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RESEARCH ARTICLE

Activated sludge process enabling highly efficient removal of heavy metal in wastewater

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Abstract

Activated sludge process was a low-cost alternative method compared to the conventional physicochemical process for the treatment of heavy metal-containing wastewater. In the present study, the removal efficiency of Pb^{2+}, Cu^{2+}, and Ni^{2+} from wastewater by a sequencing batch reactor (SBR) activated sludge system was investigated, and the mechanism was revealed by static adsorption experiment of activated sludge. The results showed that the activated sludge in the SBR system was effective in removing Pb^{2+} and Cu^{2+} from wastewater at 10 mg·L^{-1} initial concentration, with a removal efficiency of 83.1 ~ 90.0% for Pb^{2+} and 74.3 ~ 80.6% for Cu^{2+}, respectively. However, the removal efficiency for Ni^{2+} was only 0 ~ 6.2%. Static adsorption experiments showed that the adsorption capacity of activated sludge for three heavy metals was shown as $Pb^{2+} > Cu^{2+} > Ni^{2+}$. When the initial concentration was 20 mg·L^{-1}, the equilibrium adsorption capacity of activated sludge for Pb^{2+}, Cu^{2+}, and Ni^{2+} was 18.35 mg·g^{-1}, 17.06 mg·g^{-1}, and 8.37 mg·g^{-1}, respectively. The main adsorption mechanisms for Pb^{2+} and Cu^{2+} were ligand exchange, electrostatic adsorption, and surface organic complexation processes, but Ni^{2+} removal mechanism mainly included electrostatic adsorption and surface organic complexation processes, showing that Ni^{2+} removal was inhibited in the presence of Pb^{2+} and Cu^{2+}. The physicochemical properties and microbial diversity of activated sludge were greatly affected by the heavy metals in the SBR system, and genus *Rhodobacter* was found to be dominant bacteria enabling resistance to heavy metal ions.

Keywords Environmental engineering · Activated sludge process · Heavy metal · Combined pollution · Sequencing batch reactor · Biosorption

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Highlights
- Pb^{2+} and Cu^{2+} can be efficiently removed by activated sludge in the SBR system.
- the removal of Ni^{2+} was inhibited in the presence of Pb^{2+} and Cu^{2+} in the SBR system.
- the hydration radius and ligand exchange were main reasons for heavy metal removal.
- Genus *Rhodobacter* was dominant in the heavy metal-containing SBR system.

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Introduction

Due to high toxicity, accumulation, and stability, heavy metals (Cu, Cd, Cr, Hg, Pb, As, etc.) not only harm the growth, metabolism, and reproduction of animals, plants, and microorganisms (Sall et al. 2020; Shuaib et al. 2021), but also can pollute water and land resources and further cause human's health and ecological security (Briffa et al. 2020; Gupta et al. 2012). The discharge of heavy metal-containing wastewater in developing countries was still very large and has induced many serious environmental problems (Li et al. 2022a, b). According to a global survey, concentration and type of heavy metals have increased significantly in water bodies, and the situation in developing country was more serious than developed country (Gupta et al. 2012). In China, a lot of water bodies, such as Taihu Lake and Baiyangdian Lake, have been polluted by heavy metals (Ding et al. 2019; Rajeshkumar et al. 2018; Rao et al. 2018; Rodríguez-Lado

et al. 2013; Yi et al. 2011). Therefore, heavy metal pollution has been widely concerned by environment researchers.

Heavy metal-containing industrial wastewater was considered to be the main source of heavy metal pollution. Studies have found that heavy metal-containing wastewater was mainly discharged from mining, metal processing, and electroplating industries (Yi et al. 2011; Zhou et al. 2020). In China, heavy metal-containing wastewater accounts for about 20% of the total wastewater (Li et al. 2022a, b), and therefore it is significant for the water environment protection and human health to treat them effectively.

Conventional wastewater treatment methods for heavy metal removal include physicochemical and biological methods (Fu and Wang 2011; Jacob et al. 2018). Compared with physicochemical methods, biological methods have many advantages such as low cost, easy operation, and can remove COD, nitrogen, and phosphorus synchronously and efficiently (Jacob et al. 2018). Biological methods mainly rely on the adsorption of activated sludge. Heavy metals can be adsorbed on the surface of activated sludge or enriched in microbial cells and removed from wastewater through discharging of excess sludge. The biological adsorption mechanism includes electrostatic adsorption, ligand exchange, surface complexation, and precipitation. Microorganisms such as bacteria, fungi, and algae in activated sludge have a large number of functional groups (e.g., phenols, alcohol hydroxides, and carboxylic acids), which can provide biosorption sites to adsorb heavy metals in wastewater (Barros et al. 2006; Eccles 1995; Fomina and Gadd 2014). In technical specification for electroplating industry wastewater treatment (HJ 2002–2010) in China, biological treatment method was recommended for the treatment of electroplating wastewater (Li et al. 2022a, b). Furthermore, the treatment of heavy metals in wastewater using activated sludge does not involve extra energy or chemical consumption, showing great potential for the application in treating heavy metal-containing wastewater (Matyja et al. 2021).

As common domestic sewage treatment technology, the activated sludge process can adsorb heavy metals by continuously producing activated sludge. However, previous studies paid more attention to heavy metal toxicity to activated sludge rather than removal of heavy metals. Liu et al. (2022) found that the number of Gram-negative bacteria in activated sludge increased obviously under the stress condition of heavy metals Pb, As, and Hg. Bhat et al. (2020) found that activated sludge had tolerance to heavy metals, but treatment efficiency was significantly reduced when the concentration of heavy metals was high. The report from You et al. (2009) indicated that heavy metal removal could be inhibited in the complex heavy metal-containing wastewater, and the inhibited degree was Ni > Cd > Pb. Some studies have confirmed the great potential of activated sludge process to remove heavy metals from wastewater. Laurent et al. (2009) found that activated sludge can remove Cu and Cd through proton exchange, ligand exchange, and precipitation. Pagnanelli et al. (2009) found that activated sludge systems had many carboxylic and amino groups which could adsorb Cd and Pb. Activated sludge pretreated with alkali was used to adsorb heavy metals, and the maximum adsorption capacity of Cu and Cd achieved 131.6 and 93.4 $mg \cdot g^{-1}$, respectively, while the adsorption capacity of Ni was low. However, these studies did not investigate the removal efficiency by activated sludge during long-term operation, and the researches on treatment efficiency and adsorption mechanism of composite heavy metal-containing wastewater were neglected. In the long-term operation process of activated sludge, heavy metals will change the microbial community structure of activated sludge and then have an impact on the removal effect of heavy metals (Zhang et al. 2022). Therefore, the exploration of these two aspects shows great value to the increasingly serious situation of compound heavy metal wastewater pollution.

