Adsorption characteristics of Cr⁶⁺ on microalgae immobilized by different carriers

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Research Article

Keywords: Carrier, Immobilization, Adsorption, Microalgae

Posted Date: April 15th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-367902/v1>

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Abstract

To solve the problem of harvesting microalgae during heavy metal adsorption, six different carriers were selected in this study to compare the adsorption behavior of microalgae after immobilization. The optimal immobilized carrier-chitosan was obtained under the following conditions of chitosan: acetic acid (2:40), microalgae concentration (10⁸ cells/mL), and immobilization time (18 h). The optimal adsorption conditions were as follows: temperature: 30°C, pH: 5.0, adsorption dose: 1.5 g/L, initial ion concentration: 40 mg/L. Fourier transform infrared spectroscopy analysis showed that the specific functional groups of the specific microalgae played an important role in the adsorption process. The kinetic and isothermal model data showed that the adsorption process was mainly chemical adsorption and homogeneous monolayer adsorption. Moreover, X-ray photoelectron spectroscopy analysis showed that the Cr adsorption process involves the reduction of Cr(VI) to Cr(III).

Introduction

Heavy metal pollution has become a serious threat due to the intensification of industrialization and urbanization in China (Gao et al. 2018). Because of its toxicity and carcinogenicity, it poses a potential threat to human beings (Ghiassi et al. 2020, Prabhakar & Samadder 2020, Wang et al. 2018). Among the toxic heavy metals, Cr⁶⁺ is considered as a major pollutant, which is widely used in metal processing, leather tanning, electroplating, textile industry, and chromate preparation (Bharagava et al. 2018, Sanjay et al. 2020, Singh et al. 2017). Cr⁶⁺ is considered to be one of the most toxic metals found in industrial water, which can penetrate the cell wall and cause health problems, such as liver injury, pulmonary congestion, and severe diarrhea (Sanjay et al. 2020, Shalini et al. 2019, Zengying et al. 2018). Owing to the serious toxicity of Cr⁶⁺, the EU directive World Health Organization (WHO) and the United States Environmental Protection Agency (EPA) stipulate that the permissible total chromium content of standard drinking water is 0.1 mg/L, and the maximum concentration of Cr⁶⁺ in domestic water is 0.05 mg/L (Di Natale et al. 2007, Khezami & Capart 2005). The food and Agriculture Organization of the United Nations recommended that the highest level of Cr⁶⁺ in irrigation water is 0.1 mg/L. Therefore, removing heavy metal ions from water is critical.

Traditional methods to remove soluble heavy metal ions include chemical precipitation, chemical oxidation or reduction, filtration, ion exchange, electrochemical treatment, membrane technology, and evaporation recovery (Hou et al. 2018, Khalaf et al. 2019). However, these methods have a numerous drawbacks, such as incomplete removal and requirement for expensive equipment (Soltani et al. 2018). Among these technologies, chemical precipitation is one of the most common and conventional treatment methods that can effectively reduce heavy metals to an acceptable level; however, it requires several chemicals and produces secondary pollution, thereby increasing the cost (Daneshvar et al. 2002). Other treatment methods also require higher investment and operating costs (Demir & Arisoy 2007). Compared with these methods, adsorption is an effective method for wastewater treatment, because it benefits from high removal efficiency, low cost, simple operation, and strong selectivity for metals (Sasmal et al. 2020). At present, various types of low-cost adsorbents have attracted considerable

attention. In particular, the use of biosorbent microalgae to remove heavy metals from contaminated soil or water is a potential bioremediation technology (Fomina & Gadd 2014, He & Chen 2014, Huang et al. 2019). The surface of microalgae contains polysaccharides, proteins, and lipids, which can provide many functional groups (such as hydroxyl, carboxyl, carbonyl, and phosphodiester groups) to bind with heavy metal ions (Bharagava & Mishra 2017, Chen et al. 2014, Deepa et al. 2019). The living and dead cells of various microalgae show considerable adsorption capacity for heavy metal ions. Dead cells can avoid the problem that the absorption activity is affected by heavy metal ions, and therefore, it has a wide range of applications in biological adsorption. Living cells can adsorb heavy metals by adsorbing on the cell surface (biosorption) or entering the cell body (bioaccumulation) (Fomina & Gadd 2014). Dead cells can only remove heavy metals through passive surface binding biosorption process (Kadukova 2016). However, the low density of microalgae makes it difficult to separate and collect heavy metals from liquid, which limits the large-scale application of microalgae. Therefore, the immobilization of microalgae has been widely studied and has shown great potential in the treatment of wastewater containing heavy metals.

In recent years, biotechnology-based immobilized microalgae culture has been widely developed. Microalgae attached to the surface and various carriers are widely used to produce biomass and metabolites and to remove excess nutrients and soluble heavy metal ions from wastewater (Politaeva et al. 2020, Shen et al. 2020). Compared with cell suspension, immobilized microalgae have the advantages of simplifying the biomass harvesting process and enhancing the cell resistance to adverse factors (e.g., temperature, acidity, and toxic compounds).

The carriers of microorganism immobilization are divided into natural and synthetic carriers. Natural carriers are insoluble substances (e.g., wood, wool, or minerals) attached to cells in the body. Natural carriers have the advantages of hydrophilicity, biocompatibility, and easy recovery, whereas their disadvantages are poor stability and high cost. The carriers of immobilized microorganisms are usually found in loofah pulp fruit, peat, glass, plastic, wood, natural polysaccharides (e.g., agar, cellulose, alginate, carrageenan, and chitosan) and synthetic polymers (e.g., polyacrylamide, polyurethane, polyvinyl chloride, polypropylene, polysulfone, and epoxy resin) (de-Bashan & Bashan 2010). The ideal carriers of microalgae cells should not inhibit their viability, nor should they prevent mass transfer and shading. The carriers should have high mechanical, chemical, biological stability and processability. In addition, it should be cheap, safe to preserve cells, and highly hydrophilic.

In this paper, different immobilized carriers (e.g., sodium alginate, chitosan, carrageenan, diatomite, quartz sand, and polyvinyl alcohol) were used to immobilize *Synechocystis* sp. PCC6803 for the adsorption of heavy metal Cr^{6+} to study the desorption efficiency in different desorption solution following adsorption. The results provided theoretical basis for the development of high-efficiency biosorption materials for the standardized treatment of heavy metal wastewater.

