Research Article

Bioadsorptive Removal of the Pollutant Zn(II) from Wastewater by Delftia tsuruhatensis Biomass

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This investigation suggests the applicability of Delftia tsuruhatensis biomass for the removal of Zn(II) from the aqueous environment. Twenty-three zinc-resistant bacterial strains were isolated from contaminated rhizosphere soils. Selectively, the bacterium strain SA-101 was selected as the most zinc-resistant and identified by 16S rRNA sequencing as Delftia tsuruhatensis SA-101. D. tsuruhatensis SA-101 has been assigned the accession number MW629784 in the GenBank database. The optimal pH and reaction contact time for Zn(II) removal by D. tsuruhatensis SA-101 were 6.0 and 30 min, respectively. Moreover, the equilibrium and kinetic models have been applied to the Zn(II) biosorption process. The Zn(II) concentration was estimated using atomic absorption spectroscopy. The $q_{\text{max}}$ for bioadsorptive Zn(II) removal was calculated to be 90.91 ± 0.36 mg/g. The biosorption equilibrium was well fitted with the Freundlich model and the pseudo-second-order kinetic model. So, using the biomass of D. tsuruhatensis SA-101 as a biosorbent of Zn(II) from industrial wastewater represents a promising and viable alternative to chemical treatment from an environmental and economic view.

1. Introduction

The global environmental pollution with heavy metals increases by increasing the human populations and their activities. The major sources of heavy metal contamination in the aqueous environment are soil, weathering of rocks, fuel wastes, and industrial discharges [1–5]. The continuous discharge of effluents contaminated with heavy metals from the tanning, battery, and chemical industries into the aquatic environment represents a huge problem [6–10]. Chromium (Cr), copper (Cu), iron (Fe), lead (Pb), cadmium (Cd), silver (Ag), zinc (Zn), mercury (Hg), nickel (Ni), and manganese (Mn) are the commonly discharged heavy metals [11–13]. Mining and steel processing, as well as burning coal, can release zinc into the environment. The World Health Organization (WHO) declared that the acceptable limits for Zn, Cu, Cd, Pb, Fe, and Mn in the industrial effluent discharge are 5–15, 0.05–1.5, 0.1, 0.1, 0.1–1, and 0.05–0.5 ppm, respectively [14]. The high levels of heavy metals in the surrounding environment cause toxicity to all living organisms [15, 16]. For instance, exposure to zinc may cause common symptoms such as irritability, loss of appetite, and nausea in both animals and humans [6]. Most zinc is discharged into water through artificial pathways or from numerous sources, including mine drainage, industrial and municipal wastes, urban runoff, coal-fired power stations, and the burning of waste materials, but the largest input occurs from the erosion of soil particles containing Zn [17].

The major problem associated with these inorganic species is that they are nondegradable contaminants and must be removed from the substrate. There are numerous treatment methods used for heavy metal removal from the contaminated water, for instance, chemical precipitation, electrochemical treatment, ozonation, membrane separation, coagulation, flocculation, and adsorption [6, 18–21]. Nevertheless, most of the mentioned treatment methods face major problems like high operational costs and a lack of suitable ways to get rid of the residual metal sludge.
Consequently, it is necessary to find an ecofriendly, safe, sustainable, and cost-effective way to recover toxic heavy metals from the polluted substrate. Recently, heavy metals biosorption using biomass has been emerged as a safe, economical, and promising alternative way for recovering heavy metals from polluted environments [6, 7, 22, 23]. Many species of algae, fungi, and bacteria have the natural ability to adsorb heavy metals from soil and aquatic environments. Heavy metals biosorption by the microbial biomass has several advantages, including a higher concentration of chelating groups on cells surface, regeneration of the biosorbed, low chemical impurities, low operating costs, and high efficiency in detoxifying very dilute effluents [6, 7]. Several previous research studies reported that the most commonly applicable microorganisms as biosorbents are the bacterial species, in particular Gram-negative bacteria. This is due to the smaller size of their cells, the unique structure of their cell wall, their capability to grow well on different substrates under controlled conditions, and their heavy metal resistance [7, 24–27]. The high detoxifying potentiality of these tolerant Gram-negative bacteria can be employed for heavy metal recovery from industrial effluents through the biosorption process, in particular with low concentrations that could not be eliminated by other conventional methods. Several mechanisms were suggested for heavy metal recovery by bacterial biomass, such as ion exchange, physical adsorption, chelation, complex coordination, or a combination of different mechanisms [28]. The efficiency of the Gram-negative bacterial cell wall in metal biosorption is due to the presence of the outer membrane (phospholipids and proteins) besides the variety of functional groups on its surface, including carboxyl, hydroxyl, amine, phosphate, and sulphydryl groups [29, 30].