In this study, the SBR was continuously operated for 123 days to investigate the long-term treatment effects of activated sludge process in removing three kinds of heavy metals Pb^{2+}, Cu^{2+}, and Ni^{2+} from wastewater. And the adsorption mechanism was revealed based on static adsorption experiment and microbial community analysis.

Materials and methods

SBR equipment

During the dynamic removal experiment, sequencing batch reactor (SBR) was selected as the form of activated sludge system, because it can well adapt to the characteristics of large fluctuation of the hydraulic load of industrial wastewater (Uygur and Kargı 2004). The reactor consisted of a cylindrical plexiglass tank with an effective volume of 6 L (Fig. 1). Five circular holes were set on the tank wall for aeration, water entry and exit, and sample collection. The peristaltic pump was used to regulate the water in and out. The activated sludge used in SBR and static adsorption experiments was taken from a pilot-scale domestic sewage treatment system in the Renmin University of China. The sludge concentration was around 3000 $mg \cdot L^{-1}$, and the sludge sedimentation ratio was 20 ~ 30%.

Long-term operation of SBR

The simulated wastewater was designed based on the quality of the actual heavy metal-containing industrial wastewater. Table 1 showed the operation conditions of the SBR activated sludge system. The operation phase was set as follows: filling (6 min)—reaction (9.9 h)—settling

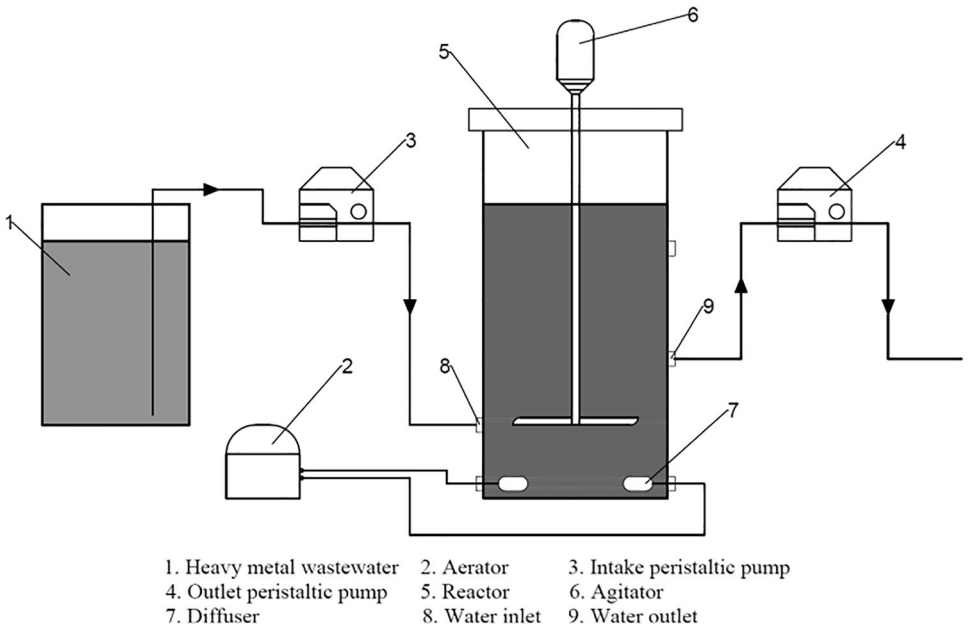

Fig. 1 Diagram of SBR equipment

Table 1 SBR operation parameter settings

Effective volume	Water exchange ratio	HRT	SRT	Operation cycle	DO	Stirring speed
6 L	0.5	24 h	8 ~ 12 d	12 h	> 2.0 mg/L at reaction stage	150 rpm

Table 2 Composition of heavy metal wastewater

Ingredient	Concentration ($mg \cdot L^{-1}$)	Chemical	Purity	Manufacturer
COD	800	Glucose	AR	Sinogram
NH_4^+-N	40	NH_4Cl	AR	Sinogram
TP	8	KH_2PO_4	AR	Sinogram
Pb^{2+}	0/3/5/10	$Pb(NO_3)_2$	AR	Macklin
Cu^{2+}	0/3/5/10	$Cu(NO_3)_2 \cdot 3H_2O$	AR	Macklin
Ni^{2+}	0/3/5/10	$Ni(NO_3)_2 \cdot 6H_2O$	AR	Macklin
pH	7.7 ± 0.2	-	-	-

(1 h)—discharging (6 min)—idling (54 min). Aeration took place only in the reaction stage.

The composition of the simulated wastewater containing heavy metals is shown in Table 2. The SBR dynamic removal experiment was divided into four stages: culture (0 $mg \cdot L^{-1}$), 3 $mg \cdot L^{-1}$, 5 $mg \cdot L^{-1}$, and 10 $mg \cdot L^{-1}$. During the culture stage, the influent had no heavy metals so that the activated sludge can adapt and reproduce. In latter three stages, the concentration of various heavy metals was 3 $mg \cdot L^{-1}$, 5 $mg \cdot L^{-1}$, and 10 $mg \cdot L^{-1}$, respectively.

Static adsorption experiment

The pH of Pb^{2+}, Cu^{2+}, and Ni^{2+} solution (20 $mg \cdot L^{-1}$) was adjusted to 6.0 using KOH and HCl. Each 25 mL of heavy metal-containing solution and 0.4 mL of dewatered activated sludge (by centrifuge) were added into 50-mL conical flasks. The activated sludge concentration was 1.0 ± 0.1 $g \cdot L^{-1}$. The mixture was then put in a shaking table to shake for 2 h at 200 r/min at 25 ℃. The sampling interval in the first 30 min was 3 min, and the sampling interval between 30 and 60 min was 10 min, and the sampling interval in the last 60 min was 15 min. Then 10 mL mixture was quickly collected, and activated sludge was separated by high-speed refrigerated centrifugation (4℃, 8000 rpm, 5 min), and the concentrations of Pb^{2+}, Cu^{2+}, and Ni^{2+} in the supernatant were measured using flame atomic absorption spectrophotometer (Z-2000). The pH, sludge concentration, adsorption time, heavy metal solution concentration, temperature, and coexisting heavy metal were adjusted to investigate the influence of different factors on the adsorption effect.

Analytical determination

The concentration of heavy metals was measured by atomic absorption spectroscopy (AAS) with flame atomic absorption spectrophotometer (Z-2000); chemical oxygen demand

(COD) was measured by rapid digestion spectrophotometry with Lianhua digestion instrument and multi-parameter water quality tester. Specific measurement methods of mixed liquid suspended solids (MLSS) and sludge volume index (SVI) were provided in Text S1.

To explore the adsorption mechanisms of activated sludge, the analyses including kinetic model fitting, isothermal adsorption model fitting, Fourier transform infrared spectroscopy (FT-IR), energy-dispersive x-ray spectroscopy (EDS), zeta potential analysis, scanning electron microscope (SEM), and 16S rDNA amplicon sequencing were carried out.

