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Removal of copper and lead ions from water using the extremophile *Deinococcus wulumuqiensis* R12

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ABSTRACT

Heavy metal pollution in the aqueous environment has become a serious problem due to increasing industrialization. Microbial preparations capable of adsorbing heavy metals are environmentally friendly and have the advantages of low energy consumption as well as high efficiency, which attracted extensive attention. Among them, radiation-resistant microorganisms showed excellent tolerance and adsorption of heavy metals. In this study, the adsorption of Cu^{2+} and Pb^{2+} from aqueous solution by the radiation-resistant strain *Deinococcus wulumuqiensis* R12 was determined. After incubation of 90 min at 32°C and pH 5.75, R12 removed 83.3% of initial 30 mg L^{-1} Cu^{2+} from the solution, corresponding to an adsorbed amount of 1.25 mg g^{-1}. At an initial Pb^{2+} concentration of 60 mg L^{-1}, and adsorbent concentration of 3.6 g L^{-1}, a maximal adsorption capacity of 12.5 mg g^{-1} and adsorption rate of 75% could be achieved after incubation at pH 5.0 and 28°C for 90 min. These results demonstrate that *D. wulumuqiensis* R12 is a biosorbent with promising potential for the large-scale control of heavy metal pollution.

Keywords: Bioremediation; Biosorption; *Deinococcus wulumuqiensis*; Heavy metal tolerance

1. Introduction

Heavy metal pollution in the aqueous environment is a serious global problem. In China, most drinking water supplies, which are mostly derived from rivers and lakes, are polluted with different heavy metals. The pollution rate of the substrate in rivers, lakes, and reservoirs is as high as 80.1%. According to the monitoring data, in the first half of 2015 alone, the heavy metals in surface waters, including As, Hg, Se, Pb, and Zn, exceeded the Chinese standard by up to 18 times. These heavy metals in the water cannot be biodegraded. They tend to enter the food chain and can be enriched hundreds of times, eventually reaching humans, and causing many health problems [1].

Heavy metal pollution in the water can be treated using physical, chemical, and biological processes. However, physical and chemical methods have high operation costs, low treatment efficiency can cause secondary pollution and other problems when treating wastewater with a heavy metal concentration of 10^{-3}–10^{3} mg L^{-1}. By contrast, biological methods are more suitable for the treatment of wastewaters with low concentrations of heavy metals and large volumes. Due to their safety, biological treatment methods have received extensive attention, mainly using algae, bacteria, fungi, or their cell extracts to adsorb or accumulate metal ions from wastewater [2–7]. The adsorptive removal of cadmium

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ions by dry biomass of *Spirulina platensis* was tested, and very high levels of removal, reaching up to 87.69% were obtained [5]. *Pseudomonas aeruginosa* B237 isolated from soils collected from a zinc mine showed the highest removal efficiencies for Cd^{2+} and Zn^{2+} [6]. *Pseudomonas* sp. NA was reported to have the ability to remove more than 110 mg L^{-1} of Cu^{2+} from wastewater within 24 h through bioreduction and biosorption at an initial Cu^{2+} concentration of 300 mg L^{-1} [7].

Although these microbes were successfully used to treat heavy metal pollution in wastewater, there are still some problems such as their low resistance to heavy metal ions, high sensitivity to changes in the actual wastewater environment, and their low ability to adsorb heavy metal ions. Therefore, it is necessary to screen microorganisms with high resistance to heavy metals and high adsorption capacity for heavy metal ions.

Radiation-tolerant microorganisms that can survive at high radiation doses (above 5,000 Gy) hold great potential for use as biosorption agents. They have important potential value in the treatment of heavy metal pollution, prevention of radionuclide leakage in nuclear power plants, and bioremediation of radioactive pollution [8]. *Deinococcus radiodurans*, a nonpathogenic, obligately aerobic soil bacterium, is the most radiation-tolerant microorganism discovered so far. As an extremophile, it exhibits strong resistance to extreme environmental conditions such as ionizing radiation, ultraviolet radiation, and drying [9]. It has also been employed to adsorb metal ions such as U^{5+} and Cr^{5+}, which are the most common heavy metals in radioactive waste [10]. A series of engineered strains have also been successfully constructed to strengthen the biosorption capacity of the wild type [11–14]. For example, Ni/Co transporter (NiCoT) genes were cloned and expressed in *D. radiodurans*, and the functional expression of these genes in bioengineered strains resulted in >60% removal of ^{60}Co (≥5 nM) within 90 min, while the biomass requirements were reduced to 1/25 of previously reported values [14]. Radiation-tolerant microorganisms are often generally adapted to survive in challenging environments affected by cold, dehydration, high heavy metal concentrations, or radioactivity because many species have multiple copies of the genome and rapid DNA repair mechanisms. For example, *D. radiodurans* can repair breaks in its chromosome within 12–24 h by a 2-step process [15,16]. These remarkable abilities make *D. radiodurans* an ideal candidate for bioremediation of water polluted by heavy metals.

