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Potassium supplement enhanced cadmium removal in a *Microcystis aeruginosa* photobioreactor: Evidence from actual and simulated wastewater

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ABSTRACT

In this study, a *Microcystis aeruginosa*-based photobioreactor (*M. aeruginosa*-based PBR) was developed for the removal of cadmium (Cd^{2+}) from diluted actual mine wastewater (DW) and Cd^{2+}-contained simulated wastewater (SW), with a uniform Cd^{2+} concentration of 0.5 mg/L. For the DW and SW, both K^+ -abundant (DWA & SWA) and K^+-insufficient (DWB & SWB) treatments were conducted. It was found that continuous supplementation of K^+ benefited Cd^{2+} removal. The Cd^{2+} removal efficiency in SWA reached 70% during the 41 days of operation, which was 20% higher than that in the SWB. The K^+ addition triggered great higher Cd^{2+} removal efficiency (90%) in the DWA in comparison to the SWA. The Cd^{2+} assimilation by *M. aeruginosa* and Cd^{2+} retention on *M. aeruginosa* surface were the primary processes involved in the PBR system. The K^+ starvation triggered a 45% and 43% loss of *M. aeruginosa* biomass in the DWA and the DWB, respectively. Hence, the Cd^{2+} removal efficiency in DWB increased significantly, and this was attributed to the increased abundance of non-living cells and enhanced bioretention of Cd^{2+}. The results revealed that continuous K^+ supplementation enhanced the Cd^{2+} removal efficiency in the *M. aeruginosa*-based PBR jointly by prompting algal cell growth, Cd^{2+} assimilation and biosorption, as well as Cd^{2+} retention on the algal cells.

1. Introduction

Cadmium (Cd^{2+}) is a common contaminant in municipal and industrial wastewaters that originates from leather tanning, electroplating, pigmentation, ore refining, wood preserving, and metal finishing (Cabral et al., 2015; Wang et al., 2020). In China, Cd^{2+} pollution events have been frequently recorded in recent decades (Xu et al., 2009), and these have brought not only brought great harm to aquatic organisms, but also have threatened water supply security and endangered food safety as well as human health by transferring through food chains (Lu et al., 2015). Cd^{2+} removal from wastewater is essential because of the adverse effects on ecosystems.

Currently, heavy metal removal from wastewater is achieved primarily by utilizing chemical and physicochemical approaches (Deng et al., 2020b), during which chemical precipitation, ion exchange, membrane filtration, flotation, and electrochemical treatments are widely employed (Lee et al., 2017). However, these methods raise some concerns, including high cost and unsatisfactory Cd^{2+} removal efficiency in wastewater with a chemical oxygen demand less than 200 mg/L (Kouzbour et al., 2019; Ni et al., 2009). Therefore, microbial-based wastewater treatment is now demanded due to the drawbacks related to conventional methods (Al Ketife et al., 2020).

Biological methods, including biosorption and bioaccumulation, are efficient alternative technologies (Rai et al., 2005). Algae is gaining increasing attention because both living and inert cells of microalgae have been successfully used to remove heavy metal contaminants, and microalgae can maintain a steadily growing biomass in wastewater with an adequate nutrient supply (Jácome-Pilco et al., 2009). This, in turn, enhances heavy metal removal. Moreover, microalgae can effectively remove nitrogen and phosphorus from wastewater to improve water

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quality (Ansari et al., 2019; Derakhshan et al., 2018). Therefore, a photobioreactor (PBR) that contains microalgae is considered to be a useful device to achieve efficient heavy metal removal (García-Galán et al., 2021; Muñoz et al., 2006). For instance, *Scenedesmus incrassatulus* has been reported to achieve a 43.5% chromium (Cr) removal in a continuous PBR (Jácome-Pilco et al., 2009), *Chlorella vulgaris* in membrane PBR achieved a maximum total Cr reduction of 50.0% (Lu et al., 2021), and 60% Cd^{2+} removal was found using *Scenedesmus obliquus* contained in a continuous PBR (Chen et al., 2012). *Microcystis aeruginosa* (*M. aeruginosa*) is one of the most widely distributed bloom-forming species in eutrophic waters (Rajasekhar et al., 2012), and it has been shown to exhibit a significant heavy metal (e.g., zinc, copper, nickel, and Cd^{2+}) tolerance and removal capacity (Deng et al., 2020a; Mehta and Gaur, 2005). Other cyanobacteria, including *Anabaena sphaerica*, *Anabaena variabilis*, and *Synechocystis* sp. have been used successfully used in a PBR for agricultural wastewater treatment (Gorain et al., 2019; Rueda et al., 2020).

Many researchers have studied the bioremediation of heavy metals using living algal cells, and it has been revealed that the removal efficiency depends on culture conditions, algal growth, and metal concentrations (Chandrashekharaiah et al., 2021; Yeo et al., 2018). Thus, it is essential to find a way to maintain the continuous proliferation of algae. Moreover, during the heavy metal removal process by microalgae, hazardous pollutants are always reduced through the biosorption procedure. This means that the microalgae can release extracellular polymeric substance (EPS) with high affinity toward heavy metals or can accumulate these heavy metals in the cell (Leong and Chang, 2020). As a consequence, this prompts the release of organic materials from algal cells or the intracellular uptake of metal ions by living algal biomass, and this may have great potential for the enhancement of the heavy metal removal. In our previous study, we demonstrated that the release of EPS could be augmented and the assimilation of Cd^{2+} was substantially enhanced with an increased abundant availability of potassium (K^+) that is essential for cell growth and proliferation in a batch cultivation experiment (He et al., 2021). These results suggested that the regulation of the K^+ level was likely to strengthen the heavy metal removal efficiency. However, this requires more evidence that includes the response of algae to Cd^{2+}, algal growth kinetics, and bio-removal capabilities, especially in actual wastewaters that contain Cd^{2+}.

For this purpose, *M. aeruginosa*-based PBR under the regulation of the K^+ concentration was established to treat actual and simulated Cd^{2+}-containing mine wastewater. The aim of this study is to determine the influence of the K^+ level on Cd^{2+} removal from wastewater using a PBR. The results provide a new method for enhancing Cd^{2+} removal from wastewater in the presence of a specific range of available K^+ using an *M. aeruginosa*-based PBR.