Results

Long-term treatment effect of heavy metal in the SBR system

Long-term treatment of heavy metals in the SBR system was divided into four stages including culture stage (0–4d), domestication stage I (3 mg·L^{-1}, 5–11 d), domestication stage II (5 mg·L^{-1}, 12–72 d), and domestication stage III (10 mg·L^{-1}, 73–123 d). The operation results are shown in Fig. 2. It was obvious that the removal rate for three kinds of heavy metals was the highest at low heavy metal concentration and gradually decreased with the increase of concentration. In the stable period of three operation stages (3 mg·L^{-1}, 5 mg·L^{-1}, and 10 mg·L^{-1}), the removal rate of Pb^{2+} achieved 100%, 91.4 ~ 96.2%, and 83.1 ~ 90.0%, respectively, the removal rate of Cu^{2+} was 87.7 ~ 90%, 81.8 ~ 85.6%, and 74.3 ~ 80.6%, respectively, and the removal rate of Ni^{2+} was 13.3 ~ 33.6%, 0.8 ~ 22.7%, and 0 ~ 6.2%, respectively. The removal efficiency of Pb^{2+} was the highest, followed by Cu^{2+} and Ni^{2+}. This result was similar to the results reported by Pagnanelli et al. (2009). This may be related to the high toxicity of Ni (You et al. 2009). Although COD removal rate, MLSS, and SVI decreased under the influence of heavy metals, they tended to be stable during the long-term operation process, indicating that the activated sludge system had adaptability and recovery capacity.

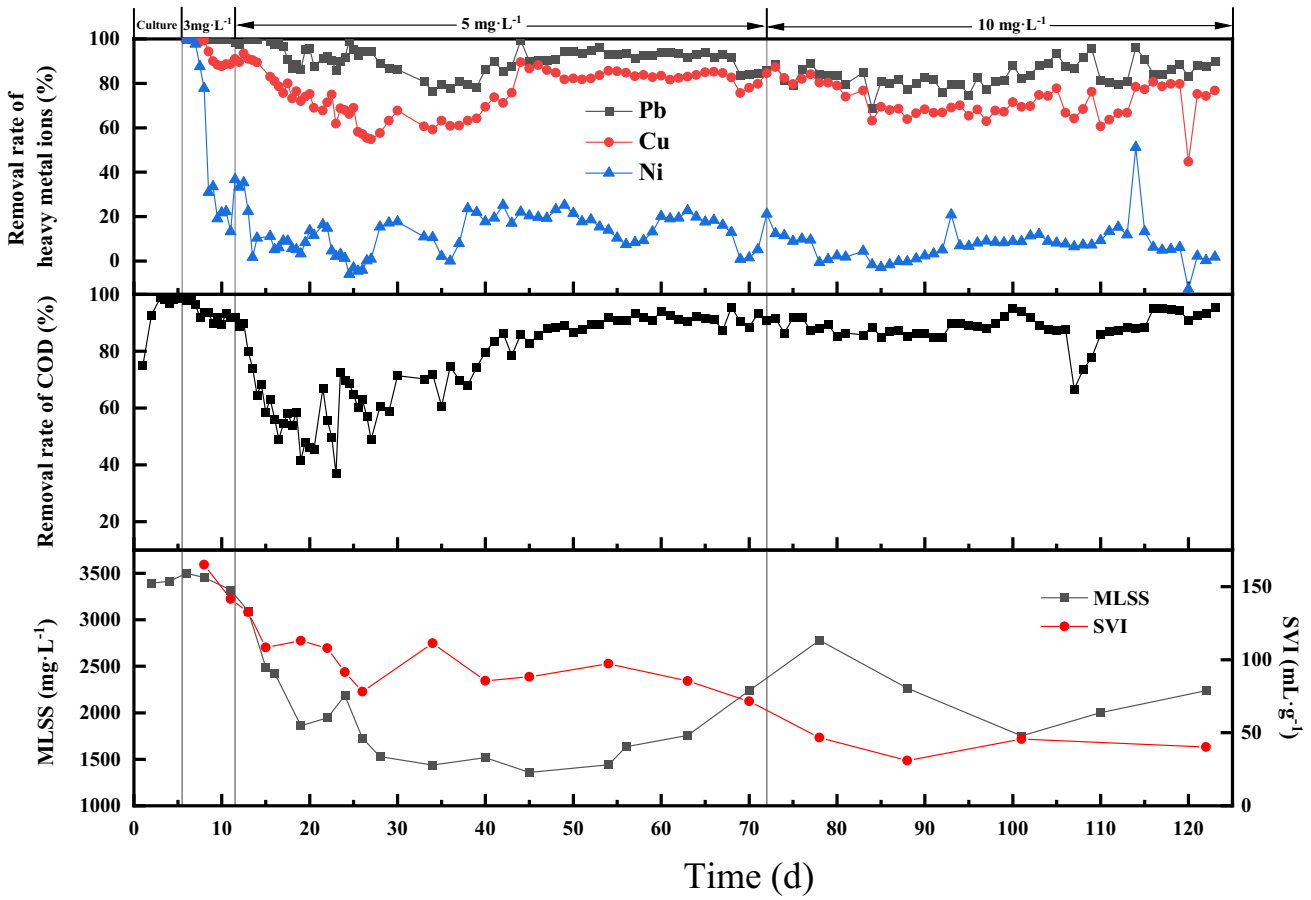

Fig. 2 Operation effect of the SBR activated sludge system

Static adsorption experiment

Adsorption effect of activated sludge on single heavy metals

As shown in Fig. 3, the removal efficiency of Pb^{2+} was the highest, followed by Cu^{2+} and Ni^{2+}. The adsorption process of Pb^{2+}, Cu^{2+}, and Ni^{2+} by activated sludge could be divided into rapid adsorption stage (0 ~ 4 min) and slow adsorption stage (4 ~ 120 min). The actual equilibrium adsorption capacities of activated sludge for Pb^{2+}, Cu^{2+}, and Ni^{2+} at initial concentration of 20 $mg{\cdot}L^{-1}$ were 18.55 $mg{\cdot}g^{-1}$, 17.27 $mg{\cdot}g^{-1}$, and 8.37 $mg{\cdot}g^{-1}$, respectively. As the initial heavy metal concentration increased from 5 to 200 $mg{\cdot}L^{-1}$, the adsorption capacity of Pb^{2+}, Cu^{2+}, and Ni^{2+} increased from 3.71 $mg{\cdot}g^{-1}$, 4.62 $mg{\cdot}g^{-1}$, and 4.65 $mg{\cdot}g^{-1}$ to 119.1 $mg{\cdot}g^{-1}$, 42.5 $mg{\cdot}g^{-1}$, and 44.5 $mg{\cdot}g^{-1}$, respectively. The increase gradually slowed down, and the removal rate of heavy metals decreased (Fig. S1). It was possible that the adsorption sites were saturated or the active adsorption of microorganisms was inhibited by the toxicity of heavy metals. With the increase of sludge concentration, the adsorption capacity decreased gradually, but the removal rate of three kinds of heavy metals showed an upward trend (Fig. S2). Increased adsorption dose led to increased adsorption sites and further led to increased removal rate of heavy metals. However, this also causes decreased adsorption capacity of unit adsorbent, suggesting that the trends in the adsorption capacities of activated sludge and removal rates of heavy metals were opposite. The activated sludge had the best removal effect on Pb^{2+}, followed by Cu^{2+}, while the removal effect on Ni^{2+} was poor. This result was consistent with the operation results in the SBR system.

