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Nanobiopolymer: Potential Applications in Bioremediation of Cadmium Contaminated Water

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Abstract

Nanobiopolymer nanotechnology, bacterial extracellular polymeric substances (EPS), has been used markedly for various water treatments. EPS has polysaccharides, proteins, and lipids, which have functional groups such as amino, sulfhydryl, carboxylic, and phosphate groups that allow binding to metal ions. The accumulation of the metal ions onto the EPS bacterial cell walls is used widely in the bioremediation of cadmium and other metals. This study objectives were extracting EPSs from the bacterium *Bacillus subtilis* 168 Cd2 and investigating their cadmium adsorption in bead column reactors after being immobilized with calcium alginate. For comparison with the bacterium's role, *B. subtilis* 168 Cd2 was used before EPS extraction and immobilized with calcium alginate, as well as a comparison with the use of calcium alginate. Cadmium adsorption from solution onto calcium alginate beads with untreated *B. subtilis* 168 Cd2 and EPS-free *B. subtilis* 168 Cd2 and under different pH (4.00, 7.00, and 7.50) were studied. The percentages of adsorption onto untreated *B. subtilis* 168 Cd2 and EPS-free *B. subtilis* 168 Cd2 were determined and were 89% and 77%, respectively. The adsorption of these were reduced when the pH increased. Scanning electron microscopy (SEM) confirmed the changes in the morphology of the adsorbents, and X-ray fluorescence (XRF) spectrometry analysis recorded the cumulative concentrations of cadmium in the adsorbents after the cadmium adsorption process. EPS in untreated *B. subtilis* 168 Cd2 significantly enhanced cadmium adsorption percentages. The fewer carboxyl and phosphate groups in EPS-free *B. subtilis* 168 Cd2 decreased cadmium adsorption. The results obtained in this study are of fundamental significance for applying nano biopolymers nanotechnology environmental biotechnology and bioremediation.

1. Introduction

Water pollution has become a significant concern of human health due to pollution induced by industrialization, urbanization, and population growth. Metal pollution is among the numerous sources of water pollution because it is toxic and undegradable [1].

In order to make contaminated waters suitable for landfill disposal, remediation techniques need to be applied. The methods for treating metal ions and remediating them from water, such as chemical methods, are unsuccessful to clean low metal concentrations and produce large amounts of slush, that needs further treatments [2],[3]. On the other hand, adsorption techniques are efficient for metal removal. Biosorbents such as plants, algae and bacteria have been used in adsorption techniques [4]. Bioremediation that is using bacteria is a more convenient method than other biosorbents to remediate metal from water due to its cost-effective technology, simplicity of installation, and use of available biomaterials that require the lowest energy requirement. Biosorption comprises the metal- surface adsorption [5], [6] that occurs via their functional groups such as carboxyl, ketones, and aldehyde groups in addition to the ion exchange, chelation, complexation, and precipitation [7]. Ion exchange is recognised for animation the essential part of biosorption, which encompasses bacterial element exchange with metal ions [8]. Subsequently, the adsorption or ion exchange routes, chelation and complexation container follow [9]. Bacterial extracellular polymeric substance (EPS), which is also referred to as the biofilm and its chemical composition depends on microbial genetics and environmental conditions. EPS has polysaccharides, proteins, and lipids, which have functional groups such as amino, sulfhydryl, carboxylic, and phosphate groups, which allow binding to metal ions [10]. The accumulation of the metal ions onto the EPS bacterial cell walls is used widely in the bioremediation of cadmium and other metals stated the benefits and weaknesses of applying EPS as it is an economical material and could be reprocessed aimed at adsorption and salvage of metal ions [3]. The EPS extracted from *B. subtilis* have been used in the adsorption of Cu (II)[11], [12], [13], [14], Al forms [15] and Cd [2], [9], [13].

