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Heavy metals biosorption in unary, binary, and ternary systems onto bacteria in a moving bed biofilm reactor

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Toxic and heavy metals cause direct and indirect damage to the environment and ultimately to humans. This study involved the isolation of indigenous bacteria from heavy metal-contaminated environments that have the ability to bioabsorb heavy metals such as cadmium, nickel, and lead. The bioabsorption process was optimized by varying parameters such as temperature, metal concentration, number of bacteria, pH, and more. The bacterial isolates were investigated in terms of morphology, biochemistry, and phylogeny, with 12 strains chosen in the initial stage and one strain chosen in the final stage. It should be remembered that the metal uptake capacity of all isolates was approximately calculated. A box and reactor were designed to house these optimized microorganisms. Based on biochemical, morphological, and molecular results, the isolated strain was found to be closely related to the *Bacillus* genus. In the first five steps of testing, the ideal pH for removing lead alone, lead with cadmium, lead with nickel, and lead ternary (with cadmium and nickel) by *Bacillus* bacteria was found to be 7, 6, 5.5, and 6.5, respectively. The absorption efficiencies for single lead (unary), lead together with nickel, cadmium (binary), and ternary (lead with cadmium and nickel) were found to be 0.36, 0.25, 0.22, and 0.21 mmol/g, respectively. The ideal temperature for lead removal was around 30 °C. The adsorption isotherm for each lead metal in different states was found to be similar to the Langmuir isotherm, indicating that the surface absorption process is a single-layer process. The kinetics of the process follow the second-order kinetic model. The amount of *Bacillus* bacteria biomass obtained during this process was approximately 1.5 g per liter.

Keywords *Bacillus*, Reactor systems, Ternary biosorption, Heavy metals

Heavy metals are introduced to the environment from both natural and anthropogenic sources. volcanic activities, weathering, erosion of minerals and rocks, and forest fires are the natural sources of these metals, while industrial operations such as mining, smelting, electroplating, painting and photography industries, agricultural fertilizers, and nuclear industries are the human resources for these heavy metals. Heavy metals are often found in agricultural and industrial chemicals, which flow into rivers, lakes, and groundwater[1]. Pollution from natural and human sources can easily spread in diverse ecosystems, and local factors enhance metal concentrations, causing negative impacts on human health and the environment[1–3]. Toxic and heavy metals are stable environmental pollutants, they are non-degradable and accumulate in the food chain, and transfer to plants and animals, causing serious threats to the environment[4,5].

Heavy metals are carcinogenic in nature and adversely affect DNA, proteins, and lipids by producing free radicals that lead to severe health and environmental problems. Lead is a soft, silvery, white, or gray metal belonging to group 14 of the periodic table. High density and poor electrical conductivity characterize lead. Although conventional chemical and physical technologies are sufficient to separate high concentrations of heavy metals, they are often ineffective in reducing their concentrations to acceptable standards. Therefore, in the case of dilute effluents, the methods mentioned are expensive and ineffective. For this reason, the separation process utilizing microorganisms, which are more efficient and cost-effective than previous methods, has attracted a great deal of attention today[6,7]. Bioabsorption of toxic and heavy metals is an energy-independent

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process (non-metabolism-dependent) in microorganisms. In biosorption, toxic metal ions are absorbed on the surface of the microbial cell or the active site of biosorbents[8,9]. The biosorption process involves several steps, such as adsorption, chelation/complexation, ion exchange, and surface deposition. Bioaccumulation is a natural biological phenomenon (metabolism-dependent) where microorganisms use proteins to uptake and sequester metal ions in the intracellular space to utilize in cellular processes[10,11].

Among the biosorbents for the removal of toxic and heavy metals, bacteria are very important[12,13]. Bacteria have been used to remove heavy metal contaminants from the environment because of their advantages, such as their small size, fast growth rate, ability to grow in a wide range of environmental conditions, as well as under controlled conditions, availability, high surface-to-volume ratio. *Bacillus*, *Escherichia*, *Micrococcus*, *Pseudomonas*, *Streptomyces*, *Acinetobacter* are examples of bacteria with high and appropriate efficiency in eliminating heavy and harmful metals[14,15].

The researchers' findings revealed that not only the species, but also the growing parameters, such as the culture environment and the physiological state or age of the organism, influence absorption performance[16,17]. Furthermore, studies have shown that environmental physicochemical conditions such as pH, ambient temperature, duration of contact between absorbent and absorbent, initial heavy metal concentration, amount of bacterial biomass, and even the use of living or dead biomass have a significant effect on the rate of metal bioabsorption by biomass[18–20] .

In this research, in addition to examining the removal of alone, binary, and ternary lead metals, and studying the parameters affecting them in laboratory conditions, a moving bed biofilm reactor (MBBR) was designed, and the removal of lead was studied in it. Among the goals of this study, both the removal of lead metal alone, and together with cadmium and nickel have been investigated.

Materials and methods

Isolation and identification of bacteria

The Mazandaran Steel Industries factory in Sari, the Lavij hot spring in Mazandaran province, and the effluent of the sausage and sausage factory in Golestan province were all sampled. Because the existing water was not uniform and varied in color between locations, two sterile samples of 50 ml Falcon size were collected from each location. The acidity of the samples was measured using a pH meter, and the presence of heavy metals was determined using an atomic absorption instrument[19].

