd, 2009; Salehizadeh and Shojaosadati, 2003). Thus, EPS must have contributed greatly to the adsorption of Cu onto periphytic biofilms, and this was in accordance with the report by Sheng et al. (2005) on EPS production of a photosynthetic bacteria *Rhodopseudomonas acidophila* in the presence of heavy metals.

In addition to adsorption onto EPS, the architectural characteristics of periphytic biofilms may also have marked effects on adsorbing metal ions (Alam et al., 2015; Donar et al., 2004). For example, the micro-spaces constructed between complex cells such as algae, and bacteria are relatively larger than those constructed by a single species (Lu et al., 2014b). This difference can provide more micro-spaces or adsorption sites for capturing Cu. In addition to micropores, the periphytic biofilms also had many heavy metal binding sites on its surface to which Cu could bind. The metal ions can adsorb onto cell walls through interactions with the negatively charged groups of cell walls such as carboxyl, amino, and hydroxyl groups (Gadd, 2009). When exposed to Cu, periphytic biofilms showed great changes in its community structure with large increases in the abundance of *Gammaproteobacteria* and *Bacteroidia* (Fig. 1A and B). *Pseudomonas* belonging to *Gammaproteobacteria* reportedly had a Cu adsorption capacity of 97 mg g^{-1} (Uslu and Tanyol, 2006) and *Bacteroidia* had a high tolerance of high heavy metal levels (Yin et al., 2015).

3.3. Cu adsorption process onto periphytic biofilms

The widely used pseudo-second-order kinetic model was used to fit the data collected from the different initial Cu concentrations and describe the adsorption process. The regression coefficients (R^2) of the model were high ($R^2 > 0.90$, Table 1) for all tested Cu concentrations demonstrating that the adsorption of Cu onto periphytic biofilms strongly followed the pseudo-second-order

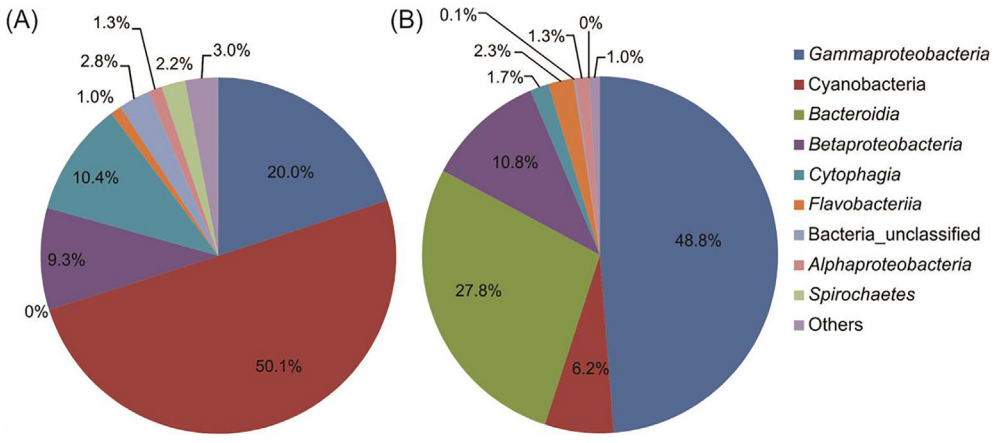

Fig. 1. The species composition of periphytic biofilms at the beginning (A) and the end of the experiment (B).