2. Materials and methods

2.1. Algae and wastewater

The nontoxic *M. aeruginosa* (FACHB-469) was obtained from the Culture Collection of Algae, the Institute of Hydrobiology, Chinese Academy of Sciences. The chromium chloride ($CdCl_2$) was purchased Aladdin Industrial Corporation (American). Algal cells were precultured in 500 mL flasks containing 300 mL of standard BG-11 medium at 25 ℃ under continuous white fluorescent light illumination (125 mmol photons m^{-2} s^{-1}) (Mohan Singh et al., 2020). The actual Cd^{2+} containing wastewater was collected from the Mining Group Co., LTD located at Xiushan County, Chongqing City, China. To avoid introducing the influence of particles and microorganisms, the raw wastewater was filtered using a 0.22 μm membrane filter to remove particles, including impurities and microorganisms, such as bacteria, fungi, and microalgae (Lu et al., 2021), and was stored at 4 ℃ until further processing. The average and standard deviation values of the principal chemical compound concentration of the actual mine wastewater are summarized in Table S1. The filtered actual mine wastewater was diluted with an equal amount of concentrated BG-11 medium (in which twice the volume of a stock solution of the standard BG-11 medium was added) to ensure a nutrition supply to *M. aeruginosa* in the PBRs. In addition, Cd^{2+} containing simulated wastewater was also processed. Briefly, 0.39 mL of the $CdCl_2$ stock solution (0.92 g/L) was added per liter of the standard BG-11 medium. The average and standard deviation values of the principal chemical compound concentration of the diluted Cd^{2+}-containing actual wastewater and simulated wastewater are shown in Table 1.

2.2. Experimental apparatus and operation

The lab-scale cylindrical PBR with an internal diameter of 10 cm and a working volume of 3.0 L was constructed from transparent plexiglass (Fig. 1). The pH of the culture liquor in the reactors was adjusted in the range of 6.5–7.0. The air was filtered using a 0.22 μm filter and supplied to the reactor from a gas distributor at an aeration rate of 0.5 L/min to provide agitation during cultivation. The experiment was conducted in air-conditioned rooms. The culture temperature was maintained at the ambient condition of 25 ± 1 ℃. The PBR was illuminated using white light emitting diode (LED) lamps placed 5 cm from the surface of the reactor, and the illumination intensity of the outer wall of the PBR was approximately 140 mmol photon m^{-2} s^{-1}. The hydraulic retention time (HRT) of the PBRs was controlled at 20 days. The *M. aeruginosa* cells in the logarithmic growth phase were collected and added into the PBRs to provide an inoculum dry biomass of 0.3 mg/mL.

The treatments in this study included DWA (contained 3 L of diluted actual wastewater with K^+ supplement), DWB (contained 3 L of diluted actual wastewater with K^+ starvation), SWA (contained 3 L of simulated wastewater with K^+ supplement), and SWB (contained 3 L of simulated wastewater with K^+ starvation). A PBR with *M. aeruginosa* cells that were incubated in standard BG-11 medium was used as control (labeled as CON). A PBR containing diluted actual wastewater or simulated wastewater, but without *M. aeruginosa* cells, were used as blank (labeled as DWBlank and SWBlank, respectively). Each treatment was performed in triplicate.

The experiment has lasted for 41 days (two HRTs). Moreover, the operation in the present study was divided into two stages (the first HRT and the second HRT). For the K^+ supplement treatment, during the entire process, KCl was introduced to provide a K^+ supply and was maintained at 17.99 mg/L K^+ (which equals to the K^+ concentration in the standard BG-11 medium) in the DWA and SWA. For the K^+-starvation treatment, the K^+ supplementation was not conducted. During stage II, K^+ starvation in DWB and SWB was conducted by replacing the K_2HPO_4 with Na_2HPO_4 (20 mL of the $Na_2HPO_4.12\ H_2O$ stock solution (8.22 g/L)) in the BG-11 medium to generate a favorable concentration of phosphorus, but without the K^+ addition. By using a comparison between DWA & DWB as well as SWA & SWB, the role of K^+ availability on the Cd^{2+} removal was deduced.

Table 1
Characteristics of the diluted wastewater and simulated wastewater used in the present study.

Properties	Diluted actual wastewater (mg/L)	Simulated wastewater (mg/L)
TN	257.92 ± 0.038	250
$N\text{-}NO_3$	252.78 ± 0.2	250
$N\text{-}NO_2$	0.54 ± 0.01	
$N\text{-}NH_4$	2.14 ± 0.01	
TP	78.342 ± 0.021	7
$P\text{-}PO_4$	7.15 ± 0.061	7
TOC	4.93 ± 0.07	
Cd^{2+}	0.5 ± 0.012	0.5 ± 0.002
K^+	18.52 ± 0.088	17.99 ± 0.004

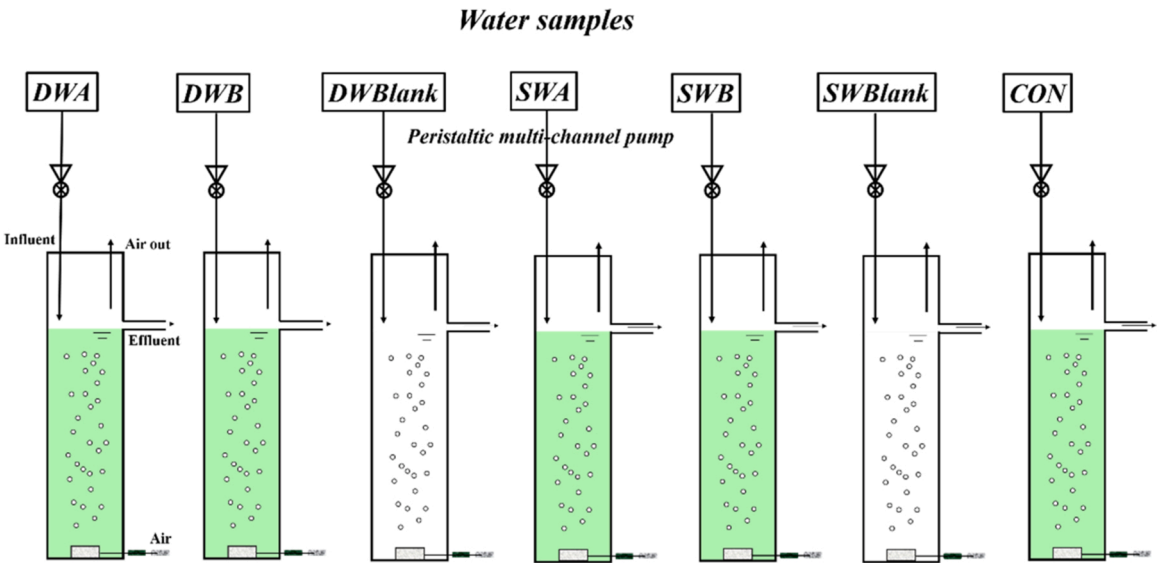

Fig. 1. Schematic diagram of the lab-scale PBR.