After bringing the samples to the laboratory, a 10^{-1}–10^{-3} dilution was prepared, and the sediment was separated by pouring the same volume of solutions into a 10 ml Falcon and centrifuging at 5000 rpm. We let it sit for ten minutes. For initial isolation, nutrient and solid LB broth (dissolve 10 g of tryptone, 5 g of yeast extract and 10 g of NaCl in one liter of distilled water and adjust the pH to 7 using 1, 0.1 M NaOH sodium hydroxide solution, 0.1, 1 M HCl hydrochloric acid) were used. The heavy metals lead, cadmium, and nickel were added to 50 mg per liter of Luria–Bertani culture medium broth. In this situation, only microbes resistant to these metals are able to survive. The culture medium was placed in an autoclave at 121 degrees Celsius for fifteen minutes. All dilutions were cultivated as pure plates on both culture media, and each sample was incubated for 48 h at 37 and 53 degrees Celsius[20].

Among the colonies formed in the culture medium, 12 colonies with distinct shapes and colors were cultivated again. Each sample was cultured three times and incubated for 24 h each time for the purpose of purification. To assure the purity of the samples, Gram staining was performed and the samples were examined under a 100 × magnification optical microscope. Gram-positive bacteria appear purple and Gram-negative bacteria appear red in this type of staining. Investigated morphological characteristics such as cell morphology, gram reaction, and colony shape. The biochemical characteristics and production of specified enzyme isolates were determined using standard procedures suggested by Priya et al. 2022[21]. Lead, cadmium, and nickel metal stock solution of 1000 mg/liter is prepared by dissolving $PbCl_2$, $NiCl_2.6H_2O$, and $CdCl_2$, $6H_2O$, in distilled water. The pH was adjusted to 7 using 0.11 M HCl and 0.11 M NaOH.

In this research, metal salts including ($PbCl_2$, $NiCl_2{\cdot}6H_2O$, and $CdCl_2$, $6H_2O$) were purchased from Merck company, made in Germany, and bacterial culture mediums were purchased from Quelab company, Canada. The devices used include the atomic absorption spectrophotometry (AAS) AA240FS in USA, heater stirrer model D 500, Iran-made Tyfazma Teb (TAT) company, pH meter Lutron (Ph-208) made in USA, centrifuge KOBOTA3300 made in Japan.

Bacteriological and biochemical characteristics of bacterial isolates

A large number of characteristics, each of which is defined by biochemical or physiological tests and on the basis of which bacteria are identified and set in their respective categories, constitute appropriate classification criteria for bacteria. Various biochemical assays were utilized to identify the isolates, some of which were utilized in this investigation[22].

Pilot design and moving bed biofilm reactor (MBBR) construction

The cube-shaped biofilm reactor with a moving and fixed platform has a total volume of 50 L and an effective volume of 20 L. At the beginning and end of the reactor are located two tanks: a storage tank at the beginning and a sedimentation and clarification tank at the end. The design quality of the reactor depends on its start-up and performance, so some parameters must be considered in the design, for example, the dimensions of the reactor, the retention time of the effluent in the system are among these, and the above items have been considered in the design of this bioreactor (Fig. 1). Information about media packing in floating and fixed modes is shown in Tables 1 and 2 and Fig. 1.

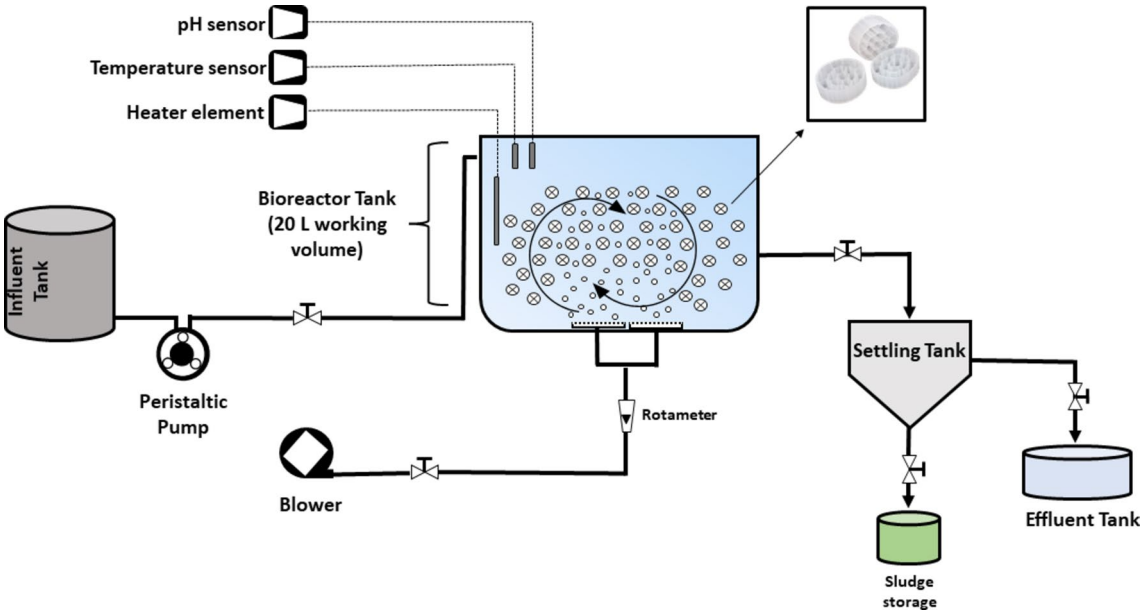

Figure 1. Schematic of moving and fixed bed biofilm bioreactor.

Parameters	Specification
Special level of total media	440 square meters per cubic meter
Special (effective) level of media	360 square meters per cubic meter
Weight per cubic meter	95 kg
The type of raw materials	Polyethylene
Approximate dimensions	10*12.5 mm
Average piece weight	0.45 g

Table 1. Technical specifications of floating packing media.

Parameters	Specification
Model	PVC-150
Media format	Wave diagonal
The type of raw materials	Polyvinyl chloride
Special level	150 square meters per cubic meter
The final thickness of the sheet	400 Micron
Media angle	60°
Weight per cubic meter	27 square meters per cubic meter
Color	Black
Maximum working temperature	60 °C
Resistance	Resistant to UV radiation
pH tolerance range	3–12

Table 2. Technical specifications of fixed packing media.