In previous studies, the extremely radiation-tolerant *Deinococcus wulumuqiensis* strain R12 was isolated from a radiation-contaminated area of Xinjiang province in western China. The strain was identified as a new species of *Deinococcus*, and it displayed notable resistance to ionizing radiation and ultraviolet radiation (>10 kGy dose of γ-irradiation, >J m^{-2} of UV irradiation) [17]. In this study, the growth characteristics of R12 were investigated and possible functional genes related to adsorption of metal ions were identified and analyzed. The tolerance of R12 to heavy metal ions was tested first, followed by an investigation of Cu^{2+} and Pb^{2+} adsorption under different conditions to evaluate the strain's potential as a biosorbent for the treatment of water bodies polluted with heavy metals. This strain expands the scope of existing microbial adsorbents and contributes new tools for the large-scale control of environmental pollution using biosorbents.

2. Materials and methods

2.1. Bacterial strains and growth conditions

D. wulumuqiensis R12 was screened in our previous work [17]. Single colonies were taken from the slant medium and used to inoculate a 250 mL flask containing 50 mL of 1/3 TGY medium (peptone 1.7 g L^{-1}, yeast extract 1.7 g L^{-1}, and glucose 3.33 g L^{-1}). The incubations were carried out at 30°C with shaking at 200 rpm. After 30 h of incubation, 1 mL of the culture was transferred into a 250 mL flask containing 50 mL of 1/3 TGY medium, followed by further cultivation for 42 h.

2.2. Tolerance of R12 to heavy metal ions

Different solutions of heavy metal ions, $Pb(NO_3)_2$, $Hg(NO_3)_2$, $Cu(NO_3)_2$, and $Na_2Cr_2O_7$ (1 mol L^{-1}) were filtered and added into autoclaved 1/3 TGY medium to prepare solid plates containing Pb^{2+}, Hg^{2+}, Cu^{2+}, or Cr^{6+}, at concentrations of 20, 40, 60, 80, 100, 120, 140, and 160 mg L^{-1}, respectively. Single colonies were streaked onto the plates and cultured at 30°C for 72 h, after which the growth of the strains was observed.

2.3. Determination of the amount of Cu^{2+} adsorbed by R12 and the adsorption rate

Preparation of biosorbents and determination of adsorption was carried out as described previously [18]. Briefly, different doses of the adsorbent were added into Cu^{2+} solutions (20–70 mg L^{-1}) with different pH (4.5–6.25) and incubated at various temperatures (22°C–37°C) for different times (15–120 min) on incubator shakers. In all experiments, controls without any added bacterial cells were also prepared. After incubation, the mixture was centrifuged at 10,000 rpm for 20 min, and the residual copper in the supernatant was analyzed by atomic absorption spectroscopy (TAS-990, China PuXi) with the following parameters: wavelength: 324.7 nm; spectral bandwidth: 0.7 nm; lamp current: 4.0 mA; acetylene flow: 1.6 L min^{-1}; combustion head height: 11.0 mm. The removal rate (q_1) and removal amount (Q_1) were calculated using Eqs. (1) and (2), respectively:

$$q_1 = \frac{C_{10} - C_1}{C_{10}} \times 100\% \quad (1)$$

$$Q_1 = \frac{(C_{10} - C_1)v_1}{m_1} \quad (2)$$

where C_{10} is the Cu^{2+} concentration in the control, mg L^{-1}; C_1 is the Cu^{2+} concentration in the sample after adsorption, mg L^{-1}; m_1 is the wet weight of the biosorbent cells, g; and v_1 is the total volume of the adsorption system, L.

2.4. Determination of the amount of Pb^{2+} adsorbed by R12 and the adsorption rate

Preparation of biosorbents and determination of adsorption were carried out as described previously [18]. The determination of adsorbed amount and adsorption rate of Pb^{2+} by R12 were performed in analogy to that of Cu^{2+}. In all experiments, samples without any added bacterial cells acted as controls. The Pb^{2+} concentration in the solution was determined by atomic absorption spectroscopy (TAS-990, China PuXi), and the parameters were as follows: wavelength: 383.3 nm; spectral bandwidth: 0.4 nm; lamp current: 2.0 mA; acetylene flow: 15 L min^{-1}; combustion head height: 20.0 mm. The removal rate (q_2) and removal amount (Q_2) were calculated using Eqs. (3) and (4), respectively:

$$q_2 = \frac{C_{20} - C_2}{C_{20}} \times 100\% \tag{3}$$

$$Q_2 = \frac{(C_{20} - C_2)v_2}{m_2} \tag{4}$$

where C_{20} is the Pb^{2+} concentration in the control, mg L^{-1}; C_2 is the Pb^{2+} concentration in the sample after adsorption, mg L^{-1}; m_2 is the wet weight of the biosorbent cells, g; and v_2 is the total volume of the adsorption system, L.

2.5. Effects of other metal ions on the adsorption of Cu^{2+} and Pb^{2+} by R12

K^+ and Na^+ (0–4.0 mmol L^{-1}) were added separately into the Cu^{2+} solution at pH 5.75 to test their influence on the adsorption of Cu^{2+} by R12. The influence of K^+ and Na^+ on the adsorption of Pb^{2+} by R12 was also tested in the same way.