The effectiveness of the biosorption process depends on the nature of the used microbial biomass, the type of metal ion, the medium pH, the contact time, the ionic strength, and the metal concentration [6, 7, 31–35].

This research aims to evaluate the potentiality and performance of D. tsuruhatensis SA-101 biomass as a biosorbent for Zn(II) from wastewater. The effects of environmental factors (reaction pH value, metal concentration, and contact time) on the biosorption process were inspected. Furthermore, the equilibrium and kinetics of zinc ion adsorption onto D. tsuruhatensis SA-101 were investigated.

2. Materials and Methods

2.1. Materials. Chemicals used in this research were purchased from Sigma-Aldrich (Germany), Merck (Germany), and Kanto Chemical (Japan) companies. Stock solution of Zn(II) was prepared from zinc nitrate hexahydrate (Zn(NO₃)₂·6H₂O) and diluted to the desired concentrations with deionized water. Solutions of 0.1 M NaOH and 0.1 M HNO₃ were prepared and used for pH adjustment of the used medium and solutions. pH was measured using a benchtop pH meter (Adwa, 1030). The concentrations of Zn(II) were assayed by the atomic absorption spectrophotometer (Model 210 VGP Buck Scientific).

2.2. Isolation of Zinc-Resistant Bacteria. Ten rhizosphere soil samples of wild grasses in Riyadh (24°42′42″N, 46°43′27″E), Saudi Arabia, were collected in sterilized plastic bottles and transported immediately to the laboratory for isolation of zinc-resistant bacteria. The zinc-resistant bacteria were isolated from soil by the pour plate method on modified tryptic soya agar (TSA) plates (pH 6.5), supplemented by 50 ppm zinc nitrate. The cultures were incubated at 37°C for 3 days and grown colonies were recovered and stored at 4°C. The pure cultures were screened on tryptic soya broth (TSB) of pH 6.5 with different Zn(II) concentrations (50–250 ppm) to detect the most resistant bacterial species and determine the minimum inhibition concentration (MIC) of Zn(II). The growth was determined by measuring the turbidity at 630 nm using the spectrophotometer (UV-9200 VIS).

2.3. Phenotypic and Genotypic Identification of the Bacterial Species SA-101

2.3.1. Phenotypic Characterization. The colony morphological characteristics were noted by the naked eye on nutrient agar (NA) plates, considering the shape, color, surface, margin, and pigmentation. Gram reaction, cell shape, and arrangement were examined microscopically. The strain was tested with different biochemical tests mentioned in Bergey’s manual of systematic bacteriology [36]. The examined biochemical tests included motility, oxidase, citrate, catalase, methyl red (MR), Voges–Proskauer (VP), urease production, nitrate reduction, indole, glucose, lactose, fructose, and mannitol fermentation.