As shown in Fig. 1, a cube-shaped rectangular MBBR reactor made of glass (35*30*30 cm with a diameter of 4 mm) was used. The working volume for this study was 20 L, with 40% of the reactor volume fled with plastic carriers (the K3 biofilm media has the following specifications: a surface area of > 620(m^2/m^3), a density of 0.95–0.96 (g/cm^3), HDPE material, a size of 25*10 mm, and a weight of 95 kg per m3). Aeration in the bioreactor was accomplished by two diffusers installed in the embedded middle-bottom of the bioreactor, which were connected to the air compressor (Hailea ACO-318; 60 L/min).

The first and second step: stabilization of *bacteria* on the reactor, artificial wastewater treatment

For this purpose, at first, bacterial biomass was prepared from overnight culture using a centrifuge at 6000 rpm for 5 min, and the amount used in the reactor was a certain amount per gram per liter. To attach bacteria to bioreactor discs, a special culture medium was designed, prepared, and poured into the bioreactor tank, and sodium alginate was added in a specific quantity per gram and liter to improve the attachment of bacteria to the discs. This stage was determined to take approximately three days for the bacteria to adhere to the disc and form a biofilm. In addition, the variables of temperature and pH were held constant; these variables were determined based on the optimal conditions for bacterial proliferation. It should be noted that the bioreactor vessel was evacuated and refilled with fresh culture medium every 24 h, allowing for continuous growth and biofilm formation.

After the formation of bacterial biofilm, the bioreactor was ready for wastewater treatment. Similarly, artificial waste water was prepared with lead alone, lead with cadmium and nickel, and ternary lead (with cadmium and nickel). To determine the optimal conditions of the bioreactor, the parameters of the hydraulic retention duration and the initial amount of cadmium absorbed by the reactor were determined in this step.

Investigating the influence of the hydraulic retention time of the bioreactor

At this stage, the parameter of hydraulic retention time in the bioreactor was checked. Likewise, synthetic wastewater containing ten milligrams per liter of toxic and heavy metals lead with cadmium and nickel, lead ternary (with cadmium + nickel) was added to the reactor, and the temperature was set to 30 °C and the pH was set to 7. From the output of the reactor, an amount of 5 ml was separated in the first 30 min to 6 h and then every two hours in 24 h, and after passing through a needle filter with a 0.22 µm mesh, toxic and heavy metals lead with cadmium and nickel, lead ternary (with cadmium + nickel).

A study on the effect of adsorbent dose on the absorption of toxic and heavy metals lead with cadmium and nickel, lead ternary (with cadmium + nickel) in bioreactors

In this step, the parameters of the initial concentration of toxic and heavy metals lead with cadmium and nickel, lead ternary (with cadmium + nickel) in the bioreactor were checked. In the same way, artificial effluent containing different amounts of salts of the above metals per mg/liter was prepared and added to the reactor, and the temperature was 30 °C and the pH was adjusted to 7. After the optimal time obtained in the previous experiment, 5 ml was separated from the output of the reactor and after passing through the filter head with a 0.22 µm mesh, the lead binary (with cadmium or nickel) and lead ternary (with cadmium + nickel) contained in it were determined by an Atomic absorption spectrometry (AAS).

Investigating the effect of the initial amount of lead with cadmium and nickel, lead ternary (with cadmium + nickel) metals in the biological reactor

This experiment was designed for the reason that in real sewage environments, if the toxic and heavy metals pollutant increases or decreases, how will it be in the package containing 5, 10, 15, 20 mg/liter of cadmium, nickel and Lead was prepared and added to the reactor, and the temperature was adjusted to 30 °C and pH to 7. After the optimal time obtained in the previous experiment, 5 ml was separated from the output of the reactor and the amount of the mentioned metals in it was checked by the atomic detector.

Investigating the effect of the aeration amount in the biological reactor

In this part of the research, the amount of wastewater containing 5 mg/L of cadmium, nickel, and lead was added to the reactor and the temperature was kept constant at 30 °C and pH equal to 7. Investigating the effect of aeration rate was tested by turning on the additional aeration motor.

Cell immobilization of selected *Bacillus* isolates on sodium alginate and its addition to fixed and movable polyethylene packings

One of the most important stages of this plan is the stabilization of bacteria and finally its use in the real environment, which is presented in detail in the previous work report and it is only enough that, at the end of the period of cell stabilization in the bioreactor, the formation of biofilm on it was seen on the surface of the media. After about two months, the number of biofilms increased. It should be noted that only a very thin layer of biofilm was observed on the external surfaces of some media. This is due to the aeration and impact of the carriers, which cause the biofilm attached to their outer surface to be torn off and become suspended biomass. For this reason, the inner surface of packing media was considered an effective surface for biofilm formation.