Adsorption effect of activated sludge on composite heavy metals

As shown in Fig. 4, the Pb^{2+} removal capacity of activated sludge was almost unaffected by the existence of Cu^{2+} and Ni^{2+} (Fig. 4a). The adsorbed Cu^{2+} capacity by activated sludge was mainly affected by Pb^{2+}, showing a decrease of 16.6% (Fig. 4b). However, the Ni^{2+} removal capacity of activated sludge was greatly inhibited by composite Pb^{2+} and Cu^{2+}, which caused a sharp decline of Ni^{2+} removal rate by 80.3% (Fig. 4c). In addition, it can be found from the adsorption effect experiments that Cu^{2+} was a main inhibition factor that caused the decrease in Ni^{2+} adsorption capacity by activated sludge. In the composite wastewater, there was competitive adsorption between different heavy metals and activated sludge, which might be closely related to the charge, atomic weight, atomic size of heavy metals, and their affinity to functional groups on the surface of activated sludge (Zhang et al. 2022).

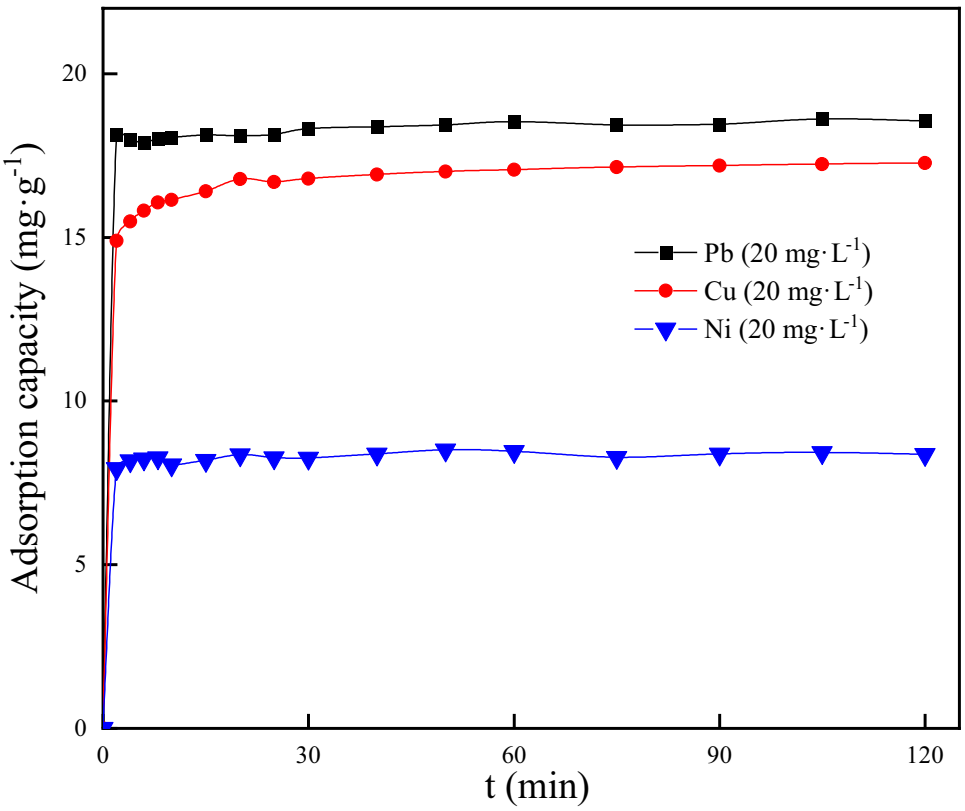

Fig. 3 Effect of adsorption time on heavy metal removal by activated sludge

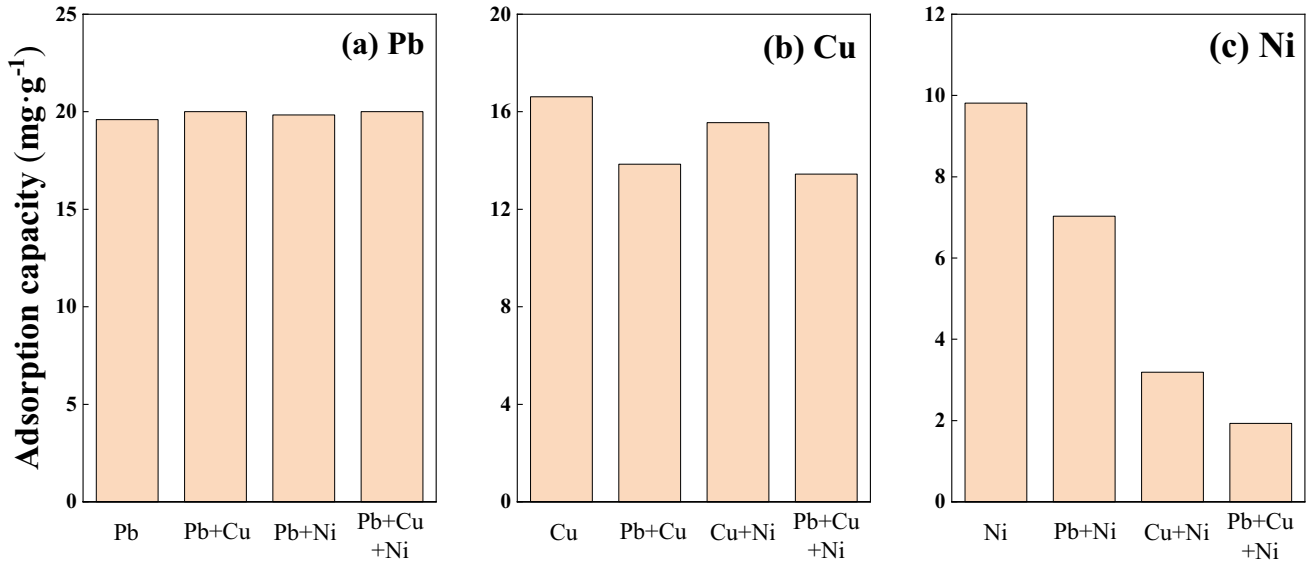

Fig. 4 Adsorption effect of heavy metals by activated sludge in different systems