3. Results and discussion

3.1. Effects of culture conditions on the growth of R12

The maximum growth of R12 was observed at pH 7–8 and a temperature of 30°C (Fig. 1). In terms of optimum temperature, R12 is therefore a mesophilic microorganism but was able to grow in a wide temperature range of 10°C–55°C. Most previous studies indicated that members of the genus *Deinococcus* have temperature optima ranging from 25°C to 37°C [19,20], and only some species screened from thermal habitats such as hot springs displayed better growth at higher temperatures [21]. The pH of the environment is an important factor affecting the permeability and stability of the plasma membrane, the solubility or ionization of the substrates, and the rate of enzymatic reactions, which in turn influence the growth of microorganisms. The pH optima of common bacteria are generally in the range of 6.5–7.5. By contrast, R12 was able to grow in a pH range of 5–12, exhibiting broad pH tolerance.

3.2. Analysis of genes related to heavy-metal tolerance in the genome of R12

In our previous study, the genome of R12 was sequenced and annotated [22]. Open reading frames (ORFs) in the genome were analyzed using Glimmer3.0 software. These ORFs were further annotated by searching against the National Center for Biotechnology Information (NCBI) non-redundant database using the BLAST algorithm. According to the annotation results, four ORFs, named orf02202, orf02204, orf02684, and orf02712, were predicted to be related to heavy metal tolerance (Table 1). According to the comparison results, three genes were predicted to encode heavy metal translocating P-type ATPases, and one was predicted to encode a heavy metal transport/detoxification protein. Alignments of protein sequences encoded by the three ORFs (orf 02202, orf02684, and orf02712) with those of heavy metal translocation P-type ATPases from *Deinococcus geothermalis* DSM 11300 (GenBank ID ABF44026.1), *Deinococcus proteolyticus* MRP (GenBank ID ADY26431.1), and *D. geothermalis* DSM 11300 (GenBank ID ABF44015.1) revealed respective similarities of 100%, 88%, and 99%. In addition, the heavy metal transport/detoxification protein that was predicted to be encoded by orf02204 was very closely related to a homolog from *D. geothermalis* DSM 11300 (GenBank ID ABF44027.1), which displayed a similarity of 98%.

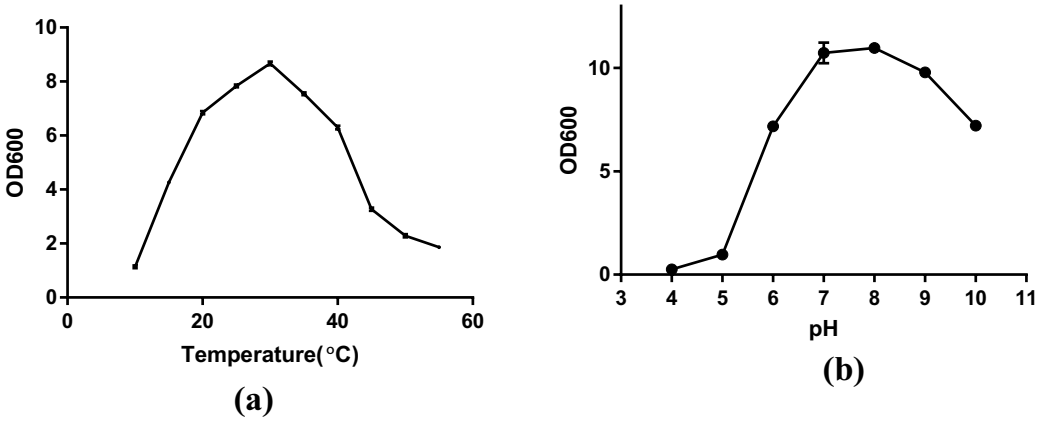


Fig. 1. Effects of (a) temperature and (b) pH on the growth of *Radiodurans wulumuqiensis* R12.

Table 1
Heavy metal tolerance genes of R12 predicted by Glimer3.0

Number	Length of the encoded protein	Description
orf02202	601	Heavy-metal translocating P-type ATPase
orf02204	64	Heavy-metal transport/detoxification protein
orf02684	642	Heavy-metal translocating P-type ATPase
orf02712	775	Heavy-metal translocating P-type ATPase

P-type ATPases are classified in the Transport Classification Database (TCDB, http://www.tcdb.org/) as belonging to the 3.A.3 the P-type ATPase (P-ATPase) superfamily and are important proteins involved in the transport of specific ions such as phospholipids across membranes. Studies have shown that these proteins are related to the heavy metal resistance of bacteria. Chien et al. [23] identified a heavy-metal translocating P-type ATPase gene from *Enterobacter* sp. and expressed it in *Escherichia coli*. The resulting recombinant strain showed increased resistance to cadmium and zinc compared to the wild-type strain. Wang et al. [24] demonstrated that P-type ATPases utilize the energy from ATP hydrolysis to pump ions across the cell membrane against a concentration gradient. Similarly, heavy metal transport/detoxification proteins are thought to participate in the uptake and efflux of heavy metals via metal-binding sites encoded in the sequence [25]. The genes encoding P-type ATPases and a heavy-metal transport/detoxification protein related to bacterial heavy-metal resistance traits were predicted in the genome of R12, which suggested that R12 might have heavy-metal tolerance.