Results and discussion

Isolation of bacteria

The laboratory cultured the samples in an environment containing heavy metals, forming various colonies on the plates. The 12 selected colonies were examined for morphological and biochemical characteristics, and their purity was confirmed using Gram staining (Table 3). To simplify the identification of the isolates grown in the enriching medium, the species isolated from the heated water of Mazandaran Lavij were labeled with W, and the species isolated from the effluents were labeled with WW and the corresponding number. Out of the 40 strains, 12 strains were examined in the first stage, and the isolate with the highest efficacy based on the preliminary tests was ultimately chosen for the actual procedure. This isolate had the highest absorption capacity for the toxic and heavy metals nickel, cadmium, and lead. Based on morphological and biochemical tests, this isolate was identified as belonging to the *Bacillus* genus. According to the minimum inhibitory concentration test and the

Prediction of bacterial genus	TSI	starch	gelatin	urea	Citrate	VP	MR	H_2S	Motility	indole	oxidase	Catalase	Pigment production	Isolate number
Bacillus	Acid/acid	+	+	+	+	+	−	+	+	+	−	+	−	WW1
Bacillus	Acid/acid	+	+	−	+	+	−	−	+	−	−	+	−	WW2
Corynebacterium	Alk/acid	−	−	−	+	−	+	+	−	+	−	+	+	WW3
Bacillus	Acid/acid	+	+	−	+	+	−	−	+	−	−	+	−	WW4
Bacillus	Acid/acid	+	+	−	+	+	−	−	+	−	−	+	−	WW5
Bacillus	Acid/acid	+	+	+	+	+	−	−	+	−	−	+	+	WW6
Bacillus	Acid/acid	+	+	−	−	+	−	−	+	−	−	+	−	WW7
Corynebacterium	Alk/acid	−	−	−	+	−	+	+	−	+	−	+	−	WW8
Bacillus	Acid/acid	+	−	−	+	+	−	−	+	−	−	+	+	WW9
Bacillus	Acid/acid	+	+	−	+	+	−	−	+	−	−	+	−	W10
Bacillus	Acid/acid	+	+	+	+	+	−	+	+	+	−	+	−	W11
Bacillus	Acid/acid	+	−	−	+	+	−	−	+	−	−	+	−	W12

Table 3. Biochemical and differential tests performed for the primary identification of isolates.

antibiogram of the selected Bacillus bacteria, this isolate was resistant to a cadmium concentration of 1500 ppm, a nickel concentration of 2200 ppm, and a lead concentration of 2400 ppm.

Kinetics of uptake of lead unary, lead binary (with cadmium or nickel), and lead ternary (with cadmium + nickel) metals by *Bacillus* bacterial isolate

The kinetics of lead metal removal from metal solutions were observed to be slow and gradual, but the studied isolate exhibited sufficient capacity to remove lead metal. The simultaneous absorption of lead metal with cadmium and lead metal with nickel reduced the removal amount, but it was still acceptable from an industrial perspective. The interference of nickel metal on lead absorption resulted in a significant reduction in metal removal from the solution. The binary process of lead metal removal in the presence of cadmium and lead in the presence of nickel showed the greatest reduction in lead removal, particularly in the presence of cadmium metal (as shown in Fig. 2).

The process was also performed in three different ways, and a significant reduction in removal was observed, especially in the case of lead removal in the presence of all three metals. However, in all cases, most of the removal occurred within the first few minutes, and the reasons for this will be discussed in the discussion and conclusion chapter.

Isotherm findings of the removal of lead metals in unary, binary, and ternary states

Perhaps isotherm with pH is the most important parameter affecting the process of removal and absorption of metal from metal solutions. As demonstrated, for the removal of cadmium individually in the solution, the rate of removal of the studied isolate increases with the concentration of the metal, and until the concentration

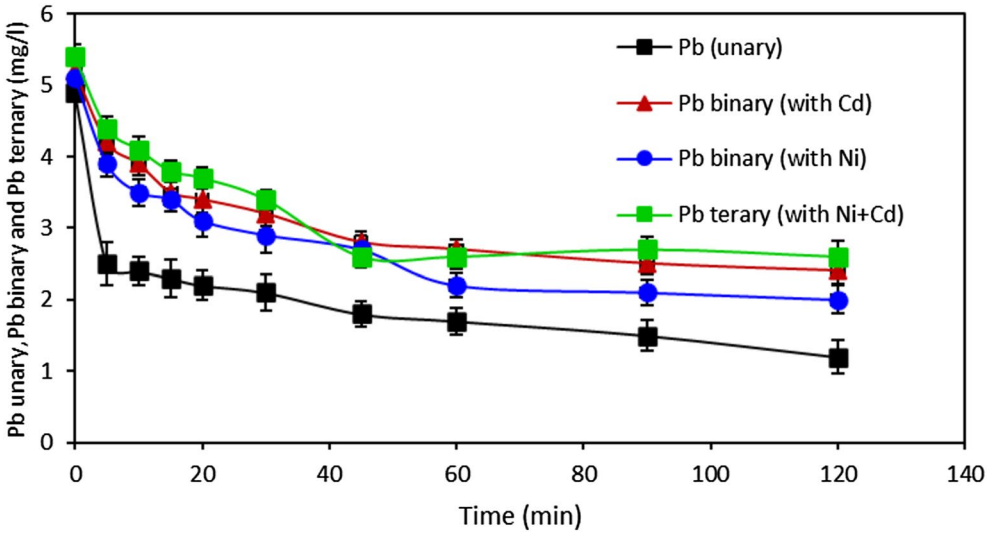

Figure 2. Kinetics of biosorption of lead metal (unary, binary with cadmium or nickel), and ternary (with cadmium or nickel).

of the metal in the solution increases, it has little effect on the metal absorption capacity. In the general state of such findings, nickel and lead had a lower absorption capacity, and their linear diagram was used to determine Langmuir isothermal parameters.

The findings of this research showed that the selected bacteria, WW6, can remove lead at a rate of 0.36 mmol/g of the bacteria's weight. This ability decreased marginally when equal amounts of nickel and cadmium were added to a vial containing lead. The decrease in the ability to remove cadmium was greater than the decrease in the ability to remove nickel, or, to put it more simply, the double state of cadmium exhibited a greater decrease in nickel absorption. In other words, there will be no change in the surface of the bacterium in response to the increase in metal density, or the change will be insignificant, resulting in a decrease in absorption after the saturation limit is reached.