2.3. Sampling and analysis

The aliquots of algae (25 mL) were filtered through 0.45 μm membranes (previously dried to a constant weight), and the algal cells on the membranes were dried in a stove at 80 ℃ for 24 h to obtain the dry biomass of *M. aeruginosa*. Additionally, the Cd^{2+} and K^{+} concentrations in the filtered supernatant were determined every two days using an atomic absorption photometer (TAS-990F, PERSEE, China). The malonaldehyde (MDA) content and superoxide dismutase (SOD) activity of *M. aeruginosa* were measured using the assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) every 10 days according to the manufacturer's protocol. The *M. aeruginosa* cells were collected by centrifugation (5000 rpm, 10 min) and were freeze-dried for the Cd^{2+} cellular distribution assay. We conducted a Fourier transform infrared spectroscopy (FTIR) analysis (Model-Shimadzu iraffinity-1, Japan) of the freeze -dried cells on days 21, 31, and 41. For the Cd^{2+} cellular distribution assay, the Cd^{2+} attached to the surface of the *M. aeruginosa* cells was characterized using X-ray photoelectron spectroscopy (XPS, ESCALAB 250Xi, Thermo Fisher, UK), and the analysis of the intracellular Cd^{2+} contents in *M. aeruginosa* has performed every 10 days following the established protocol (Deng et al., 2020a). Briefly, the centrifuged pellets were washed twice with 0.01 M EDTA before freeze-drying to remove any Cd^{2+} absorbed on the cell surface, and the collected cells were subjected to intracellular Cd^{2+} contents analysis using inductively coupled plasma atomic emission spectrometry (ICP-AES) (iCAP 6300 Duo, Thermo Fisher Scientific, USA). To avoid the influence of any extra Cd^{2+} that was not purposely added, we analyzed the original medium using ICP-AES to determine the Cd^{2+} concentration (Cd^{2+} was not detected in the BG-11 medium). All of the glassware and tubes were placed in 10% HCl for 24 h and subsequently rinsed at least five times with Milli-Q water prior to use.

2.4. Statistical analysis

We obtained all of the experimental data from three (n = 3) independent experiments at a 5% significance level. Data are presented as the mean of the triplicate ± the standard deviation. Significant differences were determined using a one-way analysis of variance (ANOVA) followed by a Dunnett post hoc test to compare the data from the treatment groups, and a $p < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Growth performance of M. aeruginosa

In the control group (CON), constant growth was observed, and the maximum dry weight reached 0.58 mg/mL on day 15. Then the dry weight tended to be stable and maintained at approximately 0.5 mg/mL since day 21 (Fig. 2). The *M. aeruginosa* cells were able to grow steadily in SWA and SWB treatments in which the initial Cd^{2+} concentration was 0.5 mg/L irrespective of K^{+} supplementation during the first 16 days. However, after eight days of operation, the dry weight of *M. aeruginosa* in SWB was significantly lower than SWA ($p < 0.05$), and this difference tended to increase with prolonged operation. It was observed that the growth rate of *M. aeruginosa* in both SWA and SWB were far lower than in the control group, indicating that the growth of *M. aeruginosa* in simulated Cd^{2+}-containing wastewater was inhibited under the current Cd^{2+} exposure dosage. *M. aeruginosa* in SWA maintained a positive growth rate untill day 41, with a final cells dry weight of 0.42 mg/mL. However, in the SWB, the cell biomass declined significantly after day 21 and reduced to 0.23 mg/mL on day 41, which was 45% lower than

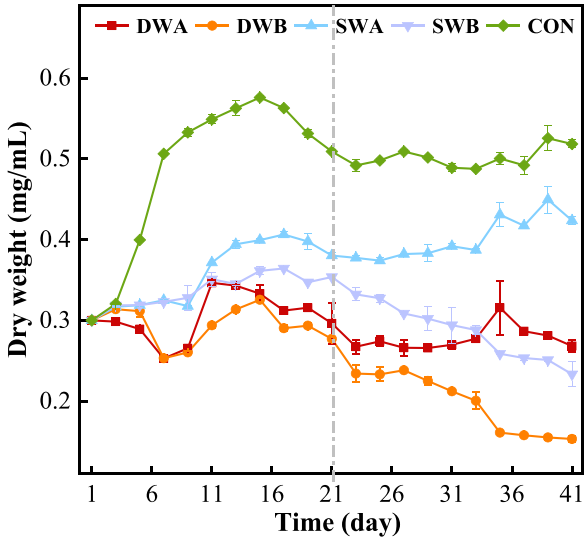

Fig. 2. Evolution of the algal biomass of *M. aeruginosa* cultivated in PBRs throughout the cultivation period.

that in the SWA. This revealed that the lack of K^+ supply caused adverse effects on *M. aeruginosa* growth, especially under the joint abiotic stress with Cd^{2+}.

Overall, the *M. aeruginosa* growth in the DWA and DWB treatments was slower than in the simulated Cd^{2+}-containing wastewater. The biomass of *M. aeruginosa* showed a significant decline until day seven, and this might have been adaptive phase for the *M. aeruginosa* to a more complex component in the diluted wastewater. During the first 21 days of cultivation, the biomass of *M. aeruginosa* in DWB was lower than in DWA. The gap was smaller, however, than that between the SWA and SWB. The final biomass in DWA was 0.27 mg/mL, which was 1.75-fold greater than in DWB. These results demonstrated that *M. aeruginosa* was feasible for treating wastewater that contains Cd^{2+}. This result was in agreement with the response of the photosynthetic efficiency. Fv/Fm, an important indicator of PSII activity (Felline et al., 2019), received the maximum inhibition (40.1% of the control) after 41 days of incubation in DWB treatment (Fig. S1), which was remarkably lower than that in DWA. The Fv/Fm dynamics in SWA and SWB shared a similar pattern with that in DWA and DWB, indicating that the K^+ starvation triggered a profound inhibition of *M. aeruginosa* growth and photosynthesis, and this could have been augmented upon the exposure to Cd^{2+}.