3.3. Effects of heavy metals on the growth of R12

The tolerance of strain *D. wulumuqiensis* R12 to heavy metal ions is shown in Table 2. Similar to the characteristics of *D. radiodurans* R1, *D. wulumuqiensis* R12 is resistant to a variety of metal ions, including Cu^{2+}, Pb^{2+}, Hg^{2+}, and Cr^{6+}. The strain maintained growth in 1/3TGY medium containing Pb^{2+} (140 mg L^{-1}) or Cu^{2+} (100 mg L^{-1}). The maximum concentration of Cr^{6+} tolerated by R12 was 80 mg L^{-1}. By contrast, the growth of R12 was significantly inhibited by Hg^{2+} at concentrations over 40 mg L^{-1}. Many studies investigated the bioremediation potential of *D. radiodurans* and its genetic modification for cleaning up heavy metals in contaminated sites [11,26–28]. Daly et al. [26] reported that *D. radiodurans* could grow in the presence of high Mn(II) concentrations, and the accumulation of Mn(II) by the strain contributed to its gamma-radiation resistance. R12 was observed to be multi-resistant to various heavy metals, making it a promising candidate for a biosorbent to remediate sites contaminated with more than one heavy metal.

Table 2
Effects of heavy metals on the growth of R12

Metal	Concentration (mg L^{-1})						
	20	40	60	80	100	120	140
Hg^{2+}	+	+	–	–	–	–	–
Cu^{2+}	+	+	+	+	+	–	–
Pb^{2+}	+	+	+	+	+	+	+
Cr^{6+}	+	+	+	+	–	–	–

+, observable growth of *Deinococcus wulumuqiensis* R12
–, no growth

3.4. Removal of copper by R12

3.4.1. Effect of pH on the removal of copper by R12

The effect of pH on the biosorption rate was investigated in a pH range of 4.5–6.25 at fixed concentrations of Cu^{2+} and R12 cells of 20 mg L^{-1} and 20 g L^{-1}, respectively. Values higher than pH 6.25 were not included because copper hydroxide would precipitate out of solution at these values. The pH was adjusted by the addition of 0.1 M HCl. The effect of pH on the adsorption of copper by R12 is shown in Fig. 2a. The adsorption rate was the lowest at pH 4.5, reaching only 30.8%. With the increase of pH, the adsorption rate of Cu^{2+} increased and reached a peak of 75% at pH 5.75. However, pH values above 5.75 led to a decrease in the removal efficiency. These results indicated that acidic conditions were detrimental for the adsorption of Cu^{2+}, and 5.75 was the optimal pH for the adsorption of copper by R12.

The bacterial cell walls play an important role in heavy metal biosorption. The cell wall of Gram-positive bacteria consists of a thick layer of peptidoglycan (PEG) and polyalcohols, that is, teichoic acids, some of which are covalently linked to lipids to form lipoteichoic acids and link the PEG to the cytoplasmic membrane [29]. According to Vijayaraghavan and Yun [29] and Din et al. [30], the functional groups of bacteria such as PEG, phospholipids, lipopolysaccharide, and various proteins are responsible for metal binding.

The pH is an important parameter that affects the solution chemistry of metal ions and the functional groups on the surface of the bacterial cell wall [31]. At low pH values, the binding sites of the cell wall are blocked by hydrogen ions that hinder the access of metal cations to the functional groups on the surface due to repulsive forces [32]. The reaction of metal ions with OH^- at high pH also influences biosorption. At high pH values, the metal ions tend to form metal hydroxides and precipitate out of solution [33]. Therefore, maximal removal of metal ions by biosorbents usually occurs at slightly acidic pH, commonly within a range of pH 5.0 to 6.25 [4,7,34].

3.4.2. Effect of contact time on the removal of copper by R12

Contact time is considered an important parameter that influences the pollutant-adsorption behavior of