Next, to check that the isothermal process follows the Langmuir model, the parameters were obtained based on the relationship of 1/qe to 1/Ce, and the parameters of this model were observed in the phases of individual, binary, and ternary absorption. Figure 3 shows the entire isothermal effect on the absorption phenomenon, which must be considered in a biosorption process in relation to the reaction between the biosorbent and adsorbents, such as toxic and heavy metals, in order to interpret the other parameters and continue the experiments. This process was predicted at the laboratory level and even on a semi-industrial and industrial scale. As shown in the table below, parameters such as qm, which represents the maximum absorption capacity, bL the Langmuir constant per liter per millimole, and R^2 are effective factors in showing which model the absorption follows. Based on the R^2 number in the Langmuir equation and comparing it with this number in the Freundlich isotherm, it was found that the isotherm of this absorption follows the Langmuir model, so that this unit for nickel absorption in Langmuir is 0.992 and the same factor in the Freundlich model is 0.961. In the same way follows the Langmuir model is used for the absorption of cadmium as well as the double states of double metals.

Findings related to the effect of pH factor on the biological absorption process of for lead unary, lead binary (with nickel or cadmium), and lead ternary, by the selected isolate WW6

The findings of this study are based on the formation of solutions with a pH level of 3 to 12 and their individual and multiple effects have been investigated. As shown in Fig. 4, the optimum pH of the aqueous solution for the removal of lead metal unary, lead binary (with nickel), lead binary (with cadmium) and ternary lead (with cadmium and nickel) was obtained as 7.5, 6, 5.5 and 6.5, respectively.

Generally, metal solutions turn into precipitates at high pH (above 9 here), after the formation of precipitates of these metals, the amount of these metals in the solution decreases, so they are less available in bacterial biomass, causing a decrease in absorption efficiency.

On the other hand, at low pH, there is a competition between metals (lead, cadmium, nickel) and positive hydrogen ions in the solution, this competition is to reach and absorb at the level of the bacterial active group, and this competition may reduce adsorption of metal on the surface of bacterial biosorbent. In binary or ternary state, competition between metals may occur to reach the surface of bacteria, on the other hand, the number of groups involved in the surface of bacteria is constant, so the competition between metals to bind to the surface groups of bacteria reduces their absorption in the comparison is with the single state of these metals. If it is lead metal unary in the aqueous solution, at a pH below 6, a decrease in its removal ability has been observed, but when the pH reaches about 7, the maximum adsorption of the metal occurs, and after that, the decrease in metal adsorption occurs with a gentler slope.

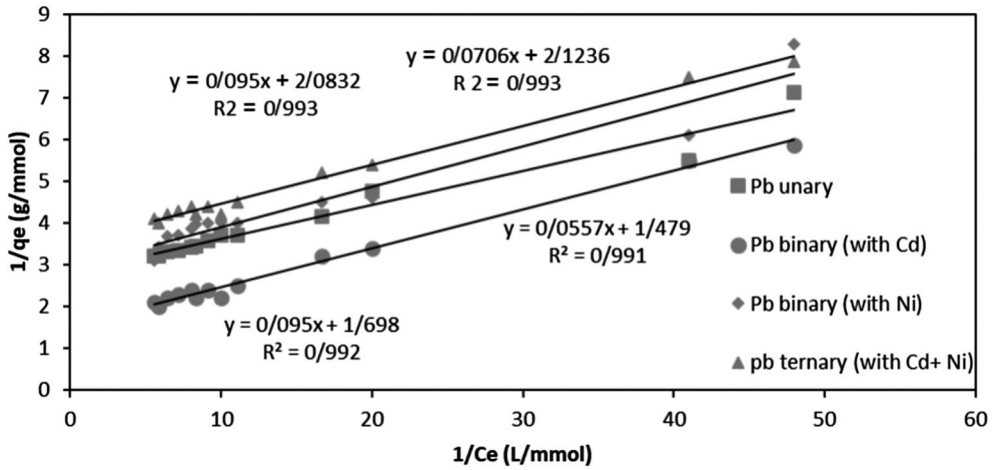

Figure 3. Investigating the isothermal process of the Langmuir model on the biosorption of cadmium, nickel and lead metals in unary and binary states linearly (qe/1 compared to Ce/1) by selected bacteria WW6.

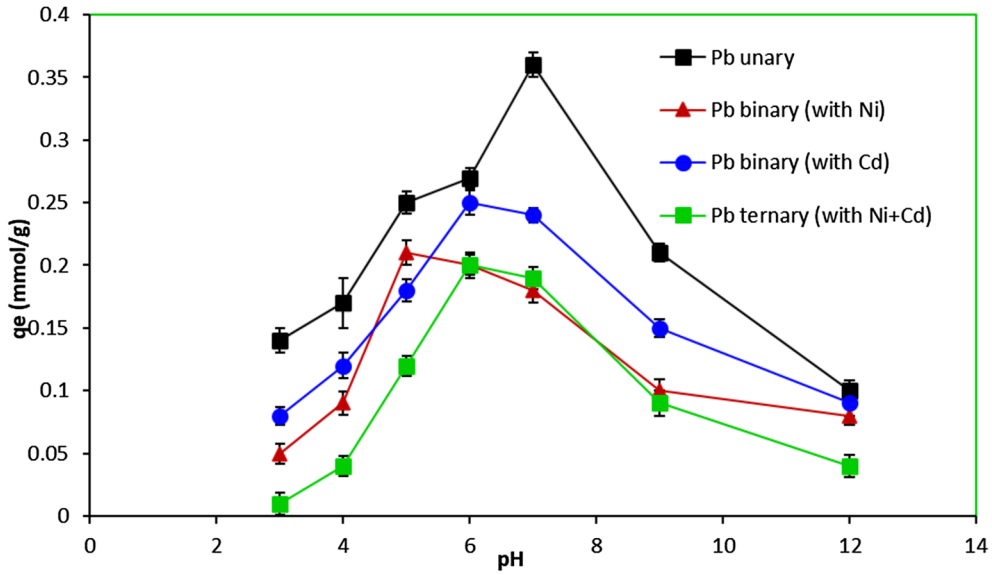

Figure 4. The effects of pH on the biosorption of biomass on the absorption of lead unary, lead binary (with nickel binary, lead with cadmium binary), and lead ternary (with nickel and cadmium) of the selected isolate WW6.