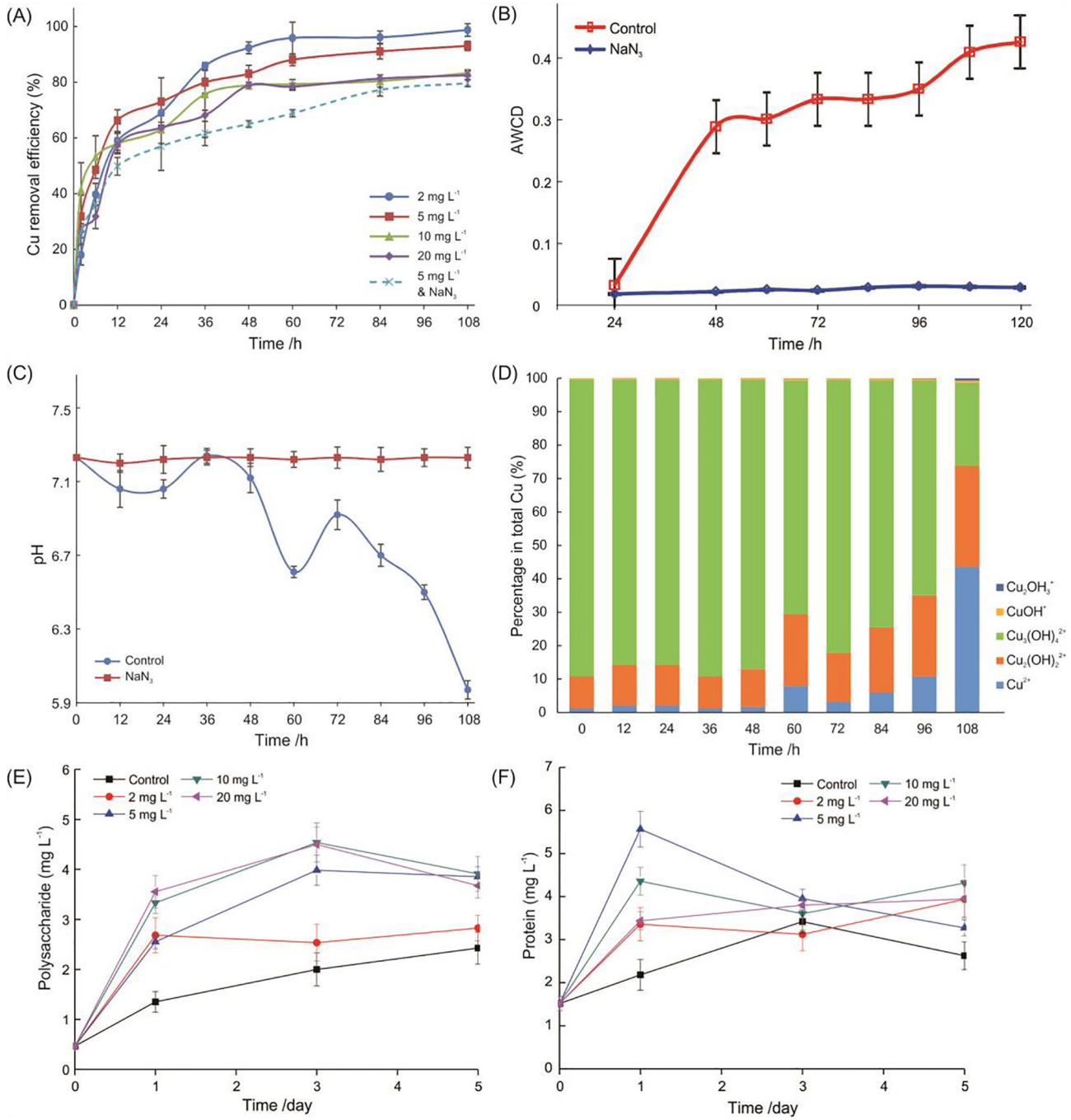

Fig. 2. A: Cu removal efficiency by periphytic biofilms at initial Cu concentrations of 2, 5, 10 and 20 mg L^{-1} and 5 mg L^{-1} with 0.5 mg L^{-1} NaN_3 over time; B: Microbial activity (represented by AWCD) of periphytic biofilms with and without 0.5 g L^{-1} NaN_3; C: pH changes during Cu removal by periphytic biofilms with and without 0.5 g L^{-1} NaN_3 at initial Cu concentration of 5 mg L^{-1}; D: The percentage of Cu-hydrates to the total Cu (II) in the aqueous phase following pH changes during Cu removal by periphytic biofilms. E: Changes of polysaccharide (mg L^{-1}) in EPS of periphytic biofilms at different Cu concentrations over time; F: Changes of protein (mg L^{-1}) in EPS of periphytic biofilms at different Cu concentrations over time.

Table 1
Kinetic model parameters for Cu adsorption by periphytic biofilms under different initial Cu concentrations.

Cu (mg L^{-1})	Pseudo-second-order kinetic model			Intra-particle diffusion model			Boyd model		
	k_2 (mg g^{-1} h^{-1})	q_2 (mg g^{-1})	R^2	k_{id} (mg g^{-1} $h^{-0.5}$)	C (mg g^{-1})	R^2	k_{id} (mg g^{-1} $h^{-0.5}$)	C	R^2
2	0.080	1.750	0.999	0.276	0.965	0.775	0.057	−0.243	0.903
5	0.059	4.325	0.999	0.570	3.707	0.743	0.084	−0.222	0.969
10	0.031	8.177	0.997	0.873	8.254	0.864	0.072	−0.126	0.895
20	0.008	16.835	0.996	2.493	10.012	0.812	0.051	−0.220	0.888