Effects of initial pH on heavy metal adsorption

With increasing pH value, the adsorption capacity of heavy metals generally increased (Fig. 5). The pH range with the best adsorption effect was from 4.0 to7.0, and the maximum adsorption capacities of Pb, Cu, and Ni were 19.37 mg·g^{-1} (at pH 4.0), 18.27 mg·g^{-1} (at pH 6.0), and 8.76 mg·g^{-1} (at pH 6.0), respectively. The increased pH reduced the zeta potential of the activated sludge (Fig. S3). Because activated sludge surface was negatively charged, under the existence of electrostatic attraction, activated sludge showed high efficiency to adsorbing positively charged heavy metal

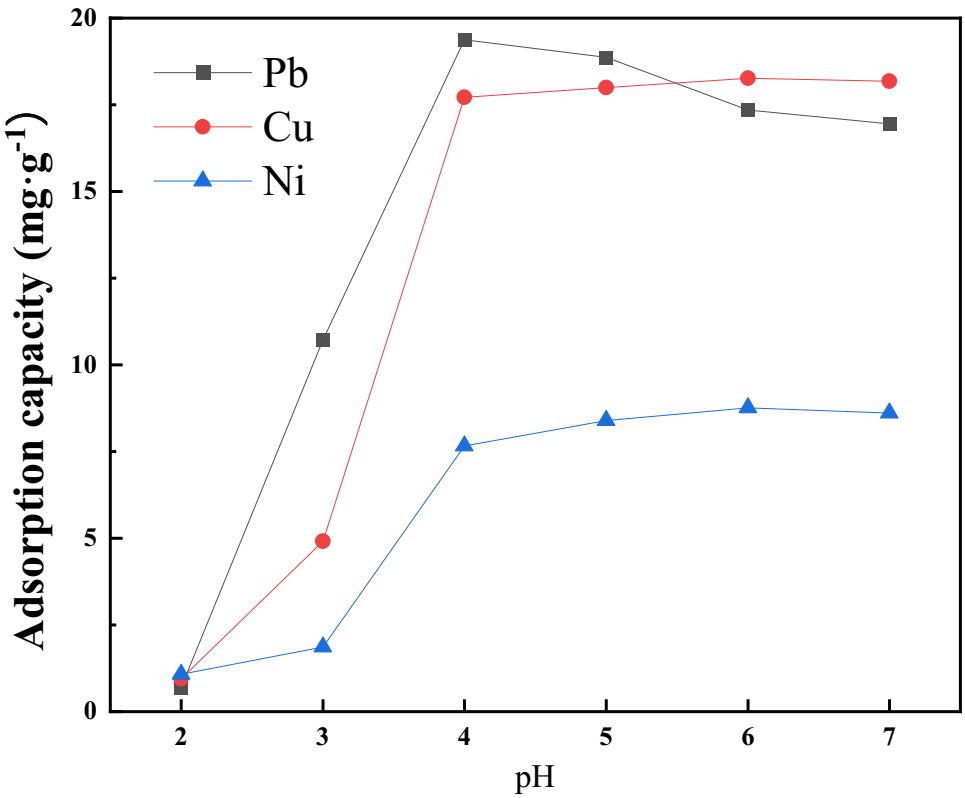

Fig. 5 Effect of pH on adsorption of heavy metal ions by activated sludge

(Morfesis et al. 2009). In acidic environment, excessive hydrogen ions will compete with heavy metals to adsorb on the surface of activated sludge, and acidic environment can also lead to the desorption of heavy metals adsorbed through ligand exchange, which can inhibit the adsorption of heavy metals (Wei et al. 2021).

Adsorption kinetic of heavy metals by activated sludge

The pseudo-first-order and pseudo-second-order models were used to fit the kinetic of the adsorption process of Pb^{2+}, Cu^{2+}, and Ni^{2+} using activated sludge (Wei et al. 2021; Xu et al. 2022). The fitting results are shown in Table 3.

The R^2 of each type of heavy metal fitted by the pseudo-second-order kinetic equation was higher than that fitted by the pseudo-first-order equation. In addition, the theoretical equilibrium adsorption capacity (Q_e) fitted by the pseudo-second-order kinetic equation was closer to the actual equilibrium adsorption capacity (Mentioned in 3.1) than that obtained by the pseudo-first-order equation. Therefore, it was considered that the pseudo-second-order kinetic equation was more suitable to describe the adsorption process of three kinds of heavy metals. It was concluded that the adsorption was mainly controlled by chemical adsorption (Aman et al. 2008), and the theoretical equilibrium adsorption capacities of Pb^{2+}, Cu^{2+}, and Ni^{2+} at the initial concentration of 20 $mg{\cdot}L^{-1}$ were 18.35 $mg{\cdot}g^{-1}$, 17.06 $mg{\cdot}g^{-1}$, and 8.37 $mg{\cdot}g^{-1}$, respectively.

Table 3 Fitting results of kinetic models

Metal	Initial concentration ($mg{\cdot}L^{-1}$)	Pseudo-first-order: $\lg(Q_e - Q_t) = \lg Q_e - \frac{k_1 t}{2.303}$		
		Q_e (mg/L)	k_1 (min^{-1})	R^2
Pb	20	18.26	2.48	0.9975
Cu	20	16.71	1.04	0.9856
	50	20.28	1.70	0.9769
Ni	20	8.31	1.55	0.9969
Metal	Initial concentration ($mg{\cdot}L^{-1}$)	Pseudo-second-order: $\frac{t}{Q_t} = \frac{t}{Q_e} + \frac{1}{k_2 Q_e^2}$		
		Q_e (mg/L)	k_2 ($g{\cdot}mg^{-1}{\cdot}min^{-1}$)	R^2
Pb	20	18.35	0.99	0.9982
Cu	20	17.06	0.16	0.9972
	50	20.74	0.20	0.9901
Ni	20	8.37	1.05	0.9981

Adsorption isotherm of heavy metals by activated sludge

Langmuir and Freundlich models were used to fit the adsorption data, and various isotherm parameters were obtained through calculation (Pagliaccia et al. 2022; Wei et al. 2021). The fitting results are shown in Table 4. Although the isothermal adsorption of Pb^{2+} was not well fitted by the two models, the adsorption process of Cu^{2+} could be fitted by Langmuir equation, and the Ni^{2+} was fitted by the Freundlich equation. With the increase of temperature, the R^2 value decreased, which was likely due to the unstable adsorption caused by the increase of temperature. The theoretical saturated adsorption capacity of three kinds of heavy metals at different temperatures could be obtained from the Langmuir model parameters. The saturated adsorption capacity of Pb^{2+} was the largest. The saturated adsorption capacities of Pb^{2+}, Cu^{2+}, and Ni^{2+} at 25 ℃ were the highest and achieved 136.21 $mg{\cdot}g^{-1}$, 41.13 $mg{\cdot}g^{-1}$, and 65.37 $mg{\cdot}g^{-1}$, respectively.