Findings related to the dosage of biosorbent (selected bacteria) on the bioabsorption ability of for lead unary, lead binary (with nickel or cadmium), and lead ternary

Surface chemistry experts suggest that reducing the density, quantity, or volume of a bio absorbent can actually increase its absorption capacity, rather than reducing the removal percentage of toxic and heavy metals. This prediction is based on the principle that as the density and number of bacteria in the solution increase, the absorption process will also increase each month. However, absorption will start to decrease when the desired quantity of bacteria in the solution exceeds a certain threshold.

Figure 5 displays the results of an experiment where selected bacterial biomass pellets were prepared at varying densities (ranging from 1.5 to 3 per liter unit) to test their biosorption capacity for lead metal and lead with nickel. For lead metal, the half biomass concentrations showed a biosorption capacity of approximately 0.23 mmol/g of bacterial weight, with an optimal absorption capacity of 0.29 mmol/g at concentration 1. At a biomass density of 1.5, the uptake rate was the highest and the maximum absorption was achieved, reaching about 0.34, followed by a decreasing trend. On the other hand, for lead with nickel, semi-biomass concentrations of about 0.24 mmol/g of bacterial weight showed an uptake capacity of 0.26 mmol/g at a concentration of 1, with an appropriate biomass density of 0.22 mmol/g of bacteria. Beyond this point, there was no increase in biological absorption, regardless of how much bacterial biomass was added (as shown in Fig. 5).

Findings related to the effect of the temperature of the solution environment on the biosorption ability of lead unary, lead binary (with nickel or cadmium), and lead ternary in the selected isolate WW6

The conditions examined in this design were 5, 15, 30, 45, and 60 °C. The reason for selecting these various temperatures is to examine the conditions of this adsorbent's use on an industrial surface and to determine if it is a suitable candidate. The results show that for cadmium and nickel, the optimal temperature for absorption was 45 °C. As the temperature rises, perhaps the action and reaction between the two bio-adsorbent compounds and the absorbent increase, and the process is associated with a rise in efficiency. The quantity of contact increases, and in the case of lead, this value was 30 °C. At both lower and higher temperatures, absorption was impacted and decreased. At temperatures between 15 and 50 °C, the removal of all three metals was acceptable, and, if used, increased the connection time between adsorbents and biosorbents.

Findings obtained to determine the active or passive mechanism of biosorption of cadmium, nickel, and lead metals by selected isolates WW6

Two compounds, sodium azide and 2-4 dinitrophenol, were employed to inactivate microorganisms using autoclaving and incubation procedures. While the former two processes deactivate the bacteria, the bacteria can still potentially recover from the merc phase. In contrast, the latter two procedures permanently eliminate the bacteria, causing it to die off completely. However, sodium azide is now recognized as a mutagenic chemical compound that halts electron transfer in the electron transfer chain of bacteria, leading to the cessation of the phosphorylation process. Essentially, electron transfer and the production of ATP are halted. In the case of 2-4 DNF, electron transfer occurs, but the phosphorylation process and ATP production do not.

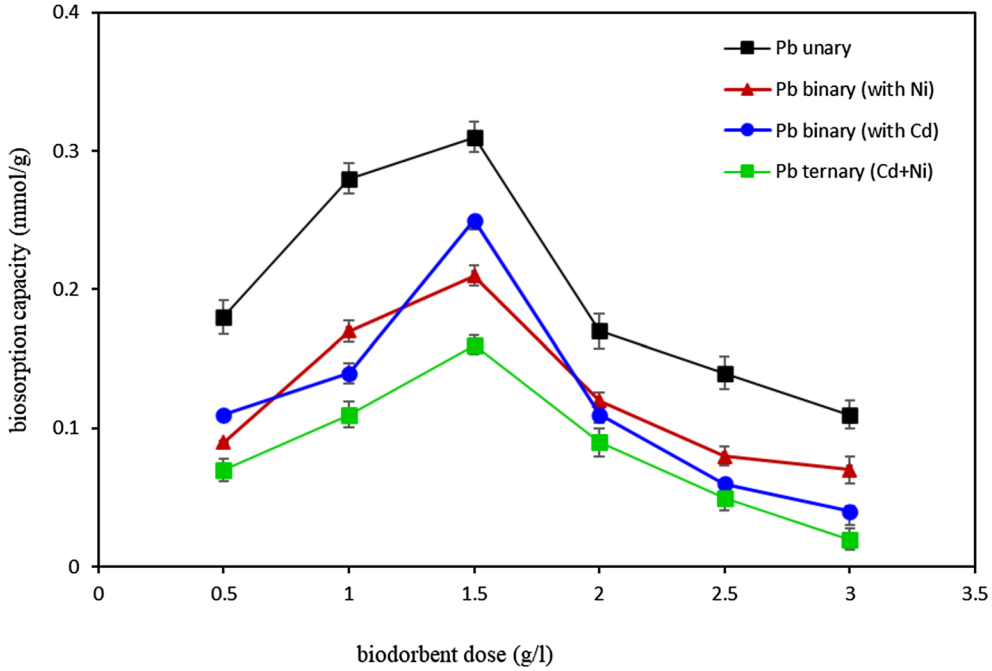

Figure 5. Investigating the process of the effect of bacterial biomass on the absorption of lead unary, lead binary (with nickel binary, lead with cadmium binary), and lead ternary (with nickel and cadmium) of the selected isolate WW6.