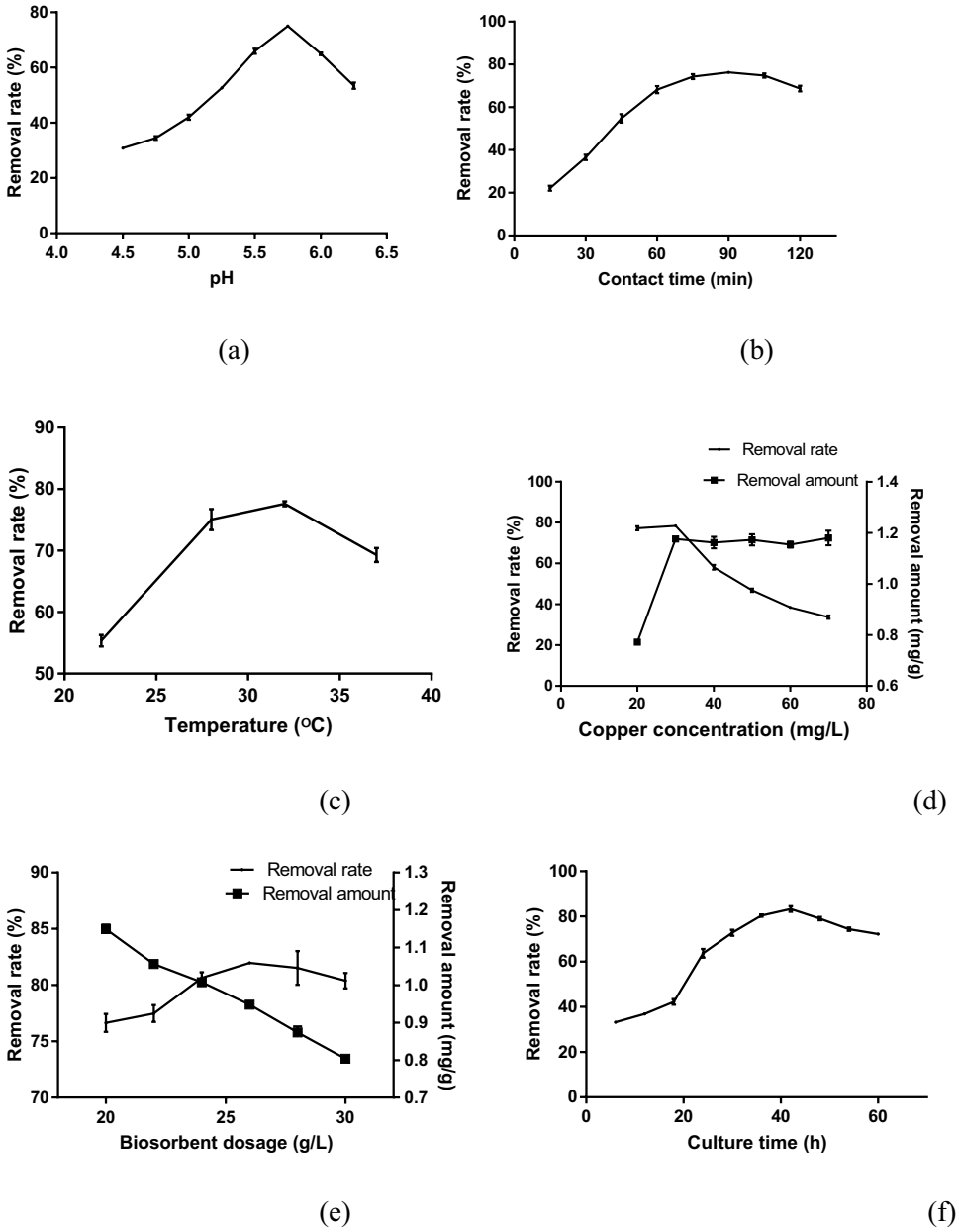


Fig. 2. Effects of adsorption parameters on the removal of Cu^{2+} by R12. Effects of (a) pH, (b) contact time, (c) temperature, (d) initial copper concentration, (e) biosorbent biomass, and (f) culture phase on the removal of Cu^{2+} by R12.

microorganisms. The influence of contact time on metal ion biosorption was examined by varying the contact time from 15 to 120 min (Fig. 2b). The rate of metal adsorption by the biosorbent increased rapidly along with the increase of contact time in the first 15–60 min. The biosorption process reached an equilibrium point after reaction for 90 min, at an adsorption rate of 76.3%. With further increase of adsorption time, the adsorption rate slowly decreased. The trend of the adsorption curve indicated that the biosorption of Cu^{2+} by R12 is likely the result of the binding of metal ions with the free chemical groups on the cell wall, such as amino, hydroxyl, and carboxyl groups, rather than metabolization by the cells. As the contact time was increased, increased desorption may have led to a decline of the adsorption ratio. As adsorption proceeds, the sorbent reaches the saturation stage, after which the adsorbed heavy metal ions tend to desorb from the binding sites and are released back into the solution [35].

3.4.3. Effect of temperature on the removal of copper by R12

The adsorption rate of Cu^{2+} by R12 was tested at 22°C, 28°C, 32°C, and 37°C to investigate the influence of temperature on the adsorption capacity (Fig. 2c). At 22°C, the adsorption rate was 55.3%. With the increase of temperature, the adsorption rate of Cu^{2+} also increased

and reached a maximum of 77.6% at 32°C. In the range of 28°C–32°C, the influence of temperature on the adsorption rate was relatively small and the corresponding curve was flat.

Temperature is one of the main factors that affect the biological elimination of pollutants by microbial strains [36]. These factors affect the general microbial metabolism by specifically promoting or inhibiting enzyme activity in cells, thereby affecting the biosorption of metal ions [37].

3.4.4. Effect of the initial copper concentration on copper removal by R12

The effect of the initial copper concentration was investigated by varying the copper concentration from 20 to 70 mg L^{-1} at a constant biosorbent concentration of 20 g L^{-1}. As sown in Fig. 2d, with the increase of the initial copper concentration from 20 to 30 mg L^{-1}, the removal rate increased from 77.2% to 78.4%. However, when further increasing the initial concentration of Cu^{2+}, the removal rate began to decrease, falling to 33.7% at an initial copper concentration of 70 mg L^{-1}. The adsorbed amount quickly increased from 0.772 to 1.176 mg L^{-1} as the copper concentration was increased from 20 to 30 mg L^{-1}, but stabilized at copper concentrations higher than 30 mg L^{-1}.