Role of sludge characteristics in adsorb heavy metals

There are sufficient polysaccharides, proteins, lipids, and other substances on the surface of microbial cells and extracellular polymeric substances (EPS) in the outer layer of activated sludge, which was beneficial to heavy metal adsorption. The previous studies showed that the functional groups in activated sludge such as carboxyl,

Table 4 Fitting results of adsorption isothermal models

Metal	Temperature (℃)	Langmuir $\frac{C_e}{Q_e} = \frac{1}{bQ_{max}} + \frac{C_e}{Q_{max}}$			Freundlich $Q_e = k_f \bullet C_e^{\frac{1}{n}}$		
		Q_{max} ($mg{\cdot}L^{-1}$)	b ($L{\cdot}mg^{-1}$)	R^2	k_f $(L/mg)^{1/n}(mg/g)$	n	R^2
Pb	15	70.20	0.27	0.6080	21.90	4.07	0.6150
	25	136.21	0.18	0.49595	28.25	2.91	0.5422
	35	99.94	0.22	0.3151	27.48	3.74	0.3607
Cu	15	22.79	1.06	0.9604	10.92	6.02	0.8835
	25	41.13	0.46	0.9971	14.77	4.46	0.8752
	35	21.90	0.75	0.8577	10.50	6.46	0.6708
Ni	15	13.95	0.14	0.9177	4.29	4.30	0.9803
	25	65.37	0.01	0.9504	2.81	1.92	0.9715
	35	19.38	0.12	0.8884	6.07	4.56	0.7204

hydroxyl, amino, amide, carbonyl, and sulfhydryl had complexation reactions with Pb^{2+}, Cu^{2+}, and Ni^{2+} (Aslan et al. 2018; Wang et al. 2006), which was also a major contribution to heavy metal removal from wastewater. The activated sludge surface before and after the adsorption was analyzed by FT-IR. Results are shown in Table 5 and Fig. S4. There were a large number of amido, hydroxyl, methylene, amide, and carboxyl groups on the surface of activated sludge, which played an essential role in the complex adsorption of Pb^{2+}, Cu^{2+}, and Ni^{2+} (Wang et al. 2006). The peak intensity of $-NH_2$ and -NH decreased obviously on activated sludge surface after the adsorption of Pb^{2+} and Cu^{2+}, indicating the action of ligand exchange. Due to the organic complexation and electrostatic adsorption of saccharides, the peak intensity of C-O and O–H on the activated sludge surface decreased after the adsorption of Pb^{2+}, Cu^{2+}, and Ni^{2+}. The results of FT-IR analysis showed that Pb^{2+}, Cu^{2+}, and Ni^{2+} could be removed by organic complexation and electrostatic adsorption. On the surface of activated sludge, Pb^{2+} and Cu^{2+} could also exchange coordination with amino groups, but Ni^{2+} was not found the process. At the same time, the saccharide component of activated sludge played an important role in the removal of the three kinds of heavy metals.

When activated sludge adsorbed heavy metal, other metal cations such as K^+, Na^+, Ca^{2+}, and Mg^{2+} may exchange with heavy metals, which caused that the adsorbed heavy metal released into the solution (Yuncu et al. 2006; Zhou et al. 2016). Activated sludge was analyzed by EDS before and after the adsorption, and the results are shown in Fig. 6. The activated sludge contained elements such as K, Na, Ca, and Mg before the adsorption. After the adsorption of heavy metals, the peaks corresponding to Pb^{2+}, Cu^{2+}, and Ni^{2+} appeared, which proved that heavy metals have been adsorbed on activated sludge. The peaks corresponding to K, Na, Ca, and Mg decreased significantly or even disappeared, and atomic percentages also decreased significantly, which showed that K, Na, Ca, and Mg cations exchanged with heavy metals during the adsorption process.

Changes of SBR system after heavy metal adsorption

The activated sludge images at the end of the culture stage and 10 $mg{\cdot}L^{-1}$ domestication stage was analyzed using SEM, and the sludge morphology is shown in Fig. 7. The microbial cells in activated sludge in the culture stage were loose and porous, and many microbial cells were distributed on the surface of the activated sludge with and obvious outlines. After the absorption of heavy metals, the microbial cells became dense, and micropores decreased greatly. The distribution of micelles decreased, and the contour was not obvious. Bacteria in the activated sludge had serious agglomeration after the absorption of heavy metals.

The 16S rDNA amplicon sequencing was performed for microbes in the activated sludge at the end of the culture stage and 10 $mg{\cdot}L^{-1}$ domestication stage using the NovaSeq sequencing platform (Fig. 8). At culture stage, although genus *Dokdonella*, *Dechloromonas*, and *Zoogloea* were dominant at the genus level, the relative abundance was only 22%, indicating a good biodiversity in the activated sludge. After the long-term adsorption of heavy metals, the relative abundance of *Rhodobacter* reached 66%, while that of other types decreased to 14%, indicating biological diversity of activated sludge decreased significantly. The same conclusion could also be illustrated by the comparison of rarefaction curves (Fig. S5).

Discussion

Activated sludge process has been proven to be effective in removing heavy metals from the wastewater in the present study. The removal of Pb^{2+} and Cu^{2+} was more effective than that of Ni^{2+} in the SBR activated sludge system. Firstly, the highly effective removal of Pb^{2+} was attributed to its small hydration ion radius. The hydration ion radius formed by heavy metal dissolved in aqueous solutions can reflect the degree of difficulty of adsorption by activated sludge. The smaller the hydration ion radius was, the easier it can

Table 5 The FT-IR changes of activated sludge before and after adsorption of heavy metals

Before adsorbent (cm^{-1})	After Pb adsorbent (cm^{-1})		After Cu adsorbent (cm^{-1})		After Ni adsorbent (cm^{-1})		Chemical bond or functional group
	Wave number	Variation	Wave number	Variation	Wave number	Variation	
3443	3422	−21	3423	−20	3442	−1	$-NH_2$, -NH, or -OH
2926	2927	1	2927	1	2927	1	$-CH_2$
1652	1653	1	1653	1	1653	1	C=C or C=O in acylamino
1543	1543	0	1543	0	1544	1	N–H in acylamino
1402	1400	−2	1400	−2	1402	0	O–H in carboxyl
1245	1245	0	1243	−2	1245	0	C-O in carboxyl or O–H in alcohols
1077	1054	−23	1053	−24	1049	−28	C-O and O–H in sugars