The autoclave treatment had the greatest effect on diminishing the absorption of lead, and all absorptions were reduced by more than half, but absorption was still observed. The effects of sodium azine, incubator, and dinitrophenol treatments were comparable. In addition to destroying bacteria, it should be noted that autoclaving impacts the structure and coating of bacteria and may cause fundamental destruction in these structures.

Studying and investigating the process of desorption of metals by metal separating compounds

Special releasing compounds, such as ethylene diamine tetra acetic acid, nitric acid, acetic acid, potassium chloride, hydrogen chloride, sulfuric acid, and sodium bicarbonate, were utilized in this process. After the contact time between the bacterium-containing metal solution and the studied metals, the bacteria were isolated and the effect of releasing agents on them was studied. The percentage of lead metal released by release agents was 79, 77, 68, and 67 for calcium chloride, potassium chloride, and ethylenediaminetetraacetic acid, respectively.

Designing two batch reactors and a semi-continuous reactor to remove the toxic metal

In the Industrial Microbiology Laboratory of the Faculty of Basic Sciences at the University of Mazandaran, a rudimentary reactor was devised and constructed for this portion of the plan, following numerous positive and negative experiments. This reactor was a moving bed biofilm reactor (MBBR), as depicted in Fig. 1. In addition, the studied microbes and the aforementioned metals were brought into contact for eight hours, and the amount of removal was measured. Obviously, the results demonstrated that for a 4-L reactor, a minimum of four grams of biomass bacteria are required, and after the study period, approximately 90 percent of the metal has been removed. In this solution, the pH was adjusted to eight and the reactor temperature was kept at room temperature.

The effect of storage time in the biological reactor MBBR with the presence of lead unary, lead binary (with cadmium or nickel), and lead ternary (with cadmium + nickel) in bioreactors

The removal percentage of lead (alone, binary, and ternary) metals at various times in the designed bioreactor is depicted. In the first four hours, there is the least quantity of the specified metals, indicating that the most removal occurred during this time. By extending the storage time from 30 min to approximately four hours, the removal of cadmium, nickel, and lead increased from 0/39, 66.75 and 62.21% to 84.41, 78.32 and 74.22%, respectively. Because the longer holding time increases the duration of reaction and bonding with the metal and enhances the performance of the reactor, the removal efficiency has improved. The removal efficacy in four hours was the maximum value, as measured by percentage. This number of six hours is extremely essential to us in terms of industrial applications, and it is also dependable and valuable. The duration of the wastewater's presence in the bioreactor is one of the most significant concerns of the toxic metal removal industry. (as shown in Fig. 6). The findings of this study indicate that the time obtained is appropriate for this procedure[23–26].

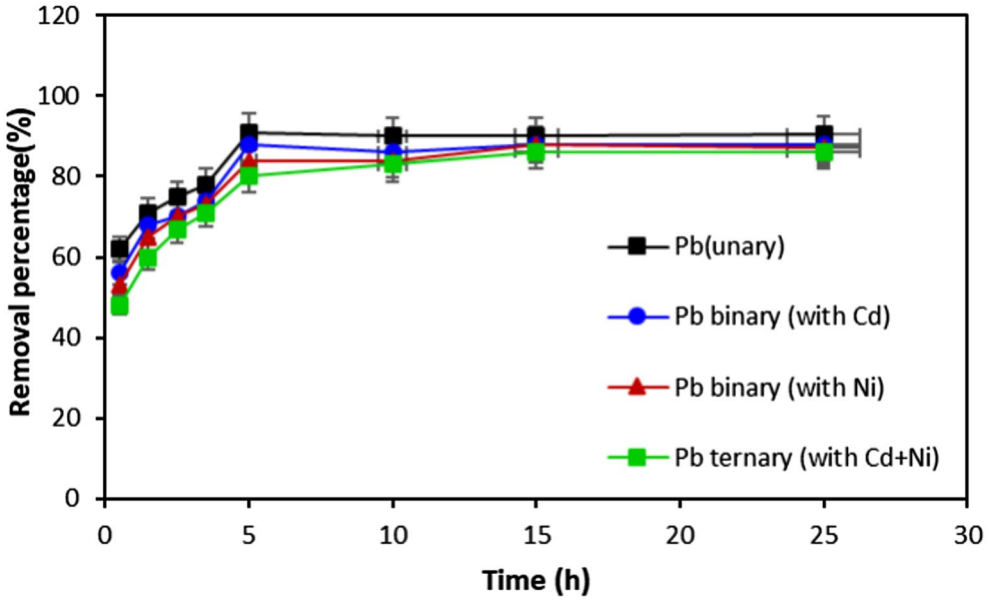

Figure 6. The effect of time on the removal of lead unary, lead binary (with cadmium and nickel) and lead ternary in the designed biological reactor.

The effect of the initial amount of lead unary, lead binary(with cadmium or nickel) and lead ternary in the biological reactor MBBR

As depicted in Fig. 7, the maximal removal percentage of lead by itself, lead (with cadmium and nickel), and lead ternary metals in the initial concentration was approximately 10 mg per liter (ppm). It was discovered that the removal of these metals decreased with increasing concentration, which may be a result of the metals' deleterious influence on the efficacy of the bacterial isolate in the reactor (Fig. 6). Residual metals such as cadmium, nickel, lead, and zinc, etc., persist in small quantities of pollution, but this small quantity cannot be removed using chemical and physical methods. Fortunately, it can be removed from effluent using a microbial approach [27–29].