Materials And Methods

2.1 Strain culture

The microalgae *Synechocystis* sp. PCC6803 was purchased from fresh water algae culture collection at the Institute of Hydrology (FACHB) of Chinese Academy of Sciences. The cells were cultured in BG-11 medium at an initial pH of 7.1 to logarithmic phase, followed by centrifugation and precipitation. During this period, algae powder was obtained by shaking the bottle twice a day (Xu et al. 2013).

2.2 Preparation of different immobilized biological adsorbents

Sodium alginate embedding method: sodium alginate (1.0 g) was heated and dissolved in sterile water (35 mL), cooling to 45°C, and was allowed to standby. Afterward, 1.0 g of freeze-dried algae powder was stirred with 3.0 mL sterile saline, pour into standby sodium alginate solution, and mix evenly. The final concentration of sodium alginate was 2.5%. The mixture was dropped using a 5 mL syringe into the 4% CaCl₂ solution of precooling, then refrigerated at 4°C for 24 h after crosslinking, and the immobilized particles were filtered out. The mixture was rinsed with normal saline and refrigerated at 4°C. Meanwhile, blank control particles without the addition of algae powder were prepared in the same way as described above.

Carrageenan embedding method: quantitative carrageenan was dissolved in sterile water, cooled to 35-50°C, and mixed evenly with dried algae powder to a final concentration of carrageenan 2%. Suspension drops were added to 4% KCl solution to form spherical solid particles, which were cross-linked at 4°C for 24 h, and then, the immobilized particles were filtered out and washed with normal saline.

Chitosan embedding method: a chitosan solution of 1:20 (w/v) was prepared with 2% (v/v) acetic acid. After full dissolution, the chitosan solution was added to 1 mol/L NaOH solution bit by bit and condensed into white particles. Chitosan beads were filtered, collected, washed to neutral with distilled water, dried at 35°C, and soaked overnight in a 50 mmol/L citric acid buffer solution with pH 5.0. The pretreated carrier chitosan of 1.0 g was added to a certain concentration of glutaraldehyde 1.0 ml. After being shaken in a water bath and placed at room temperature for 5 h, the cross-linking carrier was washed with the buffer solution and then added with a 1.0 ml physiological saline alga solution for 2 h in a water bath. The buffer solution was then adsorbed at 4°C for 5 h in a refrigerator.

Polyvinyl alcohol (PVA) fixation: A certain amount of PVA and boric acid were sterilized in normal saline. Algal powder was added at a proportion of 1%, mixed evenly, spread into thin layers, frozen for 24 h, and gel-immobilized cells were prepared. The samples were cut to approximately 4 × 4 × 4 mm fragments, and sterile saline was used for the subsequent experiment.

Diatomite fixation: the carrier was soaked in 5% HCl for 2 h, rinsed the carrier with distilled water until neutral, soaked in 5% NaOH for 2 h, rinsed with distilled water until neutral, autoclast at 121°C for 20 min, and the sterilizing carrier was mixed with the bacterial powder bacterial suspension dissolved in sterile physiological saline. Subsequently, the carrier was shaken in a water bath for 30 min at 30 rpm /min. Finally, the carrier was settled for 10 min to filter out particles.

Quartz sand fixation: the carrier was soak with 5% HCl for 2 h, rinsed with distilled water to neutralization, soaked with 5% NaOH for 2 h, rinsed with distilled water for neutralization, autoclast at 121°C for 20 min. Next, the sterilizing carrier was mixed with the bacterial suspension dissolved in sterile physiological saline, shaken in a water bath for 30 min at 30 rpm. Finally, the carrier was settled for 10 min to separate the sediment.

2.3 Adsorption experiment

Different immobilized biological adsorbents (e.g., sodium alginate, carrageenan, chitosan, diatomite, quartz sand, and PVA) were added to 100 mL of 80 mg/L Cr⁶⁺ solution and were maintained at room temperature oscillator for 3 h. After filtering the spherical particles, the supernatant was centrifuged for 10 min at 10000 rpm/min and filtered with 0.22 um membrane. The concentration of Cr⁶⁺ was detected using an inductively coupled plasma-optical emission spectrometer (ICPS-750, Perkin Elmer). Blank controls (single algae powder and blank control particles) were also used in the adsorption experiment under the same conditions.

2.4 Desorption experiment

Different immobilized biological adsorbents used in the above adsorption experiments were placed in different desorption solutions of 50 mL (e.g., 0.5 mol/L NaOH, 0.5 mol/L HCl, 0.5 mol/L NaCl, 0.5 mol/L EDTA). Under the same conditions of adsorption experiments, the biosorbents were separated following 3 h of oscillatory desorption, and the concentration of Cr⁶⁺ in the desorption solution was detected.

2.5 Calculation method

The adsorption capacity of the immobilized biosorbent was calculated using Eq. (1) as follows:

$$q = V(C_0 - C_e)/M \quad (1)$$

Where C₀, C_e are the initial and equilibrium concentration of Cr⁶⁺ in the solution (mg/L); V is the solution volume (L); and M is the dry weight (g) of the biosorbent.

Desorption efficiency is calculated using Eq. (2) as follows:

$$P_j = C_j V_j * 100\% / (C_0 - C_e) V \quad (2)$$

Where C_j and V_j represents the metal ion concentration (mg/L) in the desorption solution and the desorption volume (L), respectively.

2.6 Scanning electron microscope (SEM) analysis

SEM analysis and the EDX microanalysis were performed using a ZEISS DSM-960 microscope equipped with an EDX unit. The different biosorbents were fixed in the dark at 4°C with 2.5% phosphate buffer glutaraldehyde for 24 h and dehydrated for 10 min with 30%, 50%, 70%, 90%, and 100% ethanol. After freeze-drying, the surfaces and sections of the samples were observed using a field SEM. Additionally, the blank carrier was used as control.

2.7 Fourier transform infrared spectroscopy

Different immobilized biological adsorbents were freeze-dried and ground into powder before and after adsorption. The 1.0 mg sample was evenly mixed with 100 mg KBr and then pressed into tablets for infrared spectrum analysis (Madison, WI, USA).