The influence of the initial concentration of metal ions on the removal rate is mainly affected by two factors. Firstly, higher concentrations of metal ions reduce the transfer resistance between aqueous and solid phases and increase the probability of collision between copper ions and sorbents sites, which results in a higher uptake of metal ions [38]. Secondly, when the concentration of metal ions continues to increase, the heavy metal ions will inhibit cell growth or even destroy the microbial adsorbent, which leads to a decrease in the adsorption rate [7].

3.4.5. Effect of biomass concentration on the removal of copper by R12

The effective adsorbent dosage is a major factor determining the actual future costs of sewage treatment. As shown in Fig. 2e, with the increase of biomass concentration of R12, the removal rate first increased and then decreased. The maximal removal rate was obtained at a biomass concentration of 26 g L^{-1}, reaching 82%. For biomass dosages in the range of 20–26 g L^{-1}, the biosorption efficiency of copper ions increased from 76.6% to 82%. However, when the biomass dosage was higher than 26 g L^{-1}, the biosorption efficiency decreased.

Obviously, the number of available adsorptive or ion-exchange sites depends on the amount of adsorbent [39]. However, when the biosorbent concentration exceeds a certain threshold, it can lead to negative effects. For example, with the increase of cell concentration, the interaction between the amphiphilic groups on the cell membranes is enhanced, leading to competitive binding between functional groups, which in turn decreases the adsorption rate [27,40]. Moreover, when the biomass dosage is too high, the pH of the adsorption solution will be changed by extracellular metabolites, thereby affecting the form of heavy metal ions in the aqueous system or changing the physicochemical properties of the cell surface. Additionally, cations secreted by the cells may also compete with metal ions for binding sites, and further weaken the adsorption effect.

3.4.6. Effect of growth stage on the removal of copper by R12

The adsorption capacity of R12 at different growth stages was tested at a biosorbent concentration of 20 g L^{-1} and an initial copper concentration of 30 mg L^{-1} (Fig. 2f). After inoculation, cells grown in a liquid medium for 30–60 h absorbed over 70% of copper ions. The highest adsorption rate was 83.3% at 42 h, corresponding to an adsorbed amount of 1.25 mg L^{-1}.

According to our previous research, after incubation for 30 h, the growth of R12 reaches the stationary phase, at which point the growth rate is roughly equivalent to the death rate. During this stage, the biomass concentration reaches its maximum. When applying the same biomass concentration, later-stage cultures had a lower proportion of living cells. Thus, if the adsorption effect of living cells is greater than that of dead cells, the adsorption rate may decrease. The adsorption difference is also related to the components of the cell wall and membrane [41]. The PEG layer, the outer membrane, and the inner membrane, which are the main components of the cell wall of gram-negative bacteria, are the main sites for Cu^{2+} adsorption. The proportions of outer membrane, inner membrane, and glycan layer in microbial cells from different culture phases are different, and accordingly affect the adsorption capacity of Cu^{2+}.

3.5. Removal of Pb^{2+} by R12

At 30°C, the pH of the Pb^{2+} solution at a concentration of 60 mg L^{-1} was changed to study the effect of pH on the adsorption of Pb^{2+} by R12, as shown in Fig. 3a. With the increase of pH from 3.5 to 5, the metal removal ratio also increased, and the maximal adsorption rate reached 75% at pH 5.0. At pH values in the range of 5.5 to 6.0, the adsorption rate remained above 70%. The increase in the biosorption of Pb^{2+} with pH could be the result of competition between cations and protons for the biosorption sites on the bacterial cells [42]. Pardo et al. [43] reported that the optimal pH for Pb^{2+} sorption by the inactivated biomass of *Pseudomonas putida* was 6.0–6.5, while in another study. Pb^{2+} biosorption by *Bacillus thioparans* strain U3 was found to be highest at pH 4 [44]. The optimal pH values for the adsorption of heavy metal ions may be influenced by the state of the biosorbent (i.e., active or inactive) [43].

The effect of the Pb^{2+} ion concentration on adsorption is shown in Fig. 3b. When the metal concentration was in the range of 20–60 mg L^{-1}, the adsorption rate was approximately proportional to the lead ion concentration, and the maximal adsorption rate reached 75% at a Pb^{2+} concentration of 60 mg L^{-1}, corresponding to an adsorbed amount of 12.5 mg L^{-1}. When the concentration of Pb^{2+} exceeded 60 mg L^{-1}, the adsorbed amount did not increase correspondingly. The trend of the curve could be explained by the relationship between the number of adsorbed active sites and the amount of Pb^{2+} ions. When there were not enough Pb^{2+} ions in the solution, there were sufficient adsorption