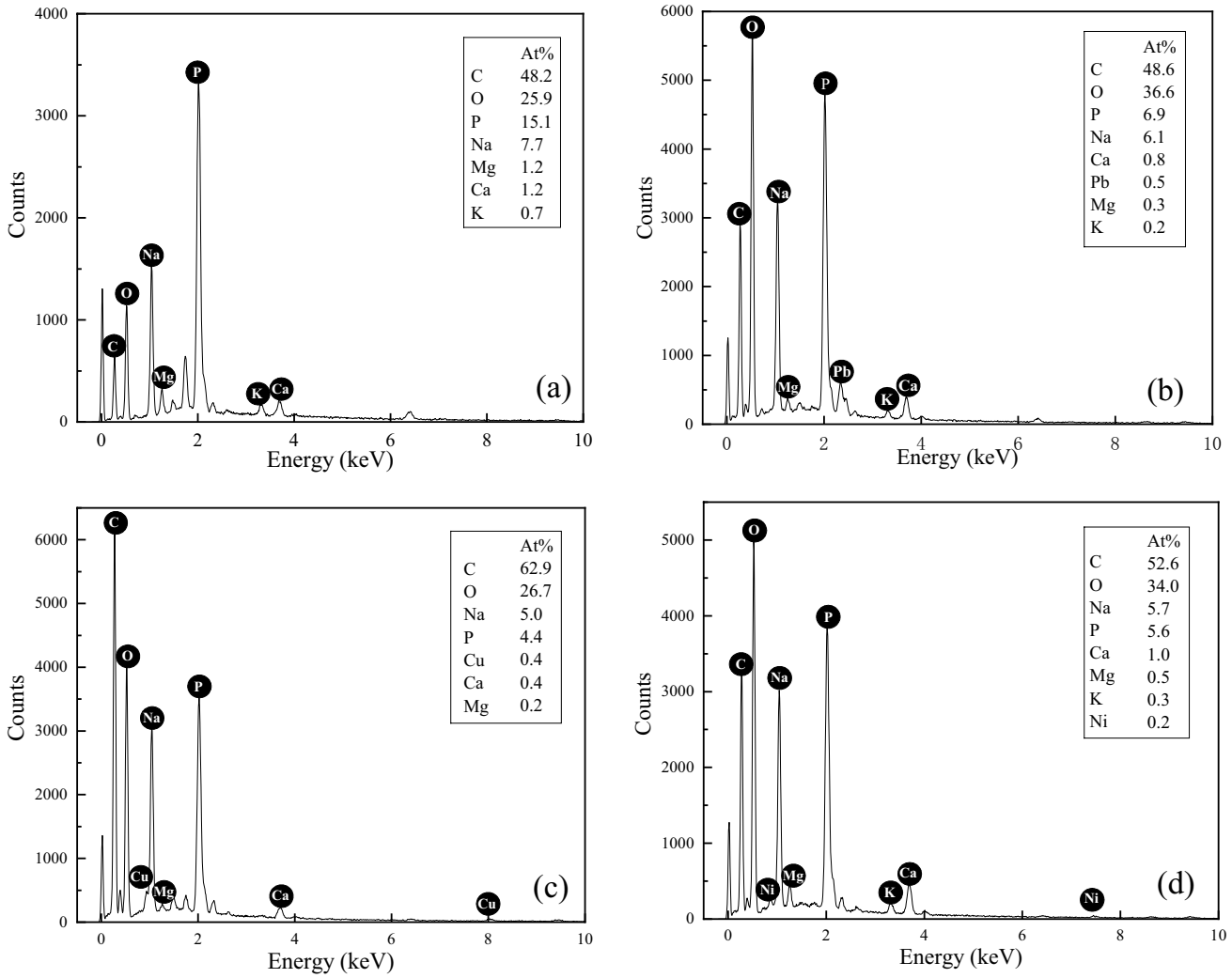

Fig. 6 EDS results of activated sludge before and after the adsorption of heavy metals**.** (a): Before adsorption; (b): After Pb^{2+}adsorption; (c) After Cu^{2+} adsorption; (d): After Ni^{2+} adsorption

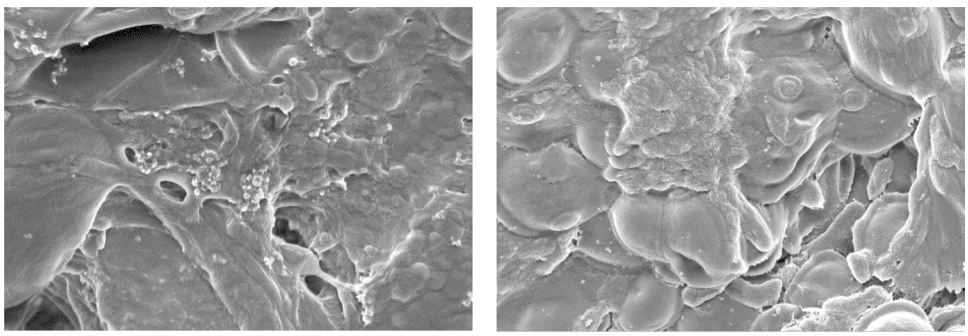

Fig. 7 SEM results of activated sludge in the SBR system**.** (a): Culture stage, (b):10 $mg{\cdot}L^{-1}$ domestication stage

be adsorbed by activated sludge (Maslova et al. 2020). The radius relationship between Pb^{2+}, Cu^{2+}, and Ni^{2+} hydration ions was $r_H(Pb^{2+}) < r_H(Cu^{2+}) \approx r_H(Ni^{2+})$ (Volkov et al. 1997). Therefore, Pb^{2+} can be adsorbed easier than Cu^{2+} and Ni^{2+}. Secondly, it was related to the differences in adsorption mechanism. Pb^{2+} and Cu^{2+} could perform ligand exchange with amino groups on the surface of activated sludge, which was also the main reason that the removal efficiency of Pb^{2+}