Our previous study showed that the IC_{50} of Cd^{2+} to *M. aeruginosa* was 5.62 mg/L (50 μM) (He et al., 2021). In the present study, *M. aeruginosa* maintained a stable biomass during the 41- day operation of PBR in SWA, indicating that *M. aeruginosa* can adapt to the low Cd^{2+} toxicity (0.5 mg/L) and maintain steady growth. Likewise, another study stated that a low Cd^{2+} concentration had little influence on the growth of *M. aeruginosa* (Deng et al., 2020a). In this study, the dynamic of the *M. aeruginosa* dry weight in SWA and SWB were far lower than that in DWA and DWB because of the coexistence of other hazardous material, such as total Cr and Pb^{2+} in the actual wastewater (Table S1),which also could have been assimilated or absorbed by the algal cells. Moreover, in comparison to DWA and SWA, the substantial reduction of biomass in DWB and SWB proved that K^+ availability played a crucial role in the resistance of *M. aeruginosa* against Cd^{2+} exposure. Hence, the higher production rate of algal biomass may be helpful for the efficient removal of Cd^{2+} in the PBR system.

3.2. Antioxidant response of *M. aeruginosa*

Environmental factors, like heavy metal stresses, can induce oxidative stress in algal cells (Costa et al., 2019). In addition, antioxidant enzymes, such as superoxide dismutase (SOD), serve as the first defense mechanism against it. During the 41 days of operation, in comparison with the control, a minimum increase in the SOD activity of *M. aeruginosa* was noted in SWA (Fig. 3a). It was observed that the SOD activity did not follow a linear relationship with operation duration in SWA, and the maximum SOD activity (24.22 U/mg protein) was noted at day 11. A similar trend was also detected in DWA. The SOD activity in SWB was higher than it was in the SWA since day 21, and it maintained a constant increase untill day 41 (29 U/mg proteins). Amongst the treatments, the maximum SOD activity was noted in DWB at day 41 (39.48 U/mg protein), which was 3.68-fold higher than the control. MDA content exhibited a positive linear relationship with operation duration in DWA, DWB, and SWB, with final MDA contents of 2.56, 6.31, and 4.8 ng/mg protein, respectively (Fig. 3b), which were 2.24, 5.35 and 4.19-fold, respectively, greater than the control. However, the maximum MDA content in SWA was observed at day 21 (2.12 ng/mg protein), which declined afterward and reached 1.83 ng/mg protein on day 41.

The increase in SOD activity of the *M. aeruginosa* cells in the treatment indicated the activation of antioxidant system in *M. aeruginosa* (Han et al., 2021). The decline of SOD in K^+ supplement treatments (DWA and SWA) suggested the adaption of *M. aeruginosa* to the Cd^{2+} exposure stress, whereas the continuous increase in SOD activity in the K^+ starvation treatments (DWB and SWB) showed that the antioxidant enzyme system could not maintain a dynamic balance of free radical generation and scavenging (Alvarez et al., 2012). Therefore, the oxidative stress tended to deteriorate with time, as proven by the dynamic of the *M. aeruginosa* dry weight in the PBR (Fig. 2). In addition, MDA is one of the oxidation products of membrane lipids, and MDA content is generally considered to be an important indicator of lipid peroxidation that reflects oxidative damage to cells under external stress (Cheng et al., 2017). Therefore, the possible reason for the significant increase in the MDA content of *M. aeruginosa* in K^+-starvation treatments

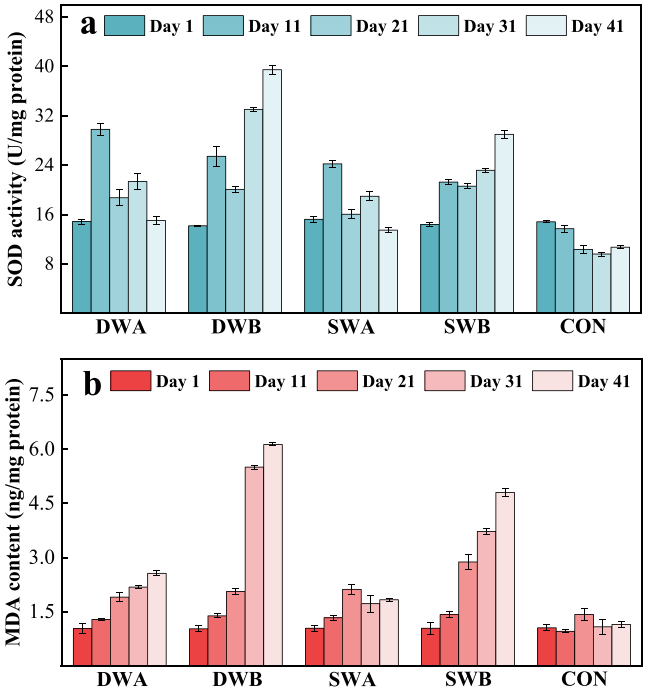

Fig. 3. SOD activity (a) and MDA content (b) in *M. aeruginosa* cells cultivated in PBRs throughout the cultivation period.

(DWB and SWB) was the unbalanced antioxidant capability and the oxidative effects caused by the heavy metal. This resulted in the peroxidation of membrane lipids and increased lipid peroxidative products, such as MDA (Gu et al., 2020). Additionally, the relatively higher MDA content in DWA and DWB than those in SWA and SWB indicated that the other components in diluted actual wastewater jointly triggered more severe oxidative damage with the Cd^{2+} exposure in the *M. aeruginosa* cells in the PBR system. This result also was supported by the dynamics of the dry weight of *M. aeruginosa* (Fig. 2).

3.3. Changes in the membrane FTIR characteristics

Phytoplankton can secrete EPS to form a protective barrier outside the algal cells to protect itself from external toxic compounds including heavy metals (Zheng et al., 2019). EPS contributes to the various functional groups on the cell surface, and it has been reported that carboxyl C—O might be the key site responsible for the bond of EPS—Cd of phytoplankton (Xie et al., 2020). On the other hand, the FTIR spectra can provide primary information about the EPS composition and functionalities of phytoplankton (Cheng et al., 2020). Typically, the broad and strong band ranging from 3200 to 3600 cm^{-1} with peaks at 1625 cm^{-1} and 1360 cm^{-1} are assigned to the -OH stretching, C-O stretching and C-OH stretching of carboxyl groups (labeled in Fig. 4). The strengthen of carboxyl groups in DWA was stronger than that in DWB, and decrease of carboxyl groups was recorded in DWA with operation duration whereas the difference was not obvious between days 21 and 31 in DWB. With a reduction of the available K^+, the strengthen of carboxyl groups in SWB was significantly decreased. This might consequently cause the impaired removal of Cd^{2+} due to a lack of the surface bonding process.

