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Biosorption of lead and cadmium from aqueous solutions by proteins extracted from E. coli cells

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Abstract

The potential use of bacterial-based protein extracts for heavy metal removal from aqueous solution was investigated. The proteins-based biosorbent were extracted from wild-type *E. coli* strain MG16755 using sonication. The metal ions adsorption efficiency was determined in batch mode. The maximum metal ions adsorption capacity was achieved after 24 hours at 37^{o}C and pH=6. Fourier-transform infrared spectroscopy (FTIR) analysis demonstrated that the protein extract is capable of interacting with heavy metal ions through its functional groups (O-H / N-H, C=O, and C-O / P-O). The equilibrium data aligned well with the Langmuir model, indicating that the protein extract's maximum adsorption capacities (Q_{max}) for Pb (II) and Cd (II) were 204 mg/g and 164 mg/g, respectively. The Dubinin-Radushkevich (DRK) isotherm model estimated the mean free energy to be 0.7 kJ/mol for both Pb (II) and Cd (II), indicating that the adsorption process is primarily physical in nature. Additionally, the adsorption of Pb (II) and Cd (II) followed pseudo-second-order kinetics most closely. The findings of this study provide fundamental recommendations for the optimal utilization of EPS from *E. coli* in bioremediation for wastewater treatment.

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1. Introduction

The US Environmental Protection Agency (USEPA) compiled in 1978 a list of 129 organic and inorganic contaminants commonly detected in wastewater that cause serious health risks in humans. The thirteen metals on this list are arsenic, mercury, beryllium, cadmium, chromium, lead, nickel, copper silver, and zinc (Balali-Mood *et al.*, 2021). Exposure to toxic metals that exceed permissible levels can lead to cumulative poisoning, cancer, and brain damage. Surveys conducted by public health services in various countries indicate that many of the population is affected by risks associated with high concentrations of metals in water supplies. Unlike organic compounds, metals are non-biodegradable, and therefore, must be eliminated from wastewater (Mahurpawar, 2015). Various methods have been introduced to reduce heavy metal contamination in effluents, including chemical precipitation, membrane separation coagulation, ion exchange, solvent extraction and reverse osmosis. When dealing with low metal concentrations, ion exchange,

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reverse osmosis, and adsorption are the most efficient approaches. Among the different adsorption techniques, activated carbon is the most extensively utilized (Chakraborty *et al.*, 2022). Fly ashes (Dobrowolski *et al.*, 2019) and zeolites (Debnath *et al.*, 2021) are examples of geological and mineral-derived sorbents that have also been used. The use of biomaterials as adsorbents is growing due to their cost-effective production, strong selectivity, and high affinity for heavy metals. These materials are commonly derived from bacterial cells (Vickers, 2017), algae, yeast (Boakye *et al.*, 2022) and fungi (Olawale, 2021)**.** Extracellular polymeric substances (EPS) are biopolymers generated by bacterial cells, comprising a diverse blend of complex compounds such as polysaccharides, proteins, phospholipids, acids, and various other substances (Shanmugam *et al.*, 2022). EPS have been sourced from various origins, including biological wastewater treatment facilities (Huang *et al.*, 2022), algae (Koçer *et al.*, 2021), and bacterial cell cultures (Czemierska *et al.*, 2016). These biopolymers contain ionizable functional groups, including amine, phosphoric, carboxyl, and hydroxyl groups, which are integral in the elimination of heavy metals. The presence of these functional groups enables multiple removal mechanisms, such as ion exchange, where metal ions are replaced by other ions from the biopolymer, and complexation, where negatively charged sites bind with metal ions to form stable complexes (Wang *et al.*, 2022).

EPS can be extracted from several types of bacteria such as *Escherichia coli* that had been used in this study. *E. coli* is a bacterial species commonly present in the intestines of humans and animals. It is a Gram-negative, rod-shaped bacterium that is typically non-pathogenic, meaning that it does not cause disease. However, there are certain strains of *E. coli*, such as O157:H7, that can lead to serious diseases including bloody diarrhea and kidney failure (Cieślik *et al.*, 2021). *E. coli* is also used in a variety of industrial applications, such as food fermentation and wastewater treatment. They are also used in the production of antibiotics, vitamins, and others (Farghali *et al.*, 2023).