Therefore, the main aim of this study was to investigate the role of EPS from *B. subtilis* 168 Cd2 in wastewater purification from cadmium. *B. subtilis* 168 Cd2 was obtained from our previous work [2] and it has a minimum inhibitory concentration (MIC) of 2 mM Cd. The objectives of the study were the use of extracting EPS from this bacterium, and the investigation of their work in purification bead reactors after being immobilized with calcium alginate. For comparison with the role of the bacterium, *B. subtilis* 168 Cd2 was used before EPS extraction immobilised with calcium alginate, as well as a comparison with the use of calcium alginate. These objectives included the observing of EPS in *B. subtilis* 168 Cd2 and the difference in the immobilised EPS before and after the adsorption under scanning electron microscopy (SEM). Conclusions were also drawn about the mechanism that led to the adsorption.

2. Materials and Methods

2.1. Bacterial Strain

The EPS-producing strain *B. subtilis* 168 Cd2 was obtained from our laboratory in previous work [2]. The strain was stored at – 80°C in E-Basel Salts (EBS) medium/fructose (10 mM) (10) supplemented with 20% (*v*/*v*) of glycerol. For experiments, frozen stock was streaked on EBS/fructose agar plates and incubated at 37 °C for 24 h. The agar plates were stored at 4°C and used within three weeks.

2.2. Cell Culture and Preparation

Growing bacterial colony of *B. subtilis* 168 Cd2 on E-Basel Salts (EBS) medium/fructose (10 mM) agar was picked up and observed under a light microscope and an inoculum prepared to get harvested cells, then collected as untreated *B. subtilis* 168 Cd2.

2.3. Preparation of EPS-free Cells

EPS were isolated using chemical extraction protocol (ethanol). Briefly, bacterial suspensions of *B. subtilis* 168 Cd2 from the early stationary growth phase were centrifuged twice at 4 °C for 30 mins at 16000 isolate EPS from other cell components and 3 volume of the cold ethanol was added to it and incubated at − 20 °C overnight. This step was repeated to get further purification, and the EPS was freeze-dried to be ready to use.

2.4. Laboratory Bench-scale Column Reactors Setup, Adsorption Experiments

The setup of the laboratory reactor is shown in Figure (1), and the schematic diagram and photo of the actual column reactor used in this study. The system is composed of a reactor (premium grade of silicone-free and polypropylene column), and the reactors were filled with immobilised beads. A peristaltic pump was used to maintain the feed level in the bioprocess bag.

Iraqi Journal of Industrial Research, Vol. 10, No. 2 (2023)

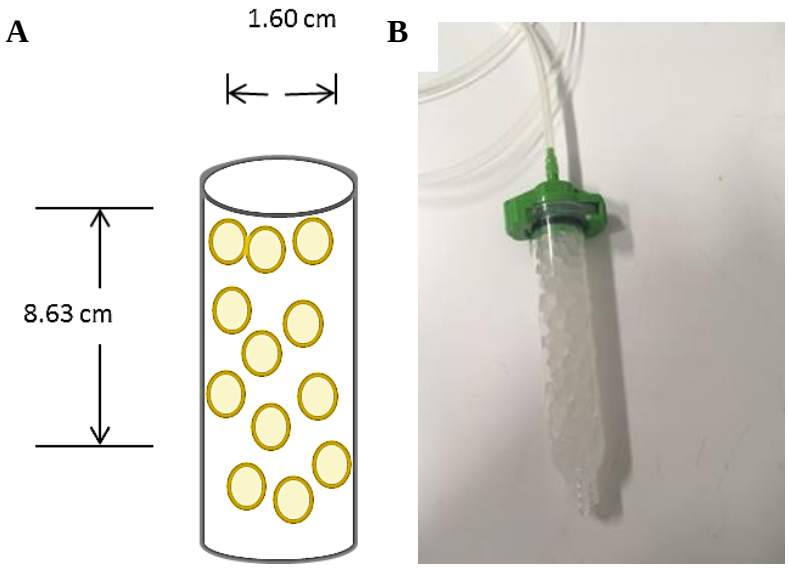

Figure (1). A- Schematic diagram, and **B-** photograph of the plastic column reactor. The column was filled with immobilised beads.

The schematic configuration and photo of a single experimental column reactor are shown in Figure (2).