When exposed to heavy metals, the impairment of hazardous assimilation is one of the primary strategies for the detoxification of phytoplankton, during which the chelation of the hazard may be the most prevalent strategy (Wu and Wang, 2014). A previous study documented a positive correlation between Cd^{2+} tolerance and EPS production of cyanobacteria (Ozturk et al., 2010). Similarly, our previous study also confirmed the strengthened secretion of EPS in the K^+ -abundant environment after Cd^{2+} exposure (He et al., 2021). In the present study, we noted the strengthening of carboxyl at days 31 and 41 in compared with day 21 in SWA, but this did not occur in SWB (Fig. 4). Moreover, the FTIR spectra illustrated that the functional groups on *M. aeruginosa* cells in DWA showed much more obvious response than DWB. In addition, the response of carboxyl groups under the different K^+ levels might trigger the changes in the Cd^{2+} retention process on the surface of *M. aeruginosa* cells.

3.4. Cd^{2+} removal

3.4.1. Cd^{2+} removal efficiency

The K^+ in the DWA and SWA groups was supplied every five days to maintain the K^+ concentration to be 17.99 mg/L (which equals to the K^+ concentration in BG-11 medium). During the first 21 days, the K^+ concentration varied in the range of 18.28–14.93 and 17.99–14.18 mg/L in DWB (Fig. 5a) and SWB (Fig. 5b) respectively. Afterward, with the

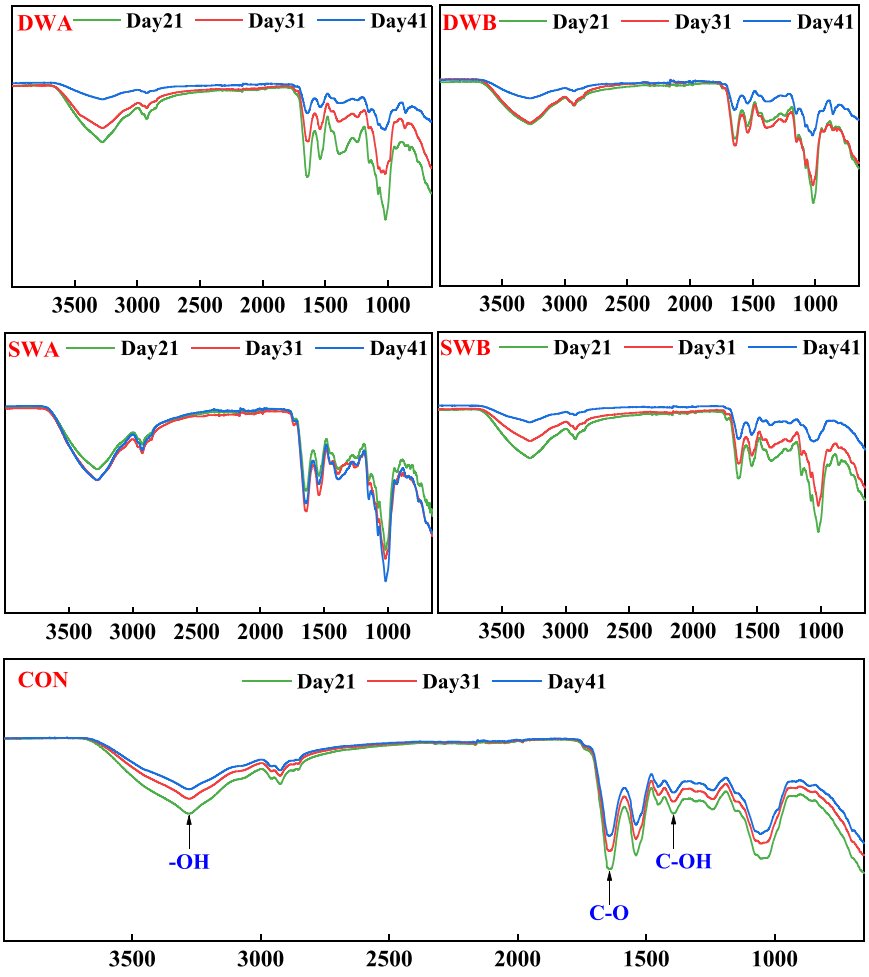

Fig. 4. The FTIR spectra of *M. aeruginosa* in the studied PBRs at days 21, 31 and 41.