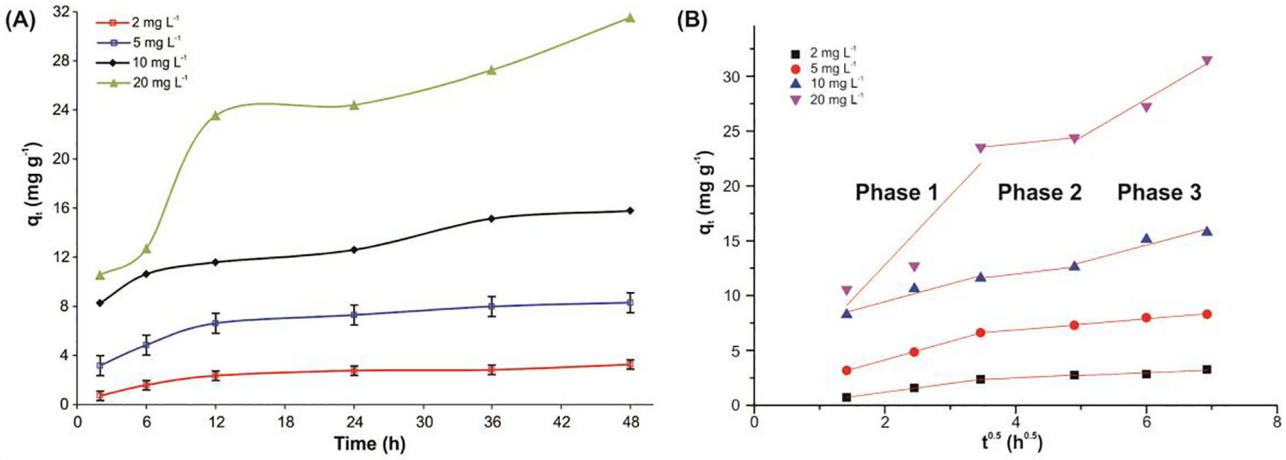

Fig. 3. A: The amount of Cu adsorbed onto periphytic biofilms over time; B: Intra-particle diffusion for Cu adsorption at initial Cu concentrations of 2–20 mg L^{-1} under an illumination of 2500 lux at 25 °C.

kinetic model. The model showed a relatively better fit at low initial Cu concentrations of 2–5 mg L^{-1} than at high Cu concentrations, probably due to Cu adsorption saturation.

In a solid-liquid adsorption system, the solute molecule transfer processes are usually characterized as intra-particle diffusion, boundary layer diffusion or both. This determines whether the controlling step of the adsorption is the intra-particle process, external diffusion processes, or both (Dawood and Sen, 2012). An intra-particle diffusion model was used to investigate the Cu adsorption process onto periphytic biofilms. The determination coefficients (R^2) of the intra-particle diffusion model were 0.743–0.864 for all Cu concentrations (Table 1, Fig. 3A), indicating that intra-particle diffusion was not the only adsorption mechanism (Tang et al., 2012). The intra-particle diffusion plots were multi-linear, containing at least three linear segments (Fig. 3B). According to the intra-particle diffusion model, if the plot of q_t versus $t^{0.5}$ presents a multi-linearity correlation, it indicates that three steps occurred during the adsorption process (Tang et al., 2012). Therefore, the Cu adsorption process by periphytic biofilms in this study could be divided into three steps: external mass transfer at the beginning (0–12 h), intra-particle diffusion in the middle period (12–24 h), and equilibrium during the last period (24–48 h). The linear portions of curves did not pass through the origin, suggesting that pore diffusion was not the step controlling the overall rate of mass transfer at the beginning of adsorption (Tang et al., 2012).

Furthermore, the intercept (C) of the intra-particle diffusion model represents the boundary layer effect (film diffusion). A high C value indicates a large contribution of surface sorption to the rate-controlling step (Özcan et al., 2005). The C values increased from 0.97 to 10.01 when the Cu concentration changed from 2 to 20 mg L^{-1} (Table 1, Fig. 3B), reflecting a larger boundary layer effect at a relatively higher Cu concentration.

3.4. Adsorption isotherms and thermodynamic parameters

Capacity of the adsorption isotherm is fundamental, and plays a crucial role in evaluating the maximum adsorption capacity. It also provides a concise overview of the steps taken by the study system, indicating how Cu can be adsorbed. The Cu adsorption capacities (q_e) at initial concentrations of 2, 5, 10 and 20 mg L^{-1} were 1.1, 3.0, 5.7 and 11.9 mg g^{-1}, respectively. These adsorption capacity data fitted well to the Freundlich, Langmuir and D-R isotherm models (R^2 = 0.656–0.993, Table 2).

As it is generally accepted that Freundlich constant (n) values between 1 and 10 represent good adsorption potential of the adsorbent (García-Calzón and Díaz-García, 2007), equilibrium data of Cu adsorption onto periphytic biofilms were fit to the linear Freundlich equation. The linear plots of ln q_e versus ln C_e were examined to determine K_F and n (Table 2). The high coefficients (R^2 = 0.993) of the plots indicated that the Cu adsorption isotherm is well described by the linear Freundlich equation while the Freundlich constant (n) of 1.11 indicates that Cu adsorption onto periphytic biofilms was favorable.