Recent reviews have highlighted the increasing use of bacterial strains such as Bacillus and Pseudomonas for heavy metal biosorption, especially due to their high tolerance to metal toxicity (Pham *et al.*, 2022; Torres, 2020). In a study by Li et al., (Li *et al.*, 2022), proteins extracted from *Escherichia coli* were utilized for lead and cadmium adsorption from contaminated water, achieving adsorption capacities of 210 mg/g for Pb(II) and 170 mg/g for Cd(II). The proteins showed a high affinity for these metal ions, driven by functional groups such as carboxyl and hydroxyl, which facilitated binding. Mathivanan et al. (Mathivanan *et al.*, 2021) explored the potential of EPS derived from *Bacillus cereus* KMS3-1 strains for the removal of Pb(II), Cu(II), and Cd(II) from wastewater. The adsorption experiments were conducted in batch mode for 150 minutes, using an initial heavy metal concentration of 50 mg/L and 0.5 g of EPS. The process reached equilibrium within 30 minutes, with adsorption capacities recorded at 39.45 mg/g for Pb(II), 36.79 mg/g for Cu(II), and 32.98 mg/g for Cd(II). Similarly, Cui et al. (Cui *et al.*, 2020b) reported that EPS extracted from *Agrobacterium tumefaciens* F2 exhibited optimal biosorption efficiency for Pb(II), Ni(II), and Cd(II) at pH 6. These studies highlight the effectiveness of bacterial proteins as an eco-friendly and efficient approach to heavy metal removal in wastewater treatment.

While many researchers have investigated the absorption of lead and cadmium using proteins from bacterial sources, the present study presents several key distinctions that advance current knowledge in this field. First, this study specifically uses proteins extracted from E. coli, a model organism with well-characterized metabolic pathways, allowing for a better understanding and potential bioengineering applications in mineral isolation. Secondly, the study provides a detailed description of the extracted proteins, including their functional groups, molecular weight distribution, and binding mechanisms. In contrast to previous work, which focused primarily on adsorption efficiency, this study incorporates Fourier transform infrared spectroscopy (FTIR) to elucidate the specific interactions between functional groups (such as carboxyl, hydroxyl, and amine) and iron and cadmium ion. Furthermore, the research investigates the role of pH, protein concentration and adsorption kinetics and provides a comprehensive evaluation of adsorption parameters that have not been explored in previous studies. By addressing these factors, the study not only confirms adsorption efficiency but provides mechanistic insights that can be applied to improve protein-based adsorbents for real-world applications. This investigation aimed to evaluate the efficacy of proteins derived from E. coli in the adsorption of Pb(II) and Cd(II) ions. The study also examined the influence of critical operational parameters, including Ph and contact time, on the biosorption efficiency. Moreover, the equilibrium data for biosorption were analyzed using several isotherm models to identify the most appropriate adsorption behavior.

2. Materials and Methods

2.1 Cultivation of *E. coli* bacterial cells

Frozen stocks of the Gram-negative wild-type *E. Coli* stress MG1665 have been streaked onto freshly prepared LB agar plates and incubated overnight at 37°C. The subsequent day, a single colony wasselected and used to inoculate 3 mL of sparkling LB broth in a tumbler tube, which turned into then incubated in a roller drum at 37°C for 16 hours. To expand the lifestyle, 50 µL of the initial

suspension was transferred into 250 mL Erlenmeyer flasks containing 50 mL of LB broth. These flasks have been then incubated in an orbital shaker water tub at one hundred fifty rpm for 24 hours at 37°C. Bacterial increase become monitored by way of measuring the optical density (OD) at 600 nm (OD600) using a UV-Vis spectrophotometer. The bacterial cells had been harvested by centrifugation at 10,000 g for 10 minutes at 4°C, and then the pellet became resuspended in a breaking buffer solution. Cell disruption become accomplished the usage of an ultrasonic probe (SONICS, USA) at a frequency of fifty kHz for 20 mins at 4°C. The resulting crude extract was centrifuged at 6,000g for half-hour at 4°C to cast off residual cell particles.