2.8 Raman spectroscopy analysis

Raman spectroscopy studied the non-polar groups of symmetrical molecules or the vibration of the molecular skeleton to produce bands, which were mainly used to identify the types of chemical substances, special structural features, or characteristic groups, which complement the infrared absorption spectrum.

2.9 Optimization of adsorption experiment conditions

2.9.1 Effect of pH

The influence of pH was investigated in range of pH 2.0-8.0. The 1.0 g/L of adsorbent and 100 mL 40 mg/L of Cr^{6+} aqueous solution was mixed uniformly at 30°C and oscillated for 30 min at 125 r/min. The solution was filtered to determine the concentration of Cr^{6+} in the solution after adsorption at each pH. The pH_{pzc} was measured using a powder addition method.

2.9.2 Effect of temperature on adsorption

The initial concentration of chromium ion was 40 mg/L; adsorption dose was 1.0 g/L; pH value was 3.0; temperature was 15, 20, 25, 30, 35, and 40°C; and oscillation adsorption was performed for 30 min at 125 r/min. The liquid was filtered to determine the concentration of Cr^{6+} in the solution after adsorption at each temperature, and the adsorption ability and adsorption efficiency were calculated.

2.9.3 Effect of initial concentration on adsorption

The initial concentration of chromium ions was 40 mg/L; pH value was 3.0; and adsorbent concentrations were 0.5, 1.0, 1.5, 2.0, and 2.5 g/L. The shaking adsorption was performed at 30°C and 125 r/min for 30 min, and the clear solution was filtered. The concentration of Cr^{6+} in the solution was determined after adsorption at different adsorbent concentrations, and the adsorption capacity and adsorption efficiency were calculated.

2.9.4 Adsorption kinetics

The adsorption experiments were performed in a series of 250 mL conical flasks. The adsorption conditions were as follows: initial 80 mg/L Cr^{6+} concentration of 100 mL, adsorption dose of 1.0 g/L, pH 3.0, and temperature of 30°C. After shaking, samples were obtained regularly and centrifuged to determine the concentration of Cr^{6+} in the supernatant.

2.9.5 Adsorption isotherms

The adsorption isothermal experiment was performed in a 250 mL conical flask of 100 mL Cr^{6+} solution. The adsorption dose was 1.0 g/L; pH was 3.0; temperature was 30°C; and initial ion concentration was 0, 10, 40, 80, 100 mg/L. After shaking adsorption for 3 h, the supernatant was centrifuged and separated to determine the concentration of Cr^{6+} in the supernatant.

2.10 X-ray diffraction (XRD) and X-ray photoelectron spectroscopy (XPS)

The change in the characteristic energy spectrum of the adsorbent before and after the adsorption of Cr^{6+} was analyzed by XRD and XPS to study its adsorption mechanism. An X-ray powder diffractometer (Japanese Dmax-rA; wavelength = 1.54 Å, Cu K α radiation) was employed to examine the crystalline structure of the sample. The XPS measurements were performed using a PHI5700 ESCA spectrometer with monochromated Al K α radiation ($h\nu$ = 1486.6 eV).

Results And Discussion

3.1 Microalgae immobilization with different carriers

3.1.1 Morphological observation and SEM before and after microalgae immobilization with different carriers

SEM was used to observe the morphological characteristics of microalgae before and after immobilization with six different carriers. As shown in Fig. 1, compared with the control group, microalgae particles were clearly attached or embedded on the surface of or inside the carriers, with the exception of carrageenan and polyvinyl alcohol. This indicated that these carriers could achieve microalgae immobilization. In addition, as indicated by Fig. 1 (a-1) and (e-1), numerous microalgae particles were observed when sodium alginate and quartz sand were used as the immobilization carriers. As shown in Fig. 1 (d-1) and (g-1), a small amount of microalgae was attached to the surface of chitosan and silica when these were used as immobilization carriers. Fig. 1 (c) and (c-1) show that the morphology of carrageenan changed substantially before and after microalgae immobilization, and irregular particle aggregates appeared. Fig. 1 (f) and (f-1) show that there were no evident microalgae particles before and after the immobilization of microalgae using polyvinyl alcohol (PVA) as a carrier. This indicated that different carriers exerted different immobilization effects on *Synechocystis* sp. PCC6803 and that the degree of immobilization of *Synechocystis* sp. PCC6803 via adsorption or entrapment differed.

Despite the biological adsorbents being immobilized with different carriers (Fig. 2), the main color of the immobilized microalgae was green. Additionally, the microalgae were embedded or adsorbed on the carriers.

3.1.2 Adsorption effect of different biosorbents

To study the effect of immobilization on the adsorption behavior of *Synechocystis* sp. PCC6803, the adsorption effect of immobilized biosorbents with different carriers was evaluated, as shown in Fig. 3. The results indicated that the adsorption capacity of different biological adsorbents were in the following order: chitosan (37.1 mg g^{-1}) > polyvinyl alcohol (24.2 mg g^{-1}) > carrageenan (19.7 mg g^{-1}) > *Synechocystis* sp. PCC6803 > diatomite (mg g^{-1}) > sodium alginate (7.6 mg g^{-1}) > quartz sand (6.2 mg g^{-1}). The adsorption capacity of Cr^{6+} adsorbents immobilized by chitosan, polyvinyl alcohol, and carrageenan were substantially higher than that of *Synechocystis* sp. PCC6803 alone. This indicated that the adsorption capacity of *Synechocystis* sp. PCC6803 can be improved after immobilization. In contrast, the adsorption capacity of adsorbents immobilized by diatomite, sodium alginate and quartz sand were substantially lower than that of *Synechocystis* sp. PCC6803 alone. This indicated that the adsorption capacity of microalgae was inhibited after immobilization and that the immobilization with different carriers has exerted effects on the adsorption behavior of *Synechocystis* sp. PCC6803. In addition, different carriers also had adsorption effect on Cr^{6+} ; however, the overall adsorption capacity was significantly lower than that of individual microalgae and their immobilized biological adsorbents.

The experimental results of Cu(II) adsorption by different biological adsorbents were shown in Fig. 4. The adsorption capacities of different biological adsorbents were in the following order: chitosan (25.98 mg g^{-1}) > *Synechocystis* sp. PCC6803 > sodium alginate (18.23 mg g^{-1}) > polyvinyl alcohol (18.21 mg g^{-1}) > carrageenan (8.43 mg g^{-1}) > diatomite (4.78 mg g^{-1}) > quartz sand (3.98 mg g^{-1}). The results showed that the adsorption capacities of other carriers for Cu(II) was lower than that of *Synechocystis* sp. PCC6803, excluding chitosan.