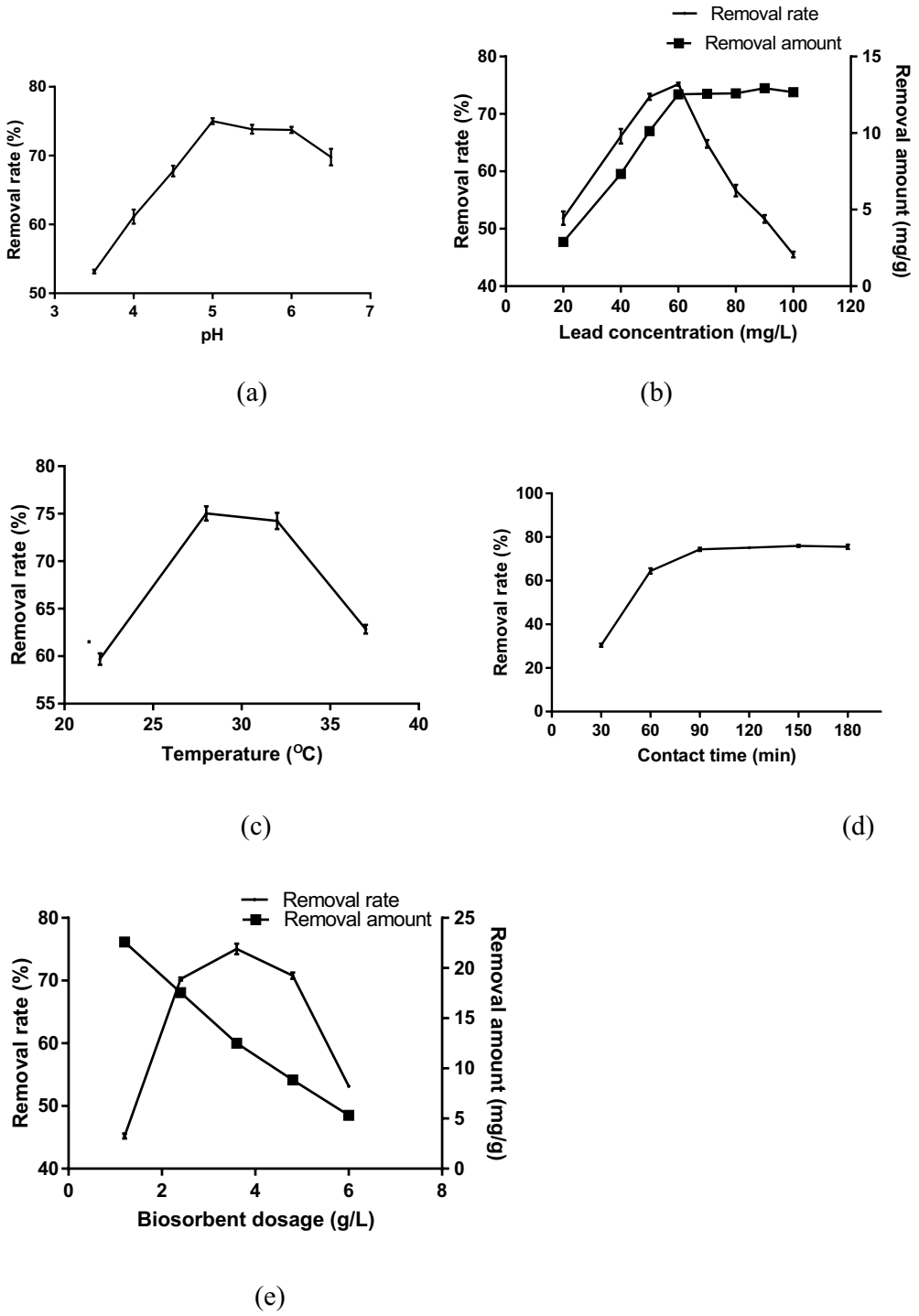
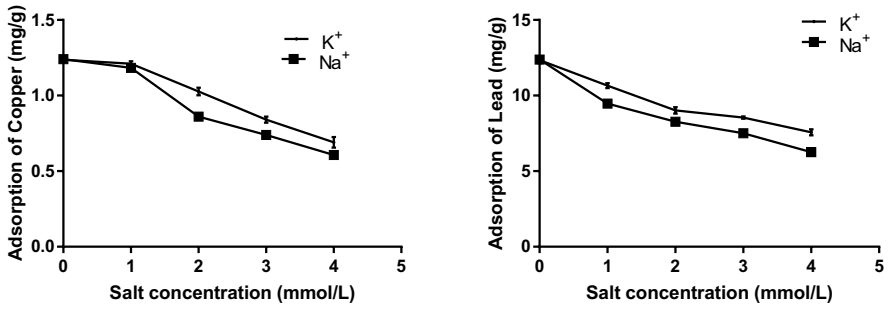


Fig. 3. Effect of adsorption parameters on the removal of Pb^{2+} by R12. Effect of pH (a), initial lead concentration (b), temperature (c), contact time (d), and biosorbent dosage (e) on the removal of Pb^{2+} by R12.

sites on the surface of the cells. Therefore, both the adsorbed amount and the adsorption rate increased with the rising concentration of Pb^{2+} ions. As the concentration of Pb^{2+} ions increased, the active adsorption sites of the cells were gradually occupied and saturated, after which the Pb^{2+} concentration had little influence on the adsorbed amount.