2.2 Extraction of protein extract from E. coli

Proteins were precipitated from the crude extract by adding ammonium sulfate (Fisher Scientific, India) to achieve 90% saturation at 4°C. The precipitated proteins were then separated by centrifugation at 6000g for 10 minutes at 4°C. The obtained pellet was dialyzed three times against phosphate-buffered saline (pH 7.4) at 4°C. Protein concentration was determined using the Bradford method, with bovine serum albumin as the standard. Finally, the protein stock was completely dried into a powdered form through freeze-drying at −65°C.

2.3 Batch Biosorption Experiments

All reagents employed in this research were of analytical grade. A stock solution (1000 mg/L) of Pb $(NO_3)_2$ and $Cd(NO_3)_2 \cdot 4H_2O$ was prepared by dissolving the respective compounds in deionized (DI) water. For batch adsorption experiments, 0.02 g of protein powder changed into combined with the metal stock solution in an Erlenmeyer flask, and the full extent changed into adjusted to 50 mL. The solution pH become maintained at 6 the usage of both zero.1 M HCl or 0.1 M NaOH. The combination turned into then incubated in a shaker water bath at three hundred rpm and 37°C for 24 hours. Following the biosorption technique, the residual heavy metal awareness was quantified the use of an atomic absorption spectrophotometer (AAS) (SHIMADZU, Japan). The adsorption ability of metals onto the E. Coli protein extract changed into decided the usage of the following equation:

$$q_e = \frac{(Co - Ce)\ V}{m} \qquad (1)$$

In this equation, q_e(mg/g) represents the amount of adsorbate absorbed, C_o denotes the initial concentration (mg/L), the equilibrium concentration of heavy metals is expressed in mg/L, V refers to the solution volume (L), and m indicates the mass of the protein extract (g).

3 Characterization of protein extract

3.1 FTIR-ATR Surface Characterization for Protein Extract

The amount of protein extract after multiplying one Colony in LB broth was found 200.0 mg/g based on the Bradford method using bovine serum albumin as the standard. The protein-based adsorbents produced by E. coli were characterized using FTIR-ATR analytical techniques to determine the chemical groups of the protein extract before the adsorption of Heavy metals.

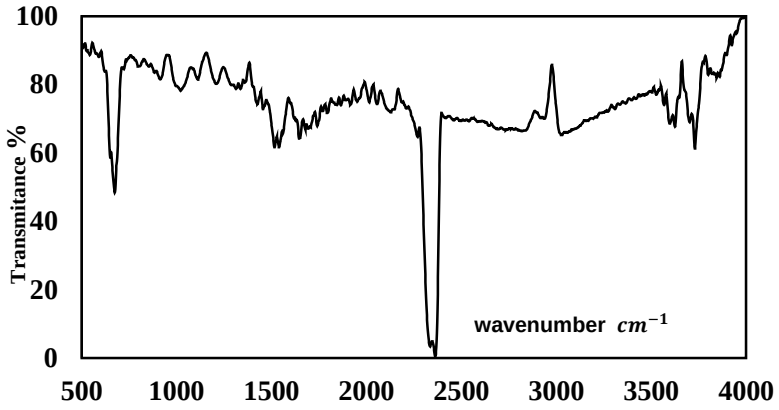

Fig. 1. FTIR spectra for EPS before heavy metal adsorption..