2.3.2. Genotypic Identification. Bacterial DNA was extracted using the Bacterial DNA Preparation kit (Jena Bioscience) based on the method of Abdel-Hamied et al. [37]. PCR amplification of 16S rRNA was conducted by the Qiagen Proof-Start Tag Polymerase kit (Qiagen, Hilden, Germany), using the primers 16SF: 5′-GAGTTTGATCCTGGCTTAG-3′ and 16SR: 5′-GGTACCTTGTTACGACTT-3′. Two μL DNA (20 ng/μL) and 12.5 μL PCR Master Mix were mixed with 20 pmol (2 μL) of each primer, then completed to 25 μL by 8.5 μL of DNAase-free water. The mixture was incubated in the thermocycler TC-3000 as follows: initial denaturation (5 min) at 94°C, annealing (30 s) at 51°C, and extension (30 s) at 72°C. A second extension was carried out for 5 min at 72°C. The resultant PCR products of 1500 bp were purified with the QIA quick gel extraction kit (Qiagen, Hilden, Germany) and subjected to cycle sequencing with dideoxy-mediated chain-termination [38]. The resulted nucleotides sequence was compared with the other sequences recorded in GenBank at the NCBI website: https://www.ncbi.nlm.nih.gov/BLAST/. Multiple sequence analysis was performed using MEGA 7.2.2. The phylogenetic tree of the bacterium SA-101 with the related species from the GenBank database was performed by the MEGA 7 program and displayed using the TREEVIEW program.
2.4. Growth Pattern of *D. tsuruhatensis* SA-101 on Different Concentrations of Zn(II). The pure culture (100 μl, 1 × 10⁶ cfu/ml) was inoculated in tryptic soya broth (TSB) contained different Zn(II) concentrations (5–140 ppm) and incubated at 130 rpm at 37°C for 60 hours. To determine the growth pattern of the bacterium, samples of the culture were withdrawn at different intervals to determine the optical density at 630 nm by spectrophotometer.

2.5. Bacterial Preparation as a Biosorbent for Zn(II). The bacterial culture was grown on TSB at 37°C for 40 hours, then the biomass was collected by centrifugation at 5000 rpm for 15 mins. The collected biomass was prepared for biosorption experiments according to Rasmey et al. [7].

2.6. Biosorption Process. Biosorption experiments of Zn(II) on dried biomass of the bacterium were carried out using a constant biosorbent dose (20 mg) in a constant volume of metal solution (20 ml) and agitation (200 rpm) for an hour at the room temperature (25 ± 2°C). The reaction mixture was centrifuged at 5000 rpm for 15 minutes and the non-biosorbed concentration of Zn(II) was assayed in the supernatant by the atomic absorption spectrophotometer (Model 210 VGP Buck Scientific). The biosorption process was also determined under different reaction conditions as follows: initial pH (2–7), incubation time (0–100 mins), and metal concentration (0–120 ppm). The quantity of biosorbed zinc ions per gram of biomass was calculated from the following equation:

\[ q_e = \frac{(C_i - C_e)V}{M}, \]  

where \( q_e \) is the metal ion concentration (mg/g) adsorbed on the biomass, \( C_i \) is the initial metal ion concentration, \( C_e \) is the final metal ion concentration, \( V \) is the medium volume, and \( M \) is the biomass weight (g).

The isotherm models of Langmuir and Freundlich were explored on the obtained biosorption results of Zn(II) by *D. tsuruhatensis* SA-101. The Langmuir equation (2) is

\[ q_{eq} = \frac{q_{max}bC_{eq}}{1 + bC_{eq}}, \]  

The linear form of Langmuir (3) is

\[ \frac{C_{eq}}{q_{eq}} = \frac{1}{q_{max}} + \frac{C_{eq}}{q_{max}}, \]  

where \( q_{max} \) represents the maximum biosorption capacity, and \( b \) (L/mg) represents the Langmuir constant. The Freundlich equation (4) is expressed as

\[ q_e = K_f C_e^{1/n}. \]  

The Freundlich linear form (5) is expressed as

\[ \ln q_e = \ln K_f + \frac{1}{n} \ln C_e, \]  

where \( K_f \) is the Freundlich constant and \( n \) is the intensity of adsorption.

The pseudo-first-order model (6) is formulated as

\[ \log (q_e - q_t) = \log q_e - \frac{K_{1,ads}}{2.303}t, \]  

where \( q_e \) and \( q_t \) (mg/g) are the biosorbed zinc quantities, \( t \) is the time in minutes, and \( k_1 \) is the rate constant (min⁻¹).