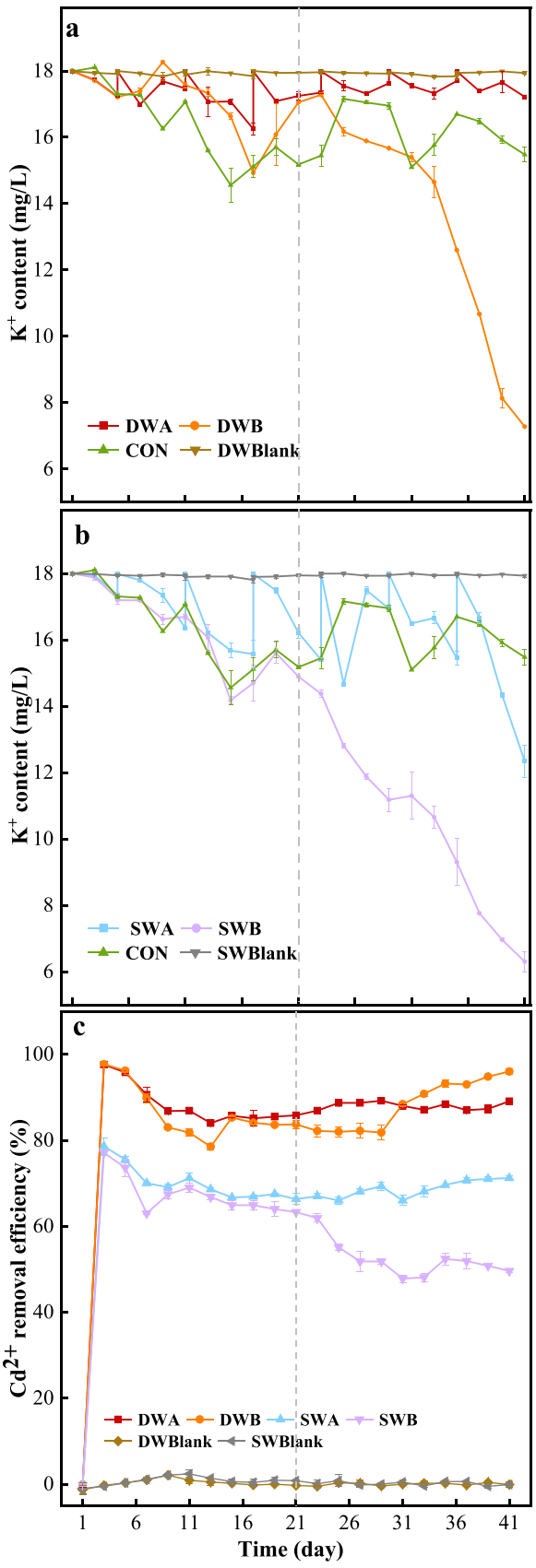

Fig. 5. Evolution of the PBRs operated during the cultivation: (a) K^+ concentration in the diluted wastewater PBR; (b) K^+ concentration in the simulated wastewater PBR; and (c) Cd^{2+} removal efficiency in the studied PBR.

inflow of K^+-free wastewaters, the concentrations of K^+ in DWB and SWB decreased significantly. The final K^+ level in SWA accounted for 71.7% of that in DWA, whereas the K^+ concentration in SWB reached 86.5% of that in DWB. The relatively higher K^+ consumption could be triggered by the higher biomass in the simulated wastewater. It should be noted that with the reduction in the K^+ level, the biomass of *M. aeruginosa* showed a sharp decrease in SWB and DWB (Fig. 2), proving that the K^+ availability was important in the cell proliferation of *M. aeruginosa* in the PBR system.

During continuous cultivation, the Cd^{2+} concentrations in the diluted and simulated wastewaters decreased significantly in the PBR (Fig. 5c). The maximum Cd^{2+} removal by *M. aeruginosa* cells achieved 97.6% and 78.6% in the DWA and SWA groups, respectively, after the beginning of the Cd^{2+} feeding (on day three) caused from the accumulation (uptake) of Cd^{2+} by the algal cells. Thereafter, the Cd^{2+} removal efficiency decreased at the average value of 13.6%, 19.2%, 12.3%, and 14.1% in DWA, DWB, SWA and SWB, respectively. With respect to the simulated wastewater, it was clear that the Cd^{2+} removal efficiency displayed a constant higher value in the continuous K^+ supplied treatments. For example, in the SWA, it reached 71.2% after 41 days of operation, which declined to 49.6% in the SWB. In the actual wastewater groups, the Cd^{2+} removal efficiency reduced from 97.6% to 85.8% before day 21 in DWA, and varied from 97.7%– 83.7% in DWB group. These findings directly proved that K^+ supplement was indispensable for Cd^{2+} removal in *M. aeruginosa*-based PBR.

With respect to heavy metal removal using the PBR, we found that cellular uptake and adsorption by the algal cells were the primary contributions (Leong and Chang, 2020), which both relied on the growth of the algae incubated in the system. In the previous study, it was revealed that once continuous K^+ supplementation was performed, the dry weight of *M. aeruginosa* cells was dramatically enhanced (SWA>SWB, DWA>DWB). Note, however, that in the SWA group, the dry weight of *M. aeruginosa* maintained stable growth, whereas in the DWA group, the dry weight of *M. aeruginosa* tended to decline after day 12. As a consequence, the dry weight in SWA was 1.58-times higher than that in DWA, indicating the *M. aeruginosa* in SWA would be much more versatile than that in DWA. However, the Cd^{2+} removal exhibited a significantly higher efficiency in DWA and DWB compared with SWA and SWB. This discrepancy may b have been the result of both algal uptake, surface retention, and the complexation of Cd^{2+} by the non-living algal cells.

3.4.2. The Cd^{2+} removal approach

3.4.2.1. Cd^{2+} retention on the algal cell surface. The high resolution XPS spectrum of Cd 3d was composed of two peaks at 405 eV and 411 eV that corresponded to Cd 3d5/2 and Cd 3d3/2, respectively. In the present study, when K^+ was deficient (DWB and SWB), the additive peak area of Cd^{2+} tended to decrease from days 21–41. In addition, by the end of the operation, the Cd^{2+} content on the surface of *M. aeruginosa* cells was 49.25% and 49.11% of that at day 21 in DWA and SWA respectively. This indicated that the Cd^{2+} retention on the cell surface was inhibited by K^+ starvation (Fig. 6). This also agreed with the observation of the decreased content of the functional groups (including carboxyl) on the surface of *M. aeruginosa* cells surface were recorded (Fig. 4). Serving as the first line of defense against Cd^{2+} toxicity, a positive correlation between EPS production and Cd^{2+} tolerance has been observed for other cyanobacteria including *Cyanothece* sp. and *Synechocystis* sp.(Mota et al., 2015; Ozturk et al., 2010). The present study confirmed that K^+ can regulate the Cd^{2+} adsorption by affecting the secretion of membranal EPS. Compared with the SWA and the SWB, however, we found a significantly lower peak area of Cd^{2+} in the DWA and DWB. This result indicated that the living cell surface retention of Cd^{2+} was not the primary process for relatively higher Cd^{2+} removal in the PBR. This may suggest that it impaired the Cd^{2+} adsorption capability of *M. aeruginosa*

in DWA and DWB, or the enhanced immigration of Cd^{2+} from cell surface to the cell (which was proven by the intracellular Cd^{2+} content in algal cells in DWA and DWR groups).

3.4.2.2. Cd^{2+} assimilation by the algal cells. The peak of the intracellular Cd^{2+} content was noted in DWB (0.512 μg/mg DW) on day 11, when the intracellular Cd^{2+} content in the simulated wastewater (SWA and SWB) was significantly lower than that in the diluted actual wastewater (DWA and DWB) (Fig. 6). In SWA, the intracellular Cd^{2+} content tended to be stable around 0.15 μg/mg DW from days 21–41. In contrast, without continuous K^+ supplement, the intracellular Cd^{2+} content decreased from 0.105 to 0.059 μg/mg DW in SWB, indicating a decreased Cd^{2+} uptake capacity of *M. aeruginosa* triggered by K^+ starvation. In the diluted actual wastewater, the intracellular Cd^{2+} content in DWB did not display a linear reduction with time, and the final intracellular Cd^{2+} content reached 0.105 μg/mg DW, which was 1.78-fold greater than it