Figure 1 shows the Fourier Transform Infrared – Attenuated Total Reflectance mode (FTIR-ATR) spectra of protein extract before heavy metals adsorption. Various functional groups were observed as shown in Fig.1, including carboxyl, hydroxyl, amine, and phosphoric groups, which are found in proteins (Huang *et al.*, 2022). These functional groups act as potential binding sites for capturing and retaining heavy metal ions. (Mohite *et al.*, 2018). The binding of lead and cadmium ions to proteins extracted from *E. Coli* is commonly stimulated with the aid of the useful companies inside the protein. FTIR evaluation identified the presence of carboxyl, hydroxyl, amine, and phosphoric companies. Which plays an important role in bonding metals through various mechanisms, including speciation bonding, electrostatic interactions, and hydrogen bonding. Studies by Zeng et al., (Zeng *et al.*, 2020) have shown that the protein extracted from *Bacillus sp. S3* contains functional groups, as mentioned previously, which play a crucial role in the process of chelation of metal ions. Protein extracted from bacteria has been extensively studied for its ability to adsorb heavy metals due to its diverse functional groups and high surface area. Also, EPS extracted from *Pseudomonas putida* showed great biosorption potential due to the composition of the unique cell envelope, which includes lipopolysaccharides, proteins, and outer membrane vesicles. The study found that EPS extract from *P. putida* exhibits similar FTIR bands to those observed in E. coli proteins, particularly in regions corresponding to key functional groups involved in metal binding. Furthermore, *P. putida* proteins showed strong adsorption of cadmium (II) ions, highlighting their potential as effective biosorbents for heavy metal treatment (Ueshima *et al.*, 2008).

3.2 The Effects of Adsorption Parameters on Metal Ion's Adsorption Efficiency

3.2.1 Effect of Contact Time

Contact time is a crucial parameter influencing the adsorption of metallic ions onto the adsorbent. The adsorption capability (q_e) of proteins extracted from E. Coli for both Pb (II) and Cd (II) ions exhibited a huge increase with prolonged contact time. Initially (0 –6 hours), the removal of Pb (II) ions changed into speedy, accompanied with the aid of a deceleration in the adsorption price after eight hours in any respect tested concentrations. The equilibrium adsorption capability (q_e)) of the protein extract become attained after 24 hours. Typically, metallic ion adsorption reaches equilibrium whilst all to be had binding sites at the adsorbent are occupied, constant with the observations of (Priyatharshini *et al.*, 2019) who mentioned that Pb(II) adsorption onto bacterial EPS stabilized after seventy two hours. Additionally, the time required for the adsorbent to reach complete saturation with metallic ions is called the equilibrium time.

3.2.2 Effect of Solution pH on metal ions adsorption

PH is a key issue in controlling the interactions between metal ions and the purposeful groups of biosorbents. Pb (II) ions generally tend to precipitate as $Pb(OH)_2$ while the pH exceeds 7, at the same time as Cd (II) ions shape $Cd(OH)_2$ at pH degrees above 7 as a result of the accelerated concentration of hydroxide (OH^-) ions within the solution (Wei *et al.*, 2016). Consequently, the influence of pH was evaluated within the range of 4 to 7. Figure 2 presents the adsorption behavior of Pb (II) and Cd (II) ions across different pH levels.