Additionally, in this study, the adsorption intensity R_L derived using the Langmuir model ranged between 0.415 and 0.877 indicating that periphytic biofilms was an effective Cu adsorbent at all initial concentrations tested (Ho et al., 2002). The equilibrium data was also described by the linear D-R isotherm model to distinguish physical adsorption from chemical adsorption (R^2 = 0.821, Table 2). The β value was 1×10^{-6} mol^2 kJ^{-2} at 0.25 g periphytic biomass indicating that Cu adsorption by periphytic biofilms was a physical process.

In many cases, the Freundlich and D-R equations have been regarded as empirical, rather than mechanistic (e.g., surface model) adsorption isotherm models (Tang et al., 2012). In this study, data collected from the adsorption process fit both empirical models well (R^2 = 0.993 and 0.821). This indicates that the Cu adsorption process by periphytic biofilms has mechanistic relevance (or surface adsorption properties) and that the model parameters included in these empirical models are of specific thermodynamic significance.

The mean sorption energy (E) can be used to distinguish between chemical and physical adsorptions. An E between 8 and 16 kJ mol^{-1} indicates that the adsorption process follows chemical ion-exchange, and an E less than 8 kJ mol^{-1} indicates that the adsorption is physical in nature (Singha and Das, 2013; Wu et al.,

Table 2
Parameters of the Langmuir, Freundlich and Dubinin-Radushkevich (D-R) Isotherm models at 25 °C.

Langmuir			Freundlich				D-R	
K_L (g^{-1})	q_m (mg g^{-1})	R^2	K_F (g^{-1})	n	R^2	β	q_m (mg g^{-1})	R^2
70.4	13.33	0.656	8.55	1.11	0.993	1.0E-06	19.83	0.821

2010). The E value of 0.7 kJ mol^{-1} in this study demonstrates that the adsorption process of Cu by periphytic biofilms was physical in nature. The thermodynamic results showed that ΔG^0 varied between −3.309 and −3.996 kJ mol^{-1} when Cu concentrations changed from 2 to 20 mg L^{-1}, indicating that Cu adsorption onto periphytic biofilms was a spontaneous process (Singha and Das, 2013; Tang et al., 2012).

3.5. Development of fiber periphyton bioreactor (FPBR) for practical application

Microorganisms in a periphytic community can self-regulate within a food web. Specifically, microalgae grow via capturing solar energy and can be eaten by protozoa; bacteria decompose the dead protozoa and microalgae; then the protozoa and bacteria debris provide nutrients for algal growth. The mass transfer cycle within periphytic biofilms could facilitate the formation of a species-rich and sustainable community structure, which can enhance its resistance to external changes such as increases in Cu concentration and maintain high metabolic activities and ecological functions in removing pollutants from wastewater. In addition, the periphytic biomass can be easily removed by scraping from the fiber and for Cu reclaim.

After 25 days growth, dense and healthy periphytic biofilms were tightly formed on the fiber substrate. It is well known that electrochemical reactions happen between matter carrying positive and negative surface charges and the surface of most photoautotrophic microorganisms in water carry negative charges (Ozkan and Berberoglu, 2013). Therefore, materials carrying positive surface charges, such as the fiber carriers, will facilitate the immobilization of periphytic biofilms for development of novel bioreactors, such as the fiber periphyton bioreactor (FPBR).

Furthermore, CEC is one key parameter defining the capacity of certain surfaces to adsorb and/or exchange cation from solution (Iturri and Buschiazzo, 2014; van Halem et al., 2012). In this FPBR, the CEC of periphytic biofilms attached to fiber substrate was 783.6 cmol kg^{-1}, which was much higher than that attached to polythene plastic (680.1 cmol kg^{-1}), a widely used substrate carrying negative surface charges. The average CEC of FPBR was also much higher than that of soil (54.7 cmol kg^{-1}), a surface on which periphytic biofilms naturally form in waterways. These results indicate that the positively charged fiber substrate greatly increased the cation density of periphytic surface and could potentially enhance its cation adsorption/exchange capacity from wastewater.

4. Conclusions

This study demonstrates that periphytic biofilms could quickly adapt to high-concentration Cu by regulating its community structure with *Gammaproteobacteria* and *Bacteroidia* being dominant species. Biosorption was the primary mechanism of Cu removal from wastewater by periphyton with numerous adsorption sites formed on its exterior surface and EPS overproduced. Cu adsorption onto periphyton fitted Freundlich, Langmuir and Dubinin-Radushkevich models well, indicating that it was a physical and spontaneous process. This study also indicates that fiber is a promising substrate in new-type periphyton bioreactor development to immobilize living bio-adsorbents (e.g., periphyton) and improve the Cation Exchange Capacity (CEC) of periphyton.

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