The experimental results of Pb(II) adsorption by different biological adsorbents were shown in Fig. 5. The comparison of adsorption capacities of different biological adsorbents was in the following order: carrageenan (25.67 mg g^{-1}) > chitosan (25.06 mg g^{-1}) > *Synechocystis* sp. PCC6803 > sodium alginate (19.21 mg g^{-1}) > diatomite (15.11 mg g^{-1}) > polyvinyl alcohol (13.67 mg g^{-1}) > quartz sand (8.56 mg g^{-1}). This showed that the adsorption capacities of other carriers for Pb(II) was lower than that of *Synechocystis* sp. PCC6803, except for chitosan and carrageenan.

The experimental results of Cd(II) adsorption by different biological adsorbents were shown in Fig. 6. The adsorption capacities of different biological adsorbents were in the following order: chitosan (24.62 mg g^{-1}) > *Synechocystis* sp. PCC6803 > sodium alginate (20.62 mg g^{-1}) > carrageenan (12.75 mg g^{-1}) > polyvinyl alcohol (4.72 mg g^{-1}) > quartz sand (3.6 mg g^{-1}) > diatomite (2.62 mg g^{-1}). This showed that the adsorption capacity of other carriers for Cd(II) was lower than that of *Synechocystis* sp. PCC6803, with the exception of chitosan.

Through a comparative study on the adsorption effects of Cr(VI), Cu(II), Pb(II), and Cd(II) before and after immobilization of microalgae with different carriers, it was evident that the effects of six carriers on the adsorption capacity of microalgae were completely different. Compared with *Synechocystis* sp. PCC6803, the adsorption effect of chitosan on the four ions was significantly improved. Meanwhile, using sodium alginate, carrageenan, polyvinyl alcohol, quartz sand, and diatomite as carriers did not increase or even decrease the adsorption capacity of microalgae, indicating that chitosan was a suitable carrier for the immobilization of *Synechocystis* sp. PCC6803.

The adsorption experiment indicated that the effects of different carriers on the adsorption behavior of *Synechocystis* sp. PCC6803 were different. Compared with *Synechocystis* sp. PCC6803 alone, immobilized microalgae with chitosan exhibited good adsorption performance for Cr(VI), Pb(II), Cu(II) and Cd(II); this indicated that a suitable immobilization carrier afford a good immobilization effect and did not affect adsorption properties. Additionally, chitosan was beneficial for immobilizing the harvesting and adsorption behavior of *Synechocystis* sp. PCC6803. Due to the immobilization of microalgae via the adsorption-cross-linking agent method, in which microalgae are immobilized using a carrier with a chemical bond, the stability of the carrier can be improved. The immobilization of diatomite and quartz sand to microalgae through weak interactions, such as hydrogen bonds and van der Waals forces, was not sufficiently strong, resulting in microalgal shedding during the cleaning process after immobilization; therefore, the adsorption capacity did not increase. Although sodium alginate, carrageenan, and polyvinyl alcohol can effectively immobilize microalgae, they do not significantly improve adsorption. This is likely because Cr(VI), Pb(II), Cu(II), and Cd(II) could not easily reach the surface of the microalgae within a short period of time after the immobilization of microalgae. In addition, the biosorbent formed by carrageenan can dissolve and thus, it does not influence the immobilization of microalgae for longer periods. Ying [54] reported the Cd(II) adsorption of immobilized microalgae with biochar, which achieved better biomass recovery and adsorption performance compared to individual microalgae and biochar.

3.1.3 Desorption effect of adsorbents in different desorption solutions

Fig. 7 showed the desorption efficiency of biological adsorbents immobilized with different carriers were different for the four desorption solutions. The results showed that the desorption efficiency of biological adsorbents were different in four desorption solutions. The desorption efficiencies of different biological adsorbents in the same desorption solution were also different. The specific desorption effects were listed in Table 1. The desorption efficiencies in the solution comprising 0.5 mol/L NaOH were as follows: chitosan > PVA > sodium alginate > carrageenan > quartz sand > diatomite. The desorption efficiencies in the solution comprising 0.5 mol/L HCl were as follows: carrageenan > diatomite > PVA > quartz sand > chitosan > sodium alginate. The desorption efficiencies of the different biological adsorbents in the solution comprising 0.5 mol/L NaCl were as follows: quartz sand > PVA > carrageenan > chitosan > diatomite > sodium alginate. Finally, the desorption efficiencies in the solution comprising 0.5 mol/L EDTA were as follows: sodium alginate > chitosan > quartz sand > diatomite > PVA > carrageenan.

Table 1 Desorption efficiency of adsorbents in different desorption solutions

adsorbents	%		0.5 mol/L NaOH		0.5 mol/L HCl		0.5 mol/L NaCl		0.5 mol/L EDTA	
	-	+	-	+	-	+	-	+	-	+
a	24.12	13.89	15.8	6.17	8.75	15.00	12.54	4.86	29.51	3.75
b	5.11	9.50	28.40	46.60	15.01	1.60	8.96	0.57	17.08	37.00
c	14.62	19.74	12.70	13.15	3.75	2.51	15.82	28.97	1.67	3.75
d	18.25	69.40	14.12	90.01	4.12	1.67	21.26	5.78	4.58	3.87
e	8.61	1.36	57.60	41.11	12.08	16.25	26.67	14.17	7.91	1.42
f	42.40	35.80	10.67	75.01	10.67	3.75	8.06	15.18	12.08	3.50
Microalgae	10.3		70.00		3.75		5.83		16.70	