Effect of aeration amount in bioreactor MBBR

To ensure the efficient purification of wastewater, the impact of aeration on the bioreactor's performance has been thoroughly evaluated. However, the findings indicated that the level of aeration did not have a significant effect on the removal of cadmium, nickel, and lead metals, as depicted in Fig. 8. The percentage removal of these metals was not significantly different at an aeration rate of 8 L per minute, which resulted in an 81.71% removal rate compared to an aeration rate of 12 L per minute. This outcome is particularly significant for the industrial process since it eliminates the need for aeration devices, resulting in substantial cost savings and reducing the expenses related to constructing and deploying the reactor in industrial centers. This has been confirmed by various studies, including Ahluwalia and Goyal D in 2006, Ahmad et al. in 2011, and Gyawali et al. in 2023[12,18,31].

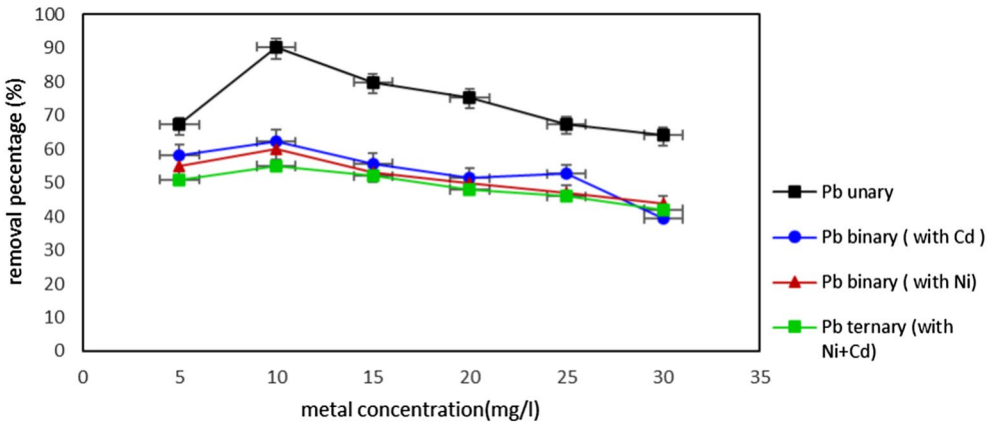

Figure 7. The effect of the amount and initial concentration of lead unary, lead binary (with cadmium and nickel) and lead ternary in the designed biological reactor on their removal percentage.

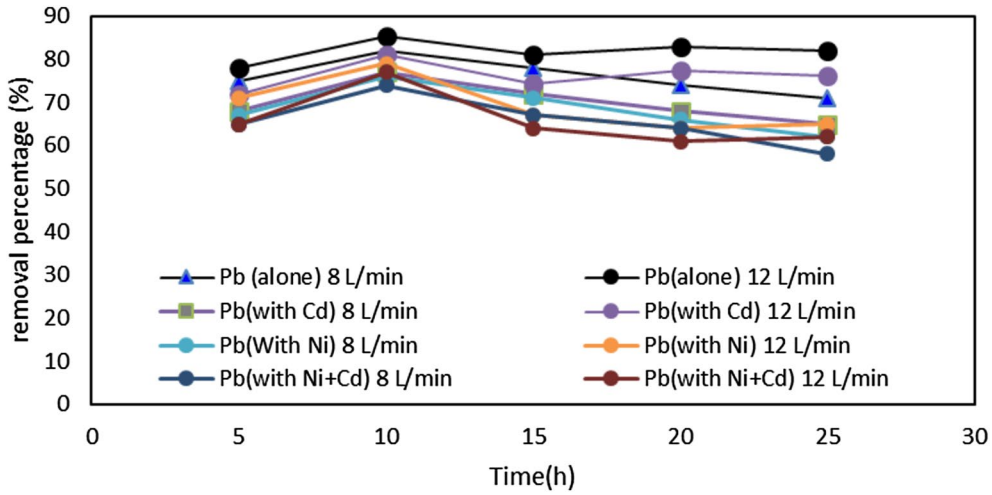

Figure 8. The effect of aeration rate on the removal of lead unary, lead binary (with cadmium or nickel) and lead ternary in the designed biological reactor on their removal percentage.

Also, in this project, the rate of removal of nickel, cadmium, and lead metals was stabilized by bacteria, and it was freely investigated that the rate of removal by bacillus bacteria fixed in the biological reactor is higher, and the percentage of metal removal is significant; this fixation has been formed on sodium alginate[30–32]. The bacterium was first immobilized on sodium alginate and then on fixed and mobile polyethylene packaging. Noteworthy is the fact that in the continuation of the experiments, the results of which will be reported, inexpensive and more appropriate stabilizers were also employed; these will be detailed in the subsequent reports[33–35] .

Conclusion and future work

The adsorption efficiencies for single lead (unary), lead together with nickel, cadmium (binary), and ternary (lead with cadmium and nickel) were found to be 0.36, 0.25, 0.22, and 0.21 mmol/g, respectively. The ideal temperature for lead removal was around 30 °C. Based on the kinetic models and experimental data, the biosorption of each heavy metal followed a second-order kinetic model and the Langmuir isotherm. Utilizing this heavy metal-resistant strain in an MBBR reactor and adhering to optimal conditions can effectively remove heavy metals from effluents. The next step for this research team is to use the MBBR reactor on an industrial scale in the industrial town of Babolsar.

Data availability

All of the raw data will be available upon request from corresponding author.

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Author contributions

S.A.A. performed the experiment, analysed the data, prepared figures, and wrote the manuscript draft. S.A.A. suggested the experiment condition and edited the manuscript. S.A.A. and M.A.N., and C.G. provided the funds and supervised the projects. All authors reviewed the manuscript.

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Competing interests

The authors declare no competing interests.

Additional information

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