The pseudo-second-order model (7) is formulated as

\[ \frac{1}{q_e} - \frac{1}{q_t} = \frac{1}{k_2t} + \frac{1}{q_t}, \]  

where \( k_2 \) is the rate constant for the pseudo-second-order model and the pseudo-second-order rate constants were detected experimentally by plotting \( t/q \) against \( t \).

3. Results

3.1. Isolation and Identification. Twenty-three zinc-resistant bacterial isolates were recovered from ten rhizosphere soil samples of wild grasses collected from Riyadh (24° 42’42” N, 46° 43’27” E), Saudi Arabia. The obtained isolates were first screened to grow and resist higher concentrations of Zn(II) metal ions (50–250 ppm) and the resulted data indicated that the isolate number SA-101 is the highly resistant bacterial species with the highest minimum inhibition concentration (MIC) of zinc metal ion (140 ppm). The bacterial strain SA-101 was selected for phenotypic and genotypic identification and for further studies of Zn(II) biosorption from solution.

A bacterial strain, SA-101, was described and characterized morphologically and biochemically (Table 1). This bacterium was Gram-negative rod-shaped of raised circular creamy colored colonies with entire edge. The cells were motile without endospores. The strain was positive for oxidase, catalase, indole, lactose, fructose, and mannitol, while it was negative for glucose, urease, nitrate reduction, methyl red, and Voges–Proskauer. The strain was not able to grow at 5°C and 45°C. Based on these phenotypic characteristics, the bacterial strain was identified as *Delftia* spp. SA-101. For identification of this bacterial strain on the species level, a partial 864 pb sequence of 16S rRNA genes of *Delftia* spp. SA-101 was compared and aligned to the sequences in the NCBI GenBank database, which revealed that this bacterial strain had 97.2% nucleotide base identity to *D. tsuruhatensis* MN229467. This strain was identified as *Delftia tsuruhatensis* SA-101. The nucleotide sequence of *D. tsuruhatensis* SA-101 has been recorded in the NCBI GenBank database under accession number MW629784. The sequence of *D. tsuruhatensis* SA-101 (MW629784) was constructed in phylogenetic tree (Figure 1) with the most related 16S rRNA gene sequences of NCBI GenBank database. The evolutionary history was assumed by the neighbor-joining method. A tree with the sum of branch length = 5.65131310 is shown. The evolutionary distances were computed using the maximum composite likelihood method and are in units of the number of base substitutions per site. All positions containing gaps and missing data were
eliminated. There are a total of 753 positions in the final dataset.

3.2. Growth Pattern of D. tsuruhatensis SA-101. The growth pattern of D. tsuruhatensis SA-101 on different concentrations of zinc(II) is shown in Figure 2. D. tsuruhatensis SA-101 was grown on TSB medium amended with different concentrations of Zn(II) (5–140ppm) in comparison with control. The bacterial strain exhibited high resistance to Zn(II) concentrations under consideration and the growth increased gradually with time in all tested Zn(II) concentrations along with the control during the first hours of incubation. The growth decreased after 32 hours of incubation. No bacterial growth was obtained at 140ppm of Zn(II). These results revealed the potential of D. tsuruhatensis SA-101 to grow with high resistance to Zn(II) metal ions.

3.3. Effect of Initial pH and Contact Time on Biosorption. The effect of initial pH on the Zn(II) biosorption by D. tsuruhatensis SA-101 was detected at an initial concentration of 20 mg/L and contact time of 60 min at 25 ± 2°C
The data revealed that with the increase in pH from 2.0–7.0, the biosorption of Zn ions has increased (Figure 3(a)). The optimum pH was found to be 6.0. The effect of contact time was detected and is presented in Figure 3(b). The high efficacy of biosorption rate and Zn(II) removal by the used bacterial biomass was faster through the first thirty minutes of the reaction.