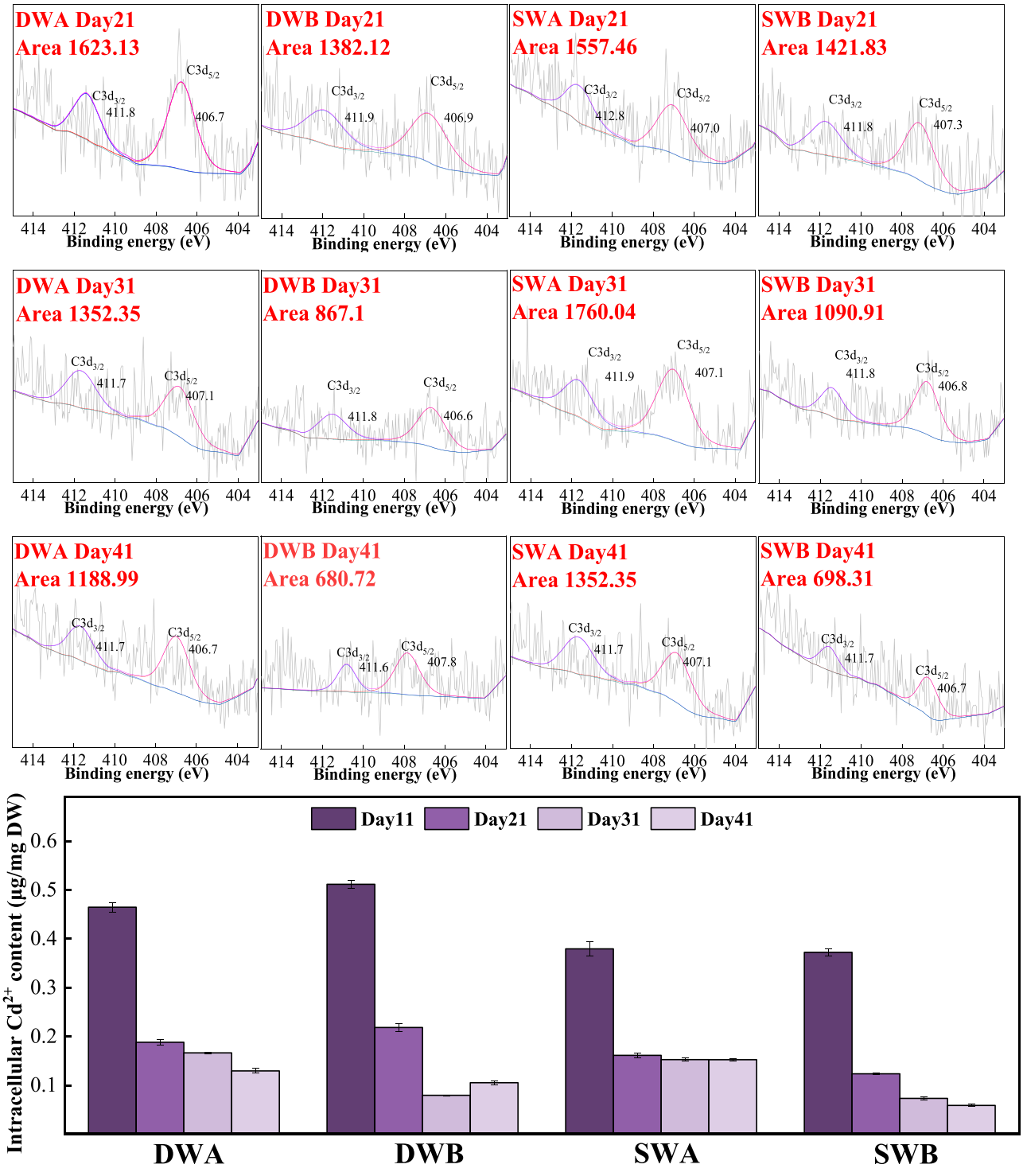

Fig. 6. The XPS spectra of Cd^{2+} on the surface and the intracellular Cd^{2+} contents of *M. aeruginosa* in the studied PBRs.

was in the SWB. These results confirmed that more Cd^{2+} was accumulated in *M. aeruginosa* cells in the diluted actual wastewater groups, and the continuous K^+ supplement tended to benefit Cd^{2+} uptake. By analyzing the results of the evolution of *M. aeruginosa* dry weight, the Cd^{2+} retention, and the Cd^{2+} accumulation together, it was found that the Cd^{2+} removal in the *M. aeruginosa* contained PBR was more obvious toward the diluted actual wastewater than the simulated wastewater.

3.4.3. Non-living algal cells in PBR benefited Cd^{2+} removal but the effects were unsustainable

The use of biological materials, including living and non-living algal cells in the removal and possibly recovery of heavy metals from industrial wastes, has gained important credibility in recent years because of their excellent performance and low cost (Montazer-Rahmati et al., 2011). Several previous studies have shown that the absorption of heavy metal ions by algal biomass may be increased by the transformation from living-cells to non-living cells (Kandasamy et al., 2021). The non-living degradation of the cell membrane offers a larger area to enhance the biosorption capacity and releases cell contents to possibly advances in cell-to-ion binding components (Roozegar and Behnam, 2019). Moreover, pretreatment with calcium chloride ($CaCl_2$), formaldehyde, and sodium hydroxide (NaOH) has been popular in the process of metal removal using living algae, and although these pretreatments will inevitably trigger loss of biomass, the altered membrane permeability can significantly enhance the metal removal efficiency (Abbas Najim and Mohammed, 2018). In the current study,we illustrated that the dry weight of *M. aeruginosa* in DWB showed a constant decrease since day 15, but a sudden reduction on day 31 (Fig. 2) In addition, the death of *M. aeruginosa* cells at day 41 indicated a failure of the *M. aeruginosa* to the undesired conditions. Therefore, the dissolved organic carbon in DWB showed a 14.2-fold increase in DWB in comparison to its initial value (Fig. S2), and this would have enhanced the complexation of Cd^{2+} by the exposed functional groups of the non-living cells. Compared with Cd^{2+} removal efficiency in DWA and DWB, the maximum removal of Cd^{2+} was observed in DWB after day 31 because of the complexation of Cd^{2+} by the non-living algal cells. This result was highly likely to be induced by the complexation of Cd^{2+} by the non-living algal cells.