3.1.4 FTIR analysis

To study the composition and changes of functional groups before and after immobilization of *Synechocystis* sp. PCC6803 with different carriers, FTIR was employed to analyze the infrared spectra of different biological adsorbents. Fig. S1 showed the changes of the infrared spectrum before and after the adsorption of *Synechocystis* sp. PCC6803. There was a broad peak at 3299.61 cm^{-1} , which is likely due to the coupling of the N-H stretching vibration and the O-H stretching vibration in the cell polysaccharides and proteins, which broaden the multiple absorption peaks (Ozturk et al. 2010). The absorption peak at 2928.30 cm^{-1} was caused by the anti-symmetrical stretching vibration of the C-H in the protein and carbohydrates on the cell wall. The absorption peak at 1644.23 cm^{-1} can be attributed to the amide I(CO-NH) C=O stretching vibration of the alkyl group in the protein and polysaccharide on the cell wall and the deformation vibration of N-H(Saavedra et al. 2018). The peak at 1450.91 cm^{-1} was due to the bending vibration of CH(Tüzün et al. 2005), and the peak at 1035.66 cm^{-1} was caused by the stretching vibration of P-OH(Pouya & Behnam 2017). Based on the infrared spectrum line (curve b) of *Synechocystis* sp. PCC6803 after adsorption of Cr^{6+} , the peak shape of the adsorbed spectrum remained mostly unchanged, as compared with that before adsorption. The absorption peaks at 3329.61 , 2928.30 , 1164.23 , 1450.91 , and 1035.66 cm^{-1} shifted to 3278.29 , 2927.66 , 1655.83 , 1451.24 , 1033.80 cm^{-1} respectively; this indicated that the structure of the adsorbents was not destroyed after adsorption of heavy metal ions. In other words, no new chemical substances were produced after adsorption. Furthermore, NH, OH, CH, C=O, CO, CN, and other groups may participate in the adsorption.

Fig. 8 showed the infrared spectra of biosorbents immobilized with different carriers after adsorbing Cr^{6+} . Sodium alginate exhibited a peak at 1060.81 cm^{-1} (C-O-C) after the immobilization of microalgae. Chitosan and quartz sand did not have any new characteristic peaks; however, the peaks shifted to varying degrees. Carrageenan exhibited a peak at 1143.74 cm^{-1} after immobilization. The characteristic peak at 3290 cm^{-1} disappeared in diatomite, and the peak of polyvinyl alcohol disappeared at 1224.75

cm⁻¹. After the adsorption of Cr⁶⁺, the characteristic peaks of different biological adsorbents shifted to different degrees. Groups belonging to the microalgae were detected after the microalgae in different carriers, indicating that the microalgae were immobilized with the carriers. Before and after adsorption, the peak shapes of different biosorbents did not change significantly, and different characteristic peaks shifted to different degrees, indicating participation in the ion adsorption process. The changes in functional groups before and after the immobilization of microalgae in different biological adsorbents and during Cr(VI) adsorption showed that all the functional groups of microalgae appeared after the microalgae were immobilized with carriers. For sodium alginate and carrageenan (Table 2), a peak at 1100 cm⁻¹ (C-O-C) was observed after immobilization; this peak was unique to the microalgae. For quartz sand, there was no algae-specific peak (3290 cm⁻¹, -OH) after the adsorption and immobilization of microalgae. This is likely due to the poor stability of the adsorption and immobilization of microalgae, which caused the microalgae to fall off after immobilizing and washing. Therefore, the hydroxyl peak belonging to the microalgae was not detected. After the adsorption of Cr(VI), the peak values of functional groups shifted in different biological adsorbents, indicating that the groups represented by the peaks (N-H, O-H, and COOH) may be involved in the adsorption process.

Table 2 FTIR spectra of before and after adsorption of different adsorbents

Carrier type	Functional groups					
	-OH	CH ₂	N-H		P-OH	
Microalgae	3299.61	2928.30	1541.40	1244.09	1035.66	/
Microalgae(Cr ⁶⁺)	3278.29	2927.64	1539.89	1243.01	1033.80	/
Alginate	3309.73	2995.34	1515.14	1238.85	/	966.30
Alginate(Cr ⁶⁺)	3305.87	2985.70	1500.56	1220.89	1066.59	914.23
Immobilized	3290.44	2972.20	1515.99	1226.68	1060.81	921.94
Immobilized(Cr ⁶⁺)	3325.16	2966.41	1506.35	1228.61	1066.59	908.44
chitosan	/	2981.84	1531.43	1284.55	1043.45	912.29
Chitosan(Cr ⁶⁺)	/	2976.06	1519.85	1230.54	1134.10	925.7
Immobilized	/	2983.77	1504.42	1274.42	1047.31	904.58
Immobilized(Cr ⁶⁺)	/	2987.63	1496.71	1224.75	1055.02	908.44
carrageenan	2995.34	/	1539.14	1359.77	/	/
Carrageenan(Cr ⁶⁺)	3008.85	/	1521.78	1326.78	1193.89	950.87
Immobilized	3022.35	/	1533.35	1338.98	1143.74	954.73
Immobilized(Cr ⁶⁺)	3018.49	/	1515.99	1328.98	1188.11	962.45
silica sand	3290.41	/	1535.16	1220.79	/	833.22
silica sand(Cr ⁶⁺)	3272.09	/	1533.15	1226.68	/	833.22
Immobilized	/	/	1554.57	1049.24	/	832.58
Immobilized(Cr ⁶⁺)	/	/	1546.86	1365.55	/	827.43
PVA	3006.92	/	1525.64	1224.75	/	954.73
PVA(Cr ⁶⁺)	/	/	1544.93	/	/	974.02
Immobilized	/	/	1539.14	/	/	956.66
Immobilized(Cr ⁶⁺)	2999.20	/	1529.49	1274.90	/	962.45

3.1.5 Raman spectral analysis of different biological adsorbents.

Both Raman spectra and FTIR have their own advantages and disadvantages, and the chemical structure information obtained from Raman and infrared spectra is complementary. To explore the changes in

functional groups of different biological carriers before and after immobilization of microalgae and before and after the adsorption of Cr^{6+} , Raman spectra of different biological adsorbents were analyzed, as shown in Fig. 9. The spectral peaks changed by different degrees before and after microalgae immobilization in different carriers (Maquelin et al. 2002). After the six carriers were immobilized with microalgae, the shape and peak of the spectrum changed to different degrees. The microalgae were immobilized by sodium alginate, chitosan, and carrageenan. The peak shape did not change significantly; however, the absorption intensity increased, indicating that the carriers did not alter the type of functional groups. When using diatomite, quartz sand and polyvinyl alcohol act as carriers to immobilize microalgae, the absorption peak of the microalgae's carbohydrate functional group ($\sim 1006\text{cm}^{-1}$) increased significantly after the adsorption of Cr^{6+} . Furthermore, the corresponding peak changed significantly, indicating that it played an important role in the adsorption process.