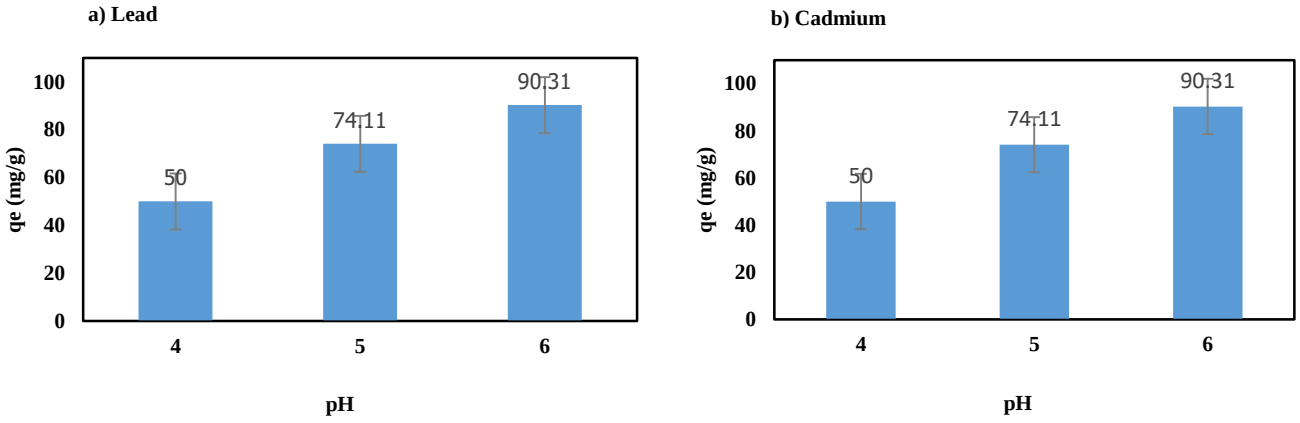

Fig. 2. Effect of solution pH on the adsorption by EPS (contact time = 24 hr., 0.02 g of protein and 50.0 mg/L lead in 50.0 ml at 37ºC).

The adsorption capacity (q_e) in mg/g showed a growing trend with growing pH levels, reaching its peak at pH 6. At lower pH values, the high concentration of hydrogen ions (H^+) and hydronium ions (H_3O^+) competes with metallic ions for to be had binding web sites on the protein, ensuing in reduced adsorption efficiency. In contrast, as the pH increases, the decreased concentration of H^+ and H_3O^+ ions minimize competition, permitting extra metal ions to attach to the binding websites and improving adsorption potential. (Mathivanan *et al.*, 2021) observed that the optimal adsorption capacity for Pb (II) ions using EPS occurred at pH 6, while (Shen *et al.*, 2021) similarly observed the highest adsorption capacity for cadmium using EPS at the same pH level.

3.3 Adsorption kinetics

Kinetic experiments had been carried out to evaluate the influence of touch time on ion elimination and to discover the capacity adsorption mechanisms (Sahnoun & Boutahala, 2018). The study involved varying the initial metal concentration while keeping the protein extract mass constant at 0.02 g, with agitation at 300 rpm and a temperature of 37°C. Samples have been accrued at particular time durations, and the ultimate heavy metal attention inside the solution wasmeasured the usage of an Atomic Absorption Spectrophotometer (AAS). The experimental information had been evaluated using non-linear fashions of pseudo-first-order (PFO) (Lagergren, 1898), pseudo-2d-order (PSO) (Azizian, 2004), as summarized in **Table 1.**

Table 1: Summarizes the kinetic models that analyzed in this study.

Kinetics model	Equation	Parameters
Pseudo-first-order	$q_t = q_e(1 - e^{-k_1 t})$	$k_1\ (min^{-1})$: PFO rate constant, representing the adsorption rate.
Pseudo-second-order	$q_t = \frac{t}{\frac{1}{q_e^2 k_2} + \frac{t}{q_e}}$	k_2 (g/mg min): PSO rate constant, indicating adsorption kinetics.

Table 2: The calculated parameters of these kinetic models for lead and cadmium removal.

Kinetic model	Parameter	Initial concentration of Pb (II) mg/L			Initial concentration of Cd (II) mg/L		
		7.00	20.00	50.00	7.00	20.00	50.00
Pseudo first order kinetic model	$q_e\ (mg\ g^{-1})$	13.53	41.74	106.58	13.96	39.1	96.09
	$k_1(min^{-1})$	0.0035	0.0031	0.003	0.0042	0.0037	0.0035
	R^2	0.9923	0.9848	0.9544	0.9809	0.9908	0.9925
pseudo second order kinetic model	$q_e\ (mg\ g^{-1})$	14.73	41.84	96.15	14.28	40.65	89.82
	k_2 g/(mg. min))	1.21×10^{-3}	2.47×10^{-4}	1.14×10^{-4}	1.42×10^{-3}	2.8×10^{-4}	1.14×10^{-4}
	R^2	0.9961	0.9888	0.9818	0.9954	0.9901	0.9823
	$q_{e\ (exp)}$ $(mg\ g^{-1})$	14.25	39.49	90.5	13.81	38.53	83.96