Heavy metal removal using living algae involves biosorption and bioaccumulation processes. The biosorption process is a fast process that occurs at the cell surface, whereas bioaccumulation is a slower metabolism-dependent process (Mehta and Gaur, 2005). *Scenedesmus abundans* has been reported to achieve a 97% removal rate of Cd^{2+} with an initial concentration of 10 mg/L in 48 h (Terry and Stone, 2002), *Chlorella pyrenoidosa* showed a 45.45% removal rate of Cd^{2+} with an initial concentration of 1 mg/L in eight days (Chandrashekharaiah et al., 2021). *Ulva lactuca* sp. achieved over a 90% Cd^{2+} removal rate with an initial concentration of 67.2 mg/L in 24 h (Bulgariu and Bulgariu, 2012). Our previous study demonstrated that *M. aeruginosa* in K^+ abundant environment can achieve a 90% Cd^{2+} removal with an initial concentration of 5.6 mg/L in 96 h (He et al., 2021). A majority of these studies were performed in suspended and immobilized batch cultures at small scales, and the results may not be satisfactory when applied to actual Cd^{2+}-containing wastewaters. A study of the Cd^{2+} removal using a turbidostatic continuous culture was firstly reported by Premazzi et al. (1978). Since then, numerous studies had been performed to investigate the efficiency of PBR for the bioremediation of heavy metal-contaminated water, and a 60% Cd^{2+} removal efficiency by *Scenedesmus obliquus* in PBR was reported by Chen et al. (2012). In the present study, the Cd^{2+} removal efficiency by *M. aeruginosa* in simulated K^+-abundant environment (SWA) achieved 70%, indicating that the *M. aeruginosa* cells might exhibit a stronger Cd^{2+} removal capacity than *Scenedesmus obliquus* (mentioned earlier). Moreover, for the dilute actual wastewater, the results of the current study confirmed that the 89% Cd^{2+} removal rate in DWA was maintained, and the biomass did not exhibit a severe decline. This indicated that this scenario did not trigger the collapse of the algal cells, and this was the maximum Cd^{2+} removal efficiency for the actual wastewater to date. Therefore, despite the evidence that a higher Cd^{2+} removal rate could be achieved in a shorter period of time in DWB than DWA by the non-living algal cells, the inhibition of *M. aeruginosa* cell growth and substantial reduction in algal biomass cause the Cd^{2+} removal in the continuous PBR to be unsustainable.

3.4.4. Continuous K^+ supplement triggered multiple response of algae in the PBR

In general, the efficiency of PBR was significantly regulated by temperature, pH, contact time, heavy metal concentration, and structural parameters (Chen et al., 2012; Yeo et al., 2018). Based on the biosorption and uptake metabolism of algae incubated in the PBR, the toxins removal efficiency was directly related to the nutrient elements supply. This can also explain the limitation of batch cultivation of lacking continuous supply of nutrients (Lu et al., 2021). Nitrogen and phosphorus are considered to be fundamental factors for algal growth, but these are also treated as contaminants in wastewaters. Therefore, some civil wastewaters can be directly treated using PBRs (Di Termini et al., 2011). As a macro-element, K^+ is essential for algae cell growth and proliferation. Our previous study found that K^+ level can enhance the Cd^{2+} biosorption and uptake by *M. aeruginosa* (He et al., 2021, 2020), but few studies have demonstrated the optimization of the heavy metal removal efficiency by regulating the contents of the vital elements for algae growth. In the present study, we proposed a new perspective for PBR optimization by regulating the K^+ level. In simulated wastewater, when the *M. aeruginosa* was exposed to only Cd^{2+}, the Cd^{2+} removal efficiency in a K^+-starvation treatment (SWB) was overall lower than that in the K^+ supplement treatment (SWA) (Fig. 5c) throughout the operation duration. This result indicated that a continuous K^+ supply in the PBR was indispensable for the growth and Cd^{2+} removal process of *M. aeruginosa*. When the K^+ level in the PBR was reduced, however, a decrease in the Cd^{2+} removal efficiency was noted, especially in SWB. The potential process underlying the performance can be illustrated as follows. When K^+ was abundant, *M. aeruginosa* was able to maintain a stable growth in the Cd^{2+}-containing inflow (Fig. 2), and the functional groups (carboxyl) on the surface of *M. aeruginosa* cells were responsible for the biosorption of Cd^{2+} onto the cells (Fig. 4). Therefore, Cd^{2+} could be continuously assimilated into the cells, and a stable Cd^{2+} removal efficiency of 70% was achieved in SWA. With the continuous reduction in thef K^+ level of in the inflow in DWB and SWB, the growth of *M. aeruginosa* was inhibited as a down-regulation of photosynthesis efficiency. Therefore, a dramatic reduction in the algal dry weight was recorded (Fig. 2). Moreover, the K^+ viability also significantly altered the Cd^{2+} biosorption ability by reducing the carboxyl content on the cell surface (Fig. 4). Consequently, the decreased biomass, restrained EPS secretion and Cd^{2+} uptake jointly caused the reduction in the Cd^{2+} removal efficiency in the K^+-deficiency PBR, emphasizing the significance of regulating the K^+ level in the PBR.

4. Conclusion

In the current study, the effect of the K^+ level on the Cd^{2+} removal efficiency from diluted actual wastewater and simulated wastewater in continuous *M. aeruginosa*-based PBR was demonstrated. We found that continuous Cd^{2+} removal could be achieved with an abundant K^+ supply, and in SWA the Cd^{2+} removal efficiency was maintained at approximately 70%. The overall higher Cd^{2+} removal rate (90%) in the diluted actual wastewater than in the simulated wastewater was caused by the existence of non-living *M. aeruginosa* cells that were triggered by other toxic components in the actual wastewater. In addition, the PBR systems using actual wastewater were more sensitive than that using simulated wastewater. Without a continuous K^+ supply, the proliferation of *M. aeruginosa* was significantly restrained, and the continuous Cd^{2+} removal capacity was destroyed quickly. Therefore, there is a great

potential for the optimization of PBR by providing a sufficient K^+ level.

Supporting information

Evolution of Fv/Fm of *M. aeruginosa* cultivated in PBR throughout the cultivation period. Dissolved organic carbon content in the PBRs at day 1 and 41.

CRediT authorship contribution statement

Mengzi Liu: Investigation, Data Curation, Writing – original draft. **Yanyan Wei:** Writing – review & editing, Funding acquisition. **Muhammad Salam**: Review & editing. **Xiaobing Yuan**: Funding acquisition. **Bingsheng Liu**: Funding acquisition. **Qiang He**: Funding review & editing. **Xuebin Hu**: Funding review & editing. **Hong Li**: Funding acquisition, Data curation, Writing – review & editing. **Yixin He**: Data curation, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2021.127719.

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