3.4. Biosorption Isotherms. Zn(II) biosorption by *D. tsuruhatensis* SA-101 biomass was detected at different metal concentrations (0.0–200 mg/L), contact time (30 min), and pH 6.0 (Figure 3(c)). The data affirm the obvious relationship between the quantity of zinc ions biosorbed by *D. tsuruhatensis* SA-101 biomass against the concentration of nonbiosorbed zinc ions. It is clear that the biosorption of Zn(II) was gradually increased with the increase of metal concentration and then became constant at the high concentrations. These obtained data were well fitted with the Langmuir model (Figure 4(a)). The Langmuir parameter values are shown in Table 2. The maximum biosorption capacity (*q*<sub>max</sub>) of Zn(II) by *D. tsuruhatensis* SA-101 biomass is 90.9 mg/g. The *b* value of *D. tsuruhatensis* SA-101 biomass for Zn(II) removal is 0.051 L/mg.

The obtained data calculated from equation no. 5 declare the linear form of the Freundlich model for Zn(II) biosorption by *D. tsuruhatensis* SA-101 biomass (Figure 4(b)). The Freundlich parameter values are summarized in Table 2. The obtained results revealed that the magnitude of *K*<sub>f</sub> and *n* prove a higher Zn(II) uptake by *D. tsuruhatensis* SA-101 biomass. The values of *K*<sub>f</sub> and *n* for Zn(II) uptake were 10.771 and 2.239, respectively. The obtained correlation coefficients revealed that both of the studied models confirm the biosorption equilibrium of Zn(II) by the *D. tsuruhatensis* SA-101 biomass.

The *q*<sub>max</sub> of Zn(II) removal by *D. tsuruhatensis* SA-101 biomass of this study was compared with different values of *q*<sub>max</sub> available in literature, given in Table 3. The *q*<sub>max</sub> for Zn(II) removal by *D. tsuruhatensis* SA-101 was in closure to many used microbial biomasses of the other previous studies.

3.5. Biosorption Kinetics. The obtained data of Zn(II) biosorption by *D. tsuruhatensis* SA-101 biomass were analyzed based on the pseudo-first-order and pseudo-second-order kinetics. The linear plot of log (*q*<sub>e</sub>−*q*<sub>t</sub>) against *t* for the pseudo-first-order model for Zn(II) removal by *D. tsuruhatensis* SA-101 biomass is presented in Figure 5(a). The correlation coefficient factor obtained for the pseudo-first-order model for *D. tsuruhatensis* SA-101 biomass was 0.513, and the resulted *q*<sub>e</sub> did not match the experimental *q*<sub>e</sub> (Table 4), which means that the pseudo-first-order model is not applicable for the present biosorption process. The linear plot of the pseudo-second-order model for Zn(II) removal by *D. tsuruhatensis* SA-101 biomass is presented in Figure 5(b). The rate constant (*k*) and the correlation coefficient (*R*<sup>2</sup>) for Zn(II) removal are shown in Table 4. The correlation coefficient for the pseudo-second-order adsorption was 0.993 at 70 mgL<sup>−1</sup> Zn(II). The biosorption capacity of *D. tsuruhatensis* SA-101 biomass for Zn(II) calculated by the pseudo-second-order was 57.1 mg/g, which is close to the value obtained by experiment. These findings declared that the pseudo-second-order is more acceptable to describe the kinetics of Zn(II) biosorption by *D. tsuruhatensis* SA-101 biomass.
4. Discussion

The study was directed to isolate and identify a bacterial species having the potential to resist and absorb Zn(II) from an aqueous medium. *Delftia tsuruhatensis* SA-101 was selected as the most zinc-resistant strain amongst 23 bacterial isolates recovered from rhizosphere soil of wild grasses. The MIC of Zn(II) showed by the strain *Delftia tsuruhatensis* SA-101 of the present study is higher than that recorded by other bacterial strains in the literature using an aqueous medium. *D. tsuruhatensis* was previously isolated and identified as a novel species from activated sludge in Tsuruhata, Kumamoto Prefecture, Japan, by Shigematsu et al. [55]. The growth pattern of this strain was studied on different concentrations of zinc ions, which indicated that this strain exhibits a high resistance for different concentrations of Zn(II), especially in the first hours of incubation. The growth pattern of different bacterial species was studied by several researchers, who reported that growth reduction occurs by increasing the metal ion concentration [6, 56, 57]. This has been elucidated: the microbial cells subjected to heavy metal stress deviate energy from growth to resist the metal toxicity.