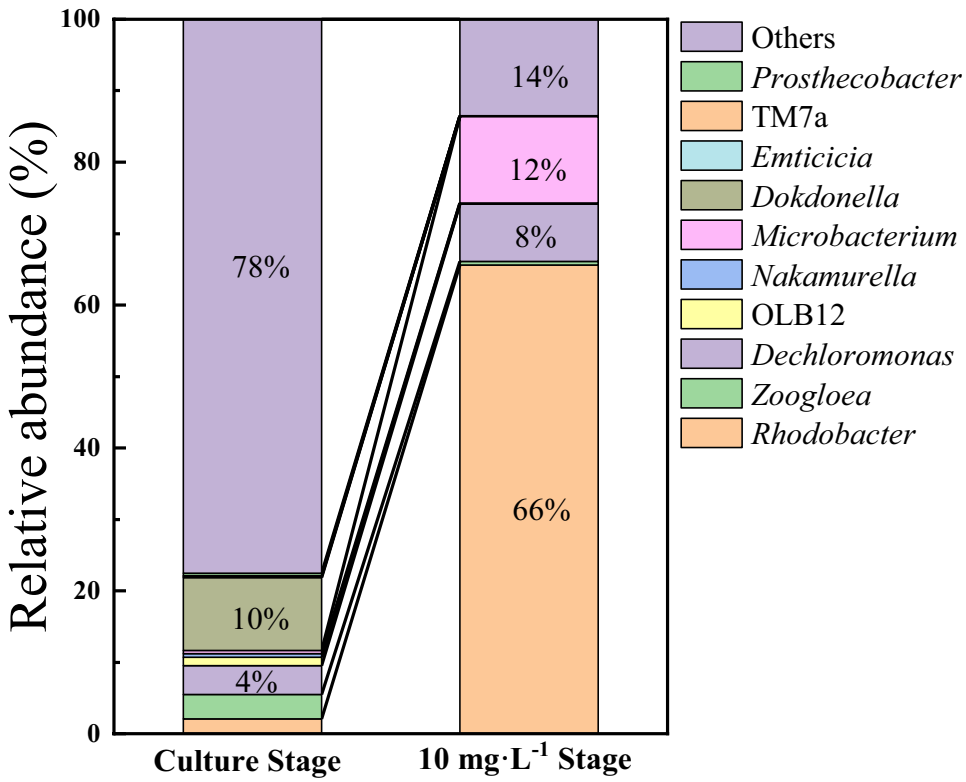

Fig. 8 Relative abundance of microorganisms in activated sludge at the genus level

and Cu^{2+} was much higher than Ni^{2+}. Although Ni^{2+} was found to have ligand exchange process with the other metal ions such as Mg^{2+}, Na^{+}, and K^{+} on the surface of activated sludge in this study, the amount of these metal ions was much lower than that of amino groups. The adsorption process of Cu^{2+} was more consistent with the Langmuir model, indicating a monolayer adsorption, which can prove that electrostatic adsorption and ligand exchange were the main mechanisms of Cu^{2+} adsorption by activated sludge. The adsorption process of Ni^{2+} was more consistent with the Freundlich model, indicating that a single ion can occupy more than one active site (Wei et al. 2021). Therefore, with the same number of active sites, the amount of Ni^{2+} adsorbed was less than that of Cu^{2+} when the adsorption was nearly saturation. On the other hand, the biotoxicity of Cu^{2+} was lower than Ni^{2+}, which made activated sludge easier to retain Cu^{2+} than Ni^{2+}. Therefore, the adsorption of Ni^{2+} by activated sludge was more difficult than Cu^{2+}. In addition, due to the high concentration of phosphate in the wastewater, part of Pb^{2+} or Ni^{2+} may be removed through the inorganic micro-precipitation mechanism, which can be proved by the higher P and O peaks (Fig. 6) after Pb^{2+} adsorption by activated sludge (Daskalakis et al. 2013).

Activated sludge mainly removes Pb^{2+} and Cu^{2+} from wastewater through ligand exchange, organic complexation, and electrostatic adsorption in the SBR system, and the removal of Ni^{2+} mainly depended on the organic complexation and electrostatic adsorption. In the treatment process of Pb^{2+}-, Cu^{2+}-, and Ni^{2+}-containing wastewater by activated sludge in the SBR activated sludge system, Pb^{2+} could quickly combine with the adsorption site on activated sludge and complete the process of adsorption, and thus the reaction of Pb^{2+} was faster than Cu^{2+} and Ni^{2+}. This was mainly because the hydration radius of Pb^{2+} was smaller than Cu^{2+} and Ni^{2+}. Ni^{2+} was hardly exchange coordination with $-NH_2$, -NH, or -OH on the surface of activated sludge, and thus Ni^{2+} has disadvantages in competitive adsorption with Pb^{2+} and Cu^{2+} during the organic complexation and electrostatic adsorption processes. Therefore, the adsorption capacity of activated sludge to Ni^{2+} was the weakest.

However, the SBR activated sludge system was significantly affected by heavy metals, showing that heavy metal showed biological toxicities to activated sludge. Heavy metals produced toxicity to activated sludge by affecting intracellular enzymes, inducing generation of reactive oxygen species (ROS), affecting cell membrane structure, and so on, which could further affect activated sludge shape and microbial community structure (Kelly et al. 2004; Li et al. 2022a, b). Generally, activated sludge enabled to be protected by secreting more EPS to adapt to heavy metal stress (Pagliaccia et al. 2022). This led to better agglomeration and settleability of the activated sludge in the SBR system. In addition, microbial community in activated sludge changed during the long-term impact of heavy metals. It was found that microbial diversity decreased significantly under the condition of the influence of heavy

metals, and genus *Rhodobacter* became the dominant in activated sludge, which was a heterotrophic microorganism that can perform photosynthesis. Genus *Rhodobacter* can degrade organic matter in wastewater under the conditions of anaerobic light or aerobic dark, and rich pigment in their cells makes them have strong antioxidant properties to cope with heavy metal stress (Lu et al. 2022; Talaiekhozani and Rezania 2017).

Conclusions

The long-term treatment of Pb^{2+}-, Cu^{2+}-, and Ni^{2+}-containing wastewater by activated sludge a SBR activated sludge system was continuously carried out for 123 days, and the mechanism of heavy metal removal by activated sludge was discussed through static adsorption experiments and sludge characteristics. Activated sludge process was effective in removing heavy metals (Pb^{2+} and Cu^{2+}) from the wastewater in the present study, activated sludge and the removal rate of Pb^{2+} and Cu^{2+} in SBR activated sludge system were 83.1 ~ 90.0% and 74.3 ~ 80.6%, respectively, at the 10 mg/L of initial concentration, while the removal rate of Ni^{2+} was less than 6.2%. The equilibrium adsorption capacities of Pb^{2+}, Cu^{2+}, and Ni^{2+} at the initial concentration of 20 $mg{\cdot}L^{-1}$ were 18.35 $mg{\cdot}g^{-1}$, 17.06 $mg{\cdot}g^{-1}$, and 8.37 $mg{\cdot}g^{-1}$, respectively. Activated sludge removed Pb^{2+} and Cu^{2+} by the ligand exchange, electrostatic adsorption, and surface organic complexation processes, while Ni^{2+} was removed by the electrostatic adsorption and surface organic processes. The Ni^{2+} removal was inhibited by Pb^{2+} and Cu^{2+} in composite heavy metal-containing activated sludge system. In the long-term treatment process of heavy metal-containing wastewater, microbial diversity of activated sludge decreased, and genus *Rhodobacter* became the dominant community with a relative abundance of 66%.

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Lu Qi and Hongchen Wang: Writing—review and editing; supervision

Zhengfang Ye and Quanlin Zhao: Project administration; funding acquisition

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Data availability The datasets used during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval Not applicable.

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