As shown in Figs. 3c and d, the highest adsorption ratio was observed at 28°C after 90 min. On this basis, various amounts of wet cell biomass were added to the 5 mL adsorption system to determine the proper biosorbent dosage. As indicated in Fig. 3e, the adsorption of Pb^{2+} by R12 increased with the increase of the biosorbent concentration, and



(a) (b)

Fig. 4. Effects of K^+ and Na^+ on the adsorption of copper (a) and lead (b) by R12.

Table 3
Comparison between the results of this work and previous reports in the literature

Metal	Removal mass (mg g^{-1})	Biosorbent	Reference
Copper	1.25	*Deinococcus wulumuqiensis*	This study
Copper	0.8	*Saccharomyces cerevisiae*	[47]
Copper	5.4	*Sphaerotilus natans*	[48]
Copper	6.6	*Pseudomonas putida*	[43]
Copper	3.65	*Aspergillus niger*	[49]
Copper	1.51	*Penicillium griseofulvum* (free)	[50]
Lead	12.5	*Deinococcus wulumuqiensis*	This study
Lead	5.0	*Penicillium* sp.	[51]
Lead	6.0	*Penicillium* sp.	[52]
Lead	5.5	*Penicillium digitatum*	[53]

the maximal adsorption rate of 75% was achieved when the biomass dosage of R12 was 3.6 g L^{-1}. Naz et al. [45] reported that 10 bacterial isolates from the sugar industry showed heavy-metal tolerance. Among them, the most effective strain was *Pseudomonas* sp., which reduced 37% of the initial 1.24 mg L^{-1} Pb^{2+}. Zhai et al. [46] prepared a biosorbent from sewage sludge, which achieved an uptake of 56% from an initial concentration of 50 mg L^{-1} Pb^{2+} ions after 120 min, as well as 59% after 180 min. Notably, R12 has the ability to remove Pb^{2+} at even higher initial concentrations, showing good potential for use in large-scale lead ion removal.

3.6. Effect monovalent metal ions on the adsorption of heavy metals by R12

Because sodium and potassium are common components of seawater, which often also requires bioremediation of heavy-metal pollution, the effects of Na^+ and K^+ (0–4.0 mmol L^{-1}) on the adsorption of Cu^{2+} and Pb^{2+} by R12 were also studied (Fig. 4). The experimental results indicated that the sodium and potassium cations affected the adsorption rates of Cu^{2+} and Pb^{2+}, especially at higher concentrations. In the case of copper (Fig. 4a), the presence of Na^+ or K^+ caused a sharp decrease of adsorption from the initial 1.24 to 0.61 and 0.69 mg g^{-1}, respectively. A similar influence on lead adsorption was also observed (Fig. 4b). It is likely that the adsorption of metal ions (such as K^+, Na^+, Cu^{2+}, and Pb^{2+}) by R12 proceeds via similar mechanisms. When they are present at the same time, the adsorption of Cu^{2+} and Pb^{2+}, therefore, decreases due to the competitive occupation of adsorption sites on the cell surface of R12 by K^+ and Na^+. Sodium is more electronegative than potassium [32], which may be the reason why the effect of sodium ions on metal biosorption by R12 was stronger than that of potassium. As a result, the addition of salts decreased the biosorption amount of copper and lead ions in the order of $K^+ < Na^+$. On the other hand, it was observed that at lower concentrations (0–1 mmol L^{-1}), the influence of K^+ and Na^+ was much lower. Considering that the average concentration of Na^+ and K^+ in seawater or other water bodies is usually lower than 0.5 mmol L^{-1}, the adsorption of copper and lead by R12 is not expected to be influenced by these monovalent metal ions at environmental concentrations.

4. Conclusions

In this study, we examined the potential of *D. wulumuqiensis* R12 as a biosorbent for heavy metals. The optimum growth temperature of R12 was determined to be 30°C, and the optimum pH was 7–8. R12 showed good tolerance to Pb^{2+}, Cr^{6+}, and Cu^{2+}. The genome of R12 was found to

contain key genes related to heavy-metal tolerance. Among them, four genes encoding three heavy metal translocation P-type ATPases and a heavy metal transport/detoxification protein were identified. After 90 min of adsorption at 32°C, 20 g L^{-1} of R12 cells removed 83.3% of 30 mg L^{-1} Cu^{2+} at pH 5.75, corresponding to an adsorbed amount of 1.25 mg g^{-1}. When the initial Pb^{2+} concentration of the solution was set to 60 mg L^{-1} and the cell concentration to 3.6 g L^{-1}, the adsorption rate of Pb^{2+} after 90 min at 28°C and pH 5.0 reached 75%, corresponding to an adsorbed amount of 12.5 mg g^{-1}. Interfering ions such as the common monovalent cations Na^{+} and K^{+} decreased the adsorption of Pb^{2+} and Cu^{2+} by R12, but the interference was not significant at environmental ion concentrations found in seawater.

A comparison of our results (Table 3) with the literature indicated that the specific uptake of lead(II) by R12 was significantly higher than in previous reports. The removal of copper(II) in this study was in the same order of magnitude as reported in the literature. As a microorganism that can survive in harsh environments, *D. radiodurans* R12 holds great potential as a biosorption agent.

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