The impact of pH on Zn ion biosorption has been examined, and it has been reported that pH value affects both metal solubility and the ionization of the available binding sites on the microbial cell wall [58, 59]. The negative charges on the bacterial cell wall play a fundamental role in the metal binding potentiality of the Gram-negative bacterial cell wall, thus increasing pH leads to increasing the negative charges on the cell surface, which supports the biosorption of the zinc cations [6, 60]. The lower efficiency of Zn(II) removal at high acidic pH values could be attributed to the competitive biosorption between hydrogen protons (H+) and Zn(II). By increasing the pH, more functional groups become available on *D. tsuruhatensis* SA-101 biomass, and as a result, they attract Zn(II) ions [49]. While in the alkaline medium (above pH 7.0), Zn(II) was precipitated thus the ions will be unavailable for the biosorption process. Wierzba [11] reported that the optimum pH for biosorption of Pb(II), Zn(II), and Ni(II) from wastewater by the biomass of *Stenotrophomonas maltophilia* and *Bacillus subtilis* were between pH 5.0 and 6.0.
Biomass is better fitted with the Langmuir model. This result is consistent with the study of Fawzy et al. [68]; for removal of Cd(II) using Oryza sativa biomass. While the current study is in contrast with the results of Fawzy et al. [69], which revealed that the Freundlich model described well the Ni(II) adsorption onto Potamogeton pectinatus. The Langmuir model reflects sorption by monolayer type and assumes that all binding sites on the biosorbent surface have the same affinity for heavy metal ions [70]. The Freundlich model represents a heterogeneous biosorption system with different binding sites [71]. The linear form of the Freundlich isotherm for the biosorption of heavy metals on the surface of biomass from different microorganisms was investigated in different studies [7, 35, 72, 73].

The biosorption of Zn(II) by D. tsuruhatensis SA-101 biomass was studied here based on the pseudo-first-order and pseudo-second-order kinetic models [74] to declare the effectiveness of this biomass as a novel biosorbent in comparison to the other used biosorbents. The pseudo-first-order and pseudo-second-order models and their linear forms were previously studied in different investigations [6, 35, 40, 42, 61, 75, 76]. Hassan et al. [35] stated that the pseudo-second-order model was fitted well for biosorption of Zn(II) by Neopestalotiopsis clavispora ASU1.

Despite the multiplicity of different substances and raw materials that have been used in the adsorption and removal of heavy metals, these materials have many serious disadvantages. The most prominent disadvantage is the high cost. While the current study encourages the use of bacteria to absorb zinc ions in an ecofriendly with low-cost way, in addition to the ability to recover (future proposed work) the adsorbed metals from the surface of the used biomass.

5. Conclusions

The current study investigated the bioadsorptive ability of D. tsuruhatensis SA-101 biomass to remove zinc ions. Our findings show that the maximum biosorption capacity (\(q_{\text{max}}\)) of this bacterium, based on the Langmuir adsorption isotherm model, was 90.91 mg/g within 30 min at a pH of 6.0. The two correlation coefficient factors (\(R^2\)) according to the Freundlich and Langmuir adsorption isotherm models were 0.974 and 0.967, respectively, which indicates that the equilibrium biosorption isotherm fitted better with the Freundlich model than the Langmuir model. Moreover, the...
adsorption kinetics were considered, which indicated that the biosorption process by *D. tsuruhatensis* SA-101 biomass fits well into the pseudo-second-order model. This study aids in proving the ability of bacterial biomass to get rid of the Zn(II) from wastewater in a safe, inexpensive, and short-term method. Also, the obtained data were in closure to many used microbial biomasses of the other previous studies. So, this research recommends the use of *D. tsuruhatensis* SA-101 biomass as a promising biosorbent for Zn(II) from the wastewater.

**Data Availability**

The data used to support the findings of this study are included within the article.

**Conflicts of Interest**

The author declares that there are no conflicts of interest.

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