3.1.6 Reusability of different biological adsorbents

Remarkable reusability is another index for evaluating the adsorption performance of adsorbents. Five consecutive adsorption-desorption cycles were employed to verify the reusability of different adsorbents such as sodium alginate, polyvinyl alcohol, diatomite, chitosan, quartz sand, and carrageenan. First, under constant conditions (298K, pH 5.0, and 150 rpm), 1.0 g of biosorbent was added and shaken for 3 h at 80 mg/L Cr^{6+} . Thereafter, the separated supernatant was used to determine the concentration of metal ions. After adding the adsorbent after separation of the supernatant to the desorption solution of 50 mL for 3 h, the regenerated biosorbent was rinsed to approximately pH 7.0, with distilled water and dried in a vacuum drying oven for the next adsorption cycle. All experiments were performed in triplicate, and the average value was calculated. The adsorption efficiency was calculated according to the following formula:

$$\text{Adsorption efficiency} = (C_0 - C_t) / C_0 * 100\%.$$

Where C_0 is the initial solution ion concentration, and C_t is the solution ion concentration at time t . From the perspective of applicability, the reusability of adsorbents is important for reducing operating costs. Therefore, the reusability of different biological adsorbents was evaluated. As shown in Fig. 10, after five adsorption-desorption cycles, excluding chitosan (approximately 70%), the adsorption efficiency of other adsorbents decreased with an increase in the recycling times. The results revealed that chitosan can be used as a reused environment-friendly adsorbent for wastewater purification after the immobilization of microalgae.

During the immobilization of microalgae cells using different carriers (chitosan, sodium alginate, carrageenan, diatomite, quartz sand, and polyvinyl alcohol), based on the morphological observations, SEM, the Cr^{6+} adsorption effect, the desorption effect, functional group compositions, and comparative studies on reusability, a biological adsorbent was formed when chitosan was employed as the carrier. Chitosan exhibited a higher microalga-immobilized efficiency and adsorption efficiency; therefore, it shows significant potential for application in water treatment.

3.2 Optimization of synthesis parameters of chitosan

To enhance the immobilization and adsorption effect, the immobilization parameters of chitosan were optimized. The experimental results were shown in Table S1. Through the orthogonal experiment of the three factors and levels on the ratio of chitosan to acetic acid, microalgae concentration, and immobilization time, it can be seen that the former was the main factor affecting the immobilization effect. The experimental results showed that the optimal synthesis parameters were chitosan: acetic acid (2.0:40), microalgae concentration (10^8 cells/mL), and immobilization time (18 h). These parameters were used to synthesize biosorbent in further experiments.

3.3 Adsorption experiments

3.3.1 Effect of pH

pH can affect the presence of ions and adsorbents in the solution. In order to explore the optimal pH of the biosorbent, a Cr^{6+} adsorption experiment was performed at pH 2.0-8.0. The results were shown in Fig. 11; as the pH increased, the removal efficiency first increased and then decreased, and the optimal pH was 7.0. This is likely because the surface groups of the adsorbent were protonated at a lower pH and the main form of Cr^{6+} in the solution was HCrO_4^- , which promoted the adsorption effect owing to electrostatic attraction. Additionally, with a further increase in pH, the surface groups of the adsorbent were deprotonated, and electrostatic repulsion reduced the adsorption efficiency.

The removal of heavy metals depends on the pH and chemical properties of the heavy metals in the solution and the zeta potential of the adsorbent. The zeta potential of the biosorbent under different attached values were shown in Fig. 11. The pHzpc (pH at zero point charge) was 6-7, and the removal rate of Cr^{6+} was somewhat high under acidic pH, reaching approximately 97% at pH 5. With an increase of pH, the removal rate of Cr^{6+} decreased gradually. When the pH of the solution was less than the pHzpc, the surface of the adsorbent was positively charged, and there was notable electrostatic attraction between the positively charged surface and the anionic Cr^{6+} . This was conducive to the adsorption of Cr^{6+} (Kera et al. 2017, Shi & Peng 2015), because Cr^{6+} existed as different anions in the solution. With an increase of pH, the surface with negative charge was disadvantageous to Cr^{6+} due to electrostatic repulsion. In addition, under alkaline pH conditions, the protons must compete with OH^- for available adsorption sites on the adsorbent surface or in the intermediate space, which also hindered the adsorption of Cr^{6+} .

3.3.2 Effect of temperature

Adsorption of adsorbent to adsorbent is a competition between adsorption and desorption. The influence of temperature on adsorption mainly includes two aspects: adsorption, an exothermic reaction that is advantageous at low temperature, and desorption, an endothermic reaction that is advantageous at high temperatures. During the exothermic "adsorption process," the greater the heat of adsorption, the greater is the influence of temperature on adsorption. However, temperature affects the solubility of the substance;

hence, it also affects adsorption. The adsorption efficiency of the biological adsorbent to Cr^{6+} at different temperatures was shown in Fig. 12. It is evident that the adsorption of Cr^{6+} on chitosan first increased and then decreased with increasing of temperature. This was because temperature affected the molecular Brownian motion, due to which the temperature of the solution increased, and the thermal motion of the molecules intensified; this led to the destruction of the adsorption balance and a decreased in adsorption capacity.

3.3.3 Effect of adsorbent dose

The adsorbent dose is one of the main factors influencing adsorption. The adsorption efficiency of Cr^{6+} on the biosorbent at the same temperature was shown in Fig. 13. As the adsorbent dosage was increased from 0.05 g to 1.5 g, the removal efficiency of Cr^{6+} in the solution increased rapidly from 46.48% to 92.54%. This was because, for an increase in the adsorption dose, more adsorption active sites were available; this rapidly increased the removal efficiency of Cr^{6+} . When the adsorption dose was greater than 0.08 g, the removal efficiency of Cr^{6+} in the solution was only slightly slow with an increase in adsorbent dosage. At this time, almost all Cr^{6+} in the solution reacted with the active sites on the adsorbent surface, and there were few residual Cr^{6+} ions in the solution. Therefore, an increase in adsorbent dose does not cause significant increase of Cr^{6+} removal efficiency. Therefore, the dosage of adsorbent was 1.5 g.