Table 2 provides the kinetic model parameters. Although the pseudo-first-order (PFO) model exhibited a high correlation coefficient (R^2), the predicted (q_e) values showed giant deviation from the experimental records, indicating its obstacles in correctly describing the adsorption kinetics of Pb (II) and Cd (II). Conversely, the pseudo-2nd-order (PSO) version supplied (q_e) values that closely matched the experimental outcomes across all examined concentrations. This indicates that the pseudo-second-order model more accurately characterizes the adsorption process, highlighting the role of chemical interactions between lead ions and proteins as the primary factor

governing the adsorption rate. (Kaleem *et al.*, 2023). The rate coefficients k_1 and k_2 are generally influenced by the initial concentration of the adsorbate. These coefficients, which function as time-scaling factors, tend to decrease as the initial adsorbate concentration increases. Consequently, higher starting concentrations of the adsorbate extend the time required to reach equilibrium, leading to lower k_1 and k_2 values (Gupta & Bhattacharyya, 2011).

3.4 Adsorption Isotherm

Once equilibrium is reached at a consistent temperature, isotherm models are applied to evaluate the adsorption performance of steel ions, analyze the interplay among the adsorbent and adsorbate, and decide key adsorption parameters. Additionally, these models facilitate the evaluation of steel ion adsorption capacities throughout diverse adsorbates the use of the same adsorbent material (Sharma *et al.*, 2019). To examine the adsorption isotherms, the equilibrium data were fitted to the non-linear forms of the Langmuir (Langmuir, 1918), Freundlich (Freundlich, 1906), and Dubinin-Radushkevich (Chen, 2015) models as shown in **Table 3.**

Table 3. Adsorption isotherm models.

Model	Equation	Parameters
Langmuir	$q_e = \frac{q_{max}\ b\ C_e}{1+bC_e}$	q_{max}(mg/g): the maximum uptake at the given conditions at all sites. b (L/mg): Langmuir constant indicating adsorbate-adsorbent affinity. C_e (mg/L): Equilibrium adsorbate concentration
Freundlich	$q_e = k_F C_e^{\frac{1}{n}}$	k_F ((mg/g) (L/mg: Freundlich adsorption coefficient, describing adsorption extent at unit equilibrium concentration. n: Adsorption intensity, indicating process favorability.
Dubinin–Radushkevich	$q_e = q_{DR} \times e^{-k_{DR}\varepsilon^2}$ $\varepsilon = RT \times \ln(1+\frac{1}{C_e})$ $E = \frac{1}{\sqrt{2k_{DR}}}$	q_{DR} (mg/g): The Dubinin-Radushkevich isotherm constant reflects the adsorbent's maximum adsorption capacity and mechanism. k_{DR} (mol^2/kJ^2): constant related to the adsorption mean free energy. Ɛ: parameter based on temperature E (kJ/mol): Mean free energy required to remove an adsorbate molecule from the adsorption site to an infinite distance.

Table 4 presents the adsorption parameters, indicating that the Langmuir model estimated the maximum adsorption capacity (Q_{max})of EPS to be 204.1 mg/g for Pb (II) and 163.93 mg/g for Cd (II). The high regression correlation coefficient (R^2) for both metals confirms that the Langmuir isotherm best describes the adsorption process, indicating monolayer adsorption without adsorbate interactions (Langmuir, 1918). The Freundlich model parameters K_F for Pb (II) and Cd (II) were 11.9, 1.27, and 11.134, respectively. Since n values exceeded one, this confirms the protein extract's strong affinity for metal ions. Additionally, the mean free energy (E) derived from the Dubinin–Radushkevich model wasmuch less than 8 kJ/mol for each metal, indicating that the adsorption procedure is in general physically in nature and governed by Van der Waals forces (Nandiyanto *et al.*, 2022). A comparison with previous studies Table 5, highlights that the biosorption capacities of 204 mg/g for Pb (II) and 164 mg/g for Cd (II) ions in this study are comparable to or exceed those reported for other biosorbents.