3.3.4 Effect of initial ion concentration

The effect of different initial ion concentrations on the adsorption effect was shown in Fig. 14. When the initial Cr^{6+} concentration was 40 mg/L, the removal efficiency of Cr^{6+} by the biosorbent was 96.22%. The initial mass concentration of tannic acid increased from 40 mg/L to 80 mg/L, and the removal efficiency of Cr^{6+} by the biosorbent gradually decreased to 27.63%. This was because, when the concentration of Cr^{6+} changed, the number of active sites that can bind to Cr^{6+} in the solution remains fixed. When the concentration of the solution was low, the active sites on the surface of the adsorbent were not completely occupied; hence, the adsorption efficiency was relatively high. With an increase in mass concentration, there were no excess active sites on the surface of the adsorbent to combine with Cr^{6+} ; therefore, the adsorption efficiency decreased significantly. Therefore, the initial concentration of Cr^{6+} should be 40 mg/L.

3.4.1 Adsorption kinetics

In order to design an efficient adsorption system and explore the main speed limiting steps of solute adsorption on the adsorbent surface, the pseudo-first-order kinetic equation and the pseudo-second-order kinetic equation were frequently used to fit the kinetic test data. The equations of pseudo-first-order dynamics and pseudo-second-order dynamics are calculated using Eq. (3) and (4).

$$\ln(q_e - q_t) = \ln q_e - k_1 t \quad (3)$$

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \quad (4)$$

Where q_e is the fitting value of equilibrium adsorption capacity (mg/g), q_t is the adsorption capacity at time t (mg/g), k_1 is the pseudo-first-order adsorption rate constant (min^{-1}), and k_2 is the pseudo-second-order adsorption rate constant ($\text{g.mg}^{-1}\text{min}^{-1}$).

Table 3 and Fig. 15 show that the value of R^2 in the pseudo-second-order kinetic equation of the adsorbent was greater than 0.99. This was better than the fitting result of the pseudo-first-order kinetic equation, indicating that Cr^{6+} was on the surface of the adsorbent. Chemical adsorption was dominant, especially ion exchange adsorption (Zargoosh et al. 2014). Based on a comparison of the measured q_e and the fitted q_e , it can be seen that the fitting result of the pseudo-second-order kinetic equation differed slightly from the measured value, which further confirmed that chemical adsorption was the dominant process (Tang et al. 2014).

Table 3 Comparison of rate constants calculated based on pseudo-first-order, pseudo-second order model

Heavy metal	Pseudo first order fit			Pseudo second order fit		
	q_e (mg g ⁻¹)	K_1	R^2	q_e (mg g ⁻¹)	K_2	R^2
Immobilized	39.828	0.027	0.992	49.6	0.0005	0.993
Microalgae	17.908	0.016	0.997	24.5	0.0005	0.998
Chitosan	5.536	0.025	0.972	6.786	0.0039	0.996

3.4.2 Adsorption isotherm

The adsorption isotherm is an important criterion for the design of the adsorption process; it was used to describe the equilibrium relationship between chitosan and adsorbate, affinity, and adsorption performance of the adsorbent. Langmuir and Freundlich equations are often used to fit the experimental data of the isothermal adsorption of solutes on the surface of adsorbents; they are shown in Eq. (5) and (6), respectively.

$$q_e = \frac{q_m k_L c_e}{1 + k_L c_e} \quad (5)$$

$$q_e = K_F C_e^{1/n} \quad (6)$$

where q_e is the equilibrium adsorption capacity (mg/g), q_m is the maximum adsorption capacity (mg/g), K_L is the adsorption constant (L/mg), and K_F and n are the Freundlich adsorption constants. Table 4 and Fig. 16 demonstrated that the correlation coefficient R^2 of the Langmuir and Freundlich isotherm equations fit with the experimental data was greater than 0.93. This indicated that the two isotherm adsorption equations can better fit the experimental data of isothermal adsorption. The adsorption principle of adsorbent surfaces was complex, including both single molecular layer adsorption and multi-layer adsorption. It was generally believed that, in the Freundlich process, $n < 0.5$ indicated that adsorption is difficult, $n = 1$ indicated linear adsorption, and $2 < n < 10$ indicated that adsorption occurs easily. The adsorption constant was greater than 2, which meant that Cr^{6+} was easily adsorbed by the adsorbent.

Table 4 The parameters for the Langmuir and Freundlich adsorption isotherm of Cr^{6+}

Heavy metal	Langmuir isotherm			Freundlich isotherm		
	q_e (mg g ⁻¹)	K_L	R_L^2	K_F	n	R_F^2
Immobilized	67.026	0.0197	0.984	3.764	2.85	0.95
Microalgae	31.787	0.10	0.997	9.66	4.2	0.98
Chitosan	16.44	0.04	0.982	1.98	2.4	0.93

3.4.3 XRD analysis

In order to explore the main components of microalgae before and after the immobilization with chitosan, XRD analysis was preformed, and the results were shown in Fig. 17. After Cr^{6+} adsorption, the interlayer peak strength decreased significantly, indicating that the interlayer structure was stretched after Cr^{6+} ion exchange.

3.4.4 XPS analysis

The adsorption mechanism of Cr^{6+} on the adsorbent was further obtained using XPS analysis. The high-resolution spectra of Cr2p, C1s, N1s, and O1s regions were presented in Fig. 18. The main element composition of biosorbent was obtained via XPS scanning at 0-1200 eV. It can be seen that the three main peaks at 280.0 eV, 400 ev, and 531.5 eV represented C(70.7%), O(25.08) and N(4.22%), respectively. After the adsorption of Cr^{6+} , a new peak (585 eV) appeared; this was the photoelectron peak of Cr^{6+} and indicated that chromium was absorbed on the surface of adsorbent. The photoelectron peaks of chromium 2p3/2 and 2p1/2 were 588.5 eV and 578.2 eV (Fig. 18(b)), respectively, which are similar to the binding energies of Cr^{3+} containing materials (Yu et al. 2013). The data showed that some of the adsorbed Cr^{6+} ions were reduced to Cr^{3+} by the biosorbent. Further analysis showed that the Cr^{3+} compound formed on the surface of the adsorbent was $Cr(OH)_3$ and not Cr_2O_3 . This is because the Cr

2p_{3/2} and 2p_{1/2} spectra cannot fit the combination of Cr₂O₃, which indicated that Cr⁶⁺ removal includes reduction of Cr⁶⁺ to Cr³⁺ during adsorption.