While the study demonstrates the potential of protein-based biosorption for the eliminating of heavy metals, a broader comparison with current adsorption methods using proteins extracted from different species of bacteria would provide deeper insights into their relative performance (Table 5). Previous studies have highlighted the effectiveness and efficiency of biosorbents, such as proteins extracted from *Pseudomonas putida*, in isolating heavy metal ions. These biomaterials contain distinct functional groups and structural features that influence adsorption capacity, selectivity, and regeneration potential (Ueshima *et al.*, 2008). In addition, while experimental results confirm the efficiency of heavy metal adsorption, practical implementation on an industrial scale requires further research. Critical aspects such as cost-effectiveness, adsorbent regeneration and feasibility of large-scale production must be addressed to determine real-world applicability. For example, evaluating the reusability of protein-based adsorbents through multiple adsorption and desorption cycles would provide insight into their long-term performance and economic feasibility. Furthermore, industrial applications should take into account regulatory requirements, environmental impact, and integration with existing wastewater treatment technologies. To

improve industrial applicability, improving the adsorption conditions and ensuring the stability of the adsorbent is essential. Also, collaboration with industries to conduct tests on a pilot scale will help verify the feasibility of protein-based sorbents for large-scale wastewater treatment.

Table 4: Adsorption isotherm parameters.

Model	Parameters	Pb (II)	Cd (II)
Langmuir	$q_{max}\,(mg\ g^{-1})$	204.1	163.9
	b($L\ mg^{-1}$)	0.057	0.0642
	R^2	0.999	0.9947
Freundlich	$k_F\,(mg\ g^{-1})\,(L\ mg^{-1})^{1/n}$	11.9	11
	N	1.27	1.34
	R^2	0.9958	0.9872
Dubinin–Radushkevich	$q_{DR}\,(mg\ g^{-1})$	69.3	66
	E (kJ/mol)	0.707	0.745
	R^2	0.8847	0.9006

Table 5: A Comparative analysis of the maximum adsorption capacity of heavy metals onto bio-sorbent materials in this study with previous studies.

Heavy metals	Q_{max} (mg/g) (Based on Langmuir isotherm)	Bio-adsorbent	Ref
Pb (II) Cd (II)	204.1 163.9	Protein extracted from *E.coli*	This study
Pb (II)	153.58	EPS extracted from *Klebsiella*	(Wei *et al.*, 2016)
Pb (II) Cd (II)	78.47 54.05	Exopolysaccharides produced by *Bacillus cereus* KMS3-1	(Mathivanan *et al.*, 2021)
Pb (II)	417.67	EPS extracted from *Pseudomonas aeruginosa N6P6*	(Kumari *et al.*, 2017)
Cd (II) Cu (II)	85.5 40.0	EPS extracted from *Aspergillus fumigatus*	(Yin *et al.*, 2011)
Pb (II) Cd (II) Ni (II)	625.00 92.59 45.05	EPS	(Cui *et al.*, 2020a)

Conclusions

This study aimed to evaluate the efficiency of proteins extracted from *E. Coli* within the removal of Pb and Cd ions at the same time as investigating the effect of key operational parameters, together with touch time and pH, on biosorption overall performance. The experimental information had been analyzed the use of pseudo-first-order and pseudo-2d-order kinetic fashions, in addition to three adsorption isotherm fashions—Langmuir, Freundlich, and Dubinin-Radushkevich (D-R)—to decide the maximum suitable version for describing the discovered adsorption behavior. The presence of negatively charged practical agencies, consisting of carboxyl, hydroxyl, amine, and phosphoric organizations, allows heavy metal attachment via automated ion exchange between the protein and metallic ions. The utility of kinetic and isothermal fashions confirmed that biosorption follows a pseudo-2d-order mechanism, with the Langmuir isotherm presenting the great fit. The findings propose that EPS derived from *E. Coli* demonstrates a better adsorption ability than other bio-adsorbents, highlighting its capacity as a powerful, fee-efficient, and practical method for heavy metal removal from wastewater.

Recommendation for future work

- Producing EPS from several types of bacteria.
- Apply the potential of using EPS as an adsorbent material for the removal of various other heavy metals.

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