The C1s spectra before and after adsorption were shown in Fig. 18(c); C-C (284.0 eV) bond and carbonyl C (287.1 eV) were clearly observed. After the adsorption of Cr ions, the percentage of groups also changed significantly (C-C decreased from 47.19% to 45.8%, whereas C=O increased from 9.75% to 17.92%), indicating that part of C-C participated in the reaction and transformed into C=O.

In the modified chitosan, the nitrogen atom mainly exists in the form of azomethyl (-C=N-) and primary amine (-NH₂). Because the electronegativity of the nitrogen atom in the -C=N- group was substantially higher than that of the -NH₂ group, the two binding energy peaks observed at 398.3 eV and 401.5 eV correspond to -C=N- and -NH₂, respectively. After the adsorption of Cr⁶⁺, the peaks of -C=N- and -NH₂ showed higher shifts due to the decrease of electron density. These results confirm that these groups were involved in the adsorption process of Cr ion.

As shown in Fig. 18(e), the O1s spectrum was divided into two peaks at 531.1 and 531.8 eV, corresponding to hydroxyl group (-OH) and carboxyl group (-COOH). After adsorption, the content of hydroxyl group (-OH) on the adsorbent increased from 27.85% to 69.32%, which may be due to the formation of new metal oxide bonds such as M-O (M=metal ion) bonds.

Conclusion

By comparing the immobilization of different carriers on microalgae, chitosan can achieve good immobilization effect and adsorption behavior. Under the optimal synthesis parameters (acetic acid (2:40), microalgae concentration (10⁸ cells/mL) and immobilization time (18 h)) and adsorption conditions, the adsorption efficiency can reach more than 98%, which had a broad industrial application prospect in the future.

Declarations

Author Contributions

Resources, L.S.; writing—original draft preparation, J.W. and R.C.; writing—review and editing, L.F., L.C., and Y.Z.; supervision, X.W. and J.L.; project administration, W.Z.; funding acquisition, L.S. All authors have read and agreed to the published version of the manuscript.

Availability of data and materials

All data and materials generated or analyzed during this study were included in this published article.

Compliance with ethical standards

Consent to participate Not applicable

Consent for publication Not applicable

Ethical approval Not applicable

Conflict of Interests The authors declare no conflict of interest.

Funding

This work was supported by the National Natural Science Foundation of China (No. 52074353), South China Key Laboratory of Applied Microbiology Open Fund Project (SKLAM006-2018), GDAS' Special Project of Science and Technology Development (2020GDASYL-20200402001), Fundamental Research Funds for the Central Universities of Central South University (2020zzts725), Hunan Provincial Innovation Foundation for Postgraduate (CX20200323).

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Figures

Figure 1

SEM of before and after immobilized microalgae with different carriers.((a), (b), (c), (d), (e), (f) and (g) were the SEM of control group carrier, chitosan, sodium alginate, carrageenan, diatomite, quartz sand, polyvinyl alcohol and silica; (a-1), (b-1), (c-1), (d-1), (e-1), (f-1) and (g-1) were the SEM of the corresponding chitosan, sodium alginate, carrageenan, diatomite, quartz sand, polyvinyl alcohol, silica immobilizing microalgae; (O) and (O-1) were the different magnification SEM of *Synechocystis* sp. PCC 6803)

Figure 2

Biological adsorbents on different carriers (A and A-1: sodium alginate, B: chitosan, C and c-1: carrageenan, D: diatomite, E: quartz sand, F: polyvinyl alcohol. The left side was the single carrier, and the right side was the immobilized microalgae.)

Figure 3

Adsorption capacity of Cr(VI) by six adsorbents ((a): sodium alginate, (b): chitosan, (c): carrageenan, (d): diatomite, (e): quartz sand, (f): polyvinyl alcohol)

Figure 4

Adsorption capacity of Cu(II) by different adsorbents ((a): sodium alginate, (b): chitosan, (c): carrageenan, (d): diatomite, (e): quartz sand, (f): polyvinyl alcohol)

Figure 5

Adsorption capacity of Pb(II) by different adsorbents ((a): sodium alginate, (b): chitosan, (c): carrageenan, (d): diatomite, (e): quartz sand, (f): polyvinyl alcohol)

Figure 6

Adsorption capacity of Cd(II) by different adsorbents ((a): sodium alginate, (b): chitosan, (c): carrageenan, (d): diatomite, (e): quartz sand, (f): polyvinyl alcohol)

Figure 7

Desorption efficiency of different adsorbents in different desorption solutions ((a): 0.5 mol/L NaOH, (b): 0.5 mol/L HCl, (c): 0.5 mol/L NaCl, (d): 0.5 mol/L EDTA)

Figure 8

FTIR spectra of the different adsorbents before and after adsorption of Cr^{6+} ((a), (b), (c), (d), (e), (f), (g)) were the carriers of sodium alginate, chitosan, carrageenan, diatomite, quartz sand,

Figure 9

Raman spectroscopy of the different adsorbents before and after adsorption of Cr^{6+} ((a), (b), (c), (d), (e), (f) were the carriers of sodium alginate, chitosan, carrageenan, diatomite, quartz sand, polyvinyl alcohol)

Figure 10

Effects of cycle number on Cr6+ adsorption of different adsorbents

Figure 11

Effect of pH on adsorption of Cr6+ onto adsorbent (right) and Zeta potential (left) as a function of biosorbent

Figure 12

Effect of temperature on the adsorption of Cr^{6+} by immobilized microalgae

Figure 13

Effect of adsorbent dose on the adsorption of Cr^{6+} by immobilized microalgae

Figure 14

Effect of initial concentration on the adsorption of Cr6+ by immobilized microalgae

Figure 15

Adsorption kinetic plots of Cr6+

Figure 16

The Langmuir and Freundlich isotherm for Cr⁶⁺ adsorption with the immobilized microalgae

Figure 17

XRD patterns of biosorbent before and after Cr⁶⁺ adsorption

Figure 18

XPS spectra for biosorbent before and after reacting with Cr(VI). (a) Survey scans the spectral region from 0 to 1400 eV; (b-e) high-resolution XPS Cr2p, C1s, N1s, O1s narrow scans as function of electron binding.

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