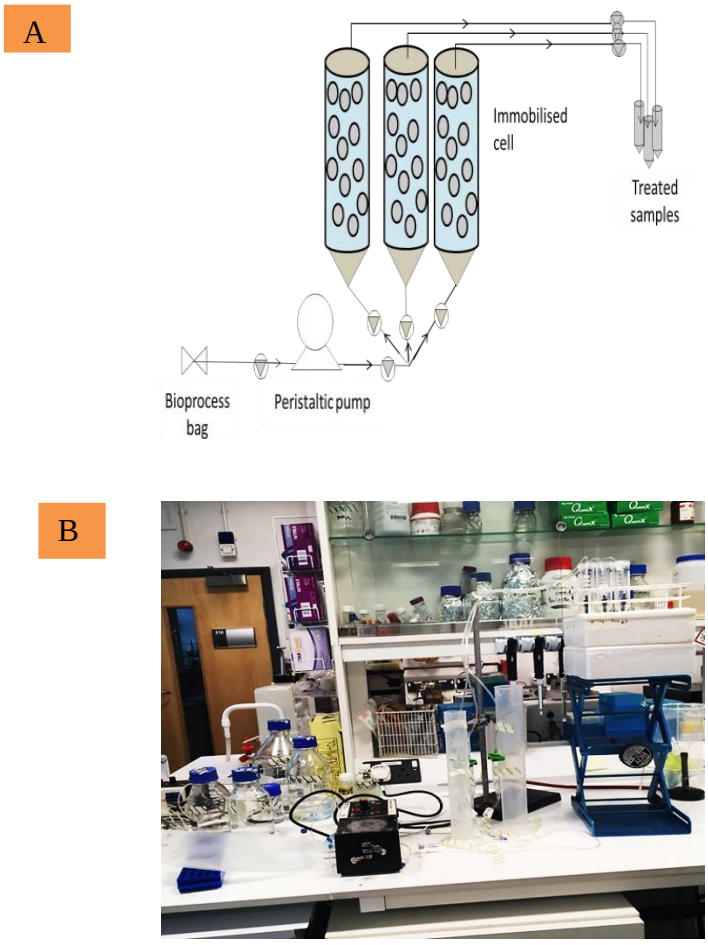

Figure (2). A- Scheme, and **B-** photograph of the actual single experimental column reactor used in this study.

In the adsorption experiments of cadmium, EBS/F with an insignificant concentration of 1.8 mM Cd, selected to be below the MIC of *B. subtilis* 168 Cd2, was used to be cleaned by five reactors:

Ca-alginate beads holding, untreated *B. subtilis* 168 Cd2

Ca-alginate beads holding EPS-free *B. subtilis* 168 Cd2,

Control Ca-alginate beads without untreated *B. subtilis* 168 Cd2 or EPS-free *B. subtilis* 168 Cd2.

Reactors were operated for 48 hours, and eluanta samples were collected every two hours to determine the total concentration of cadmium. The immobilisation of untreated *B. subtilis* 168 Cd2 or EPS-free *B. subtilis* 168 Cd2 in the Ca-alginate matrix was carried out according to [8]. The amount of adsorbed cadmium was regularised according to [9].

2.5. Scanning Electron Microscopy (SEM) and X-ray Fluorescence (XRF) Spectrometry Analysis

For observing the colony of untreated *B. subtilis* 168 Cd2, and the differences in the beads before and after the adsorption, the beads were fixed using our previous protocols [8], [10]. Then the samples were dried using a critical point drier (EMTECH K850), coated with gold, and analyzed using an SEM analyzer (JEOL-JSM-6610LV). As the un-flat section of the beads is not suitable for EDX analysis that is equipped with SEM to analyze the constituents (cadmium before and after the adsorption) of the beads, our previous protocol of using XRF for direct analysis of beads was used [9].

2.6. Chemical Analysis

The adsorption of cadmium from EPS/F was evaluated after decisive the concentrations of cadmium residual in the EPS/F eluants using inductively coupled plasma mass spectrophotometry (ICP-MS, Thermo Scientific, X Series 2), after acidifying with 1% nitric acid. For precision of the instrument, indium was used as an internal standard for ICP-MS, in addition to establish limit of detection (LOD) and the recovery of the Cd analysis.

3. Results and Discussion

3.1. Chemical Properties of EPS

It could be possible to obtain the chemical properties of EBS of *B. subtilis* 168 from other previously studied *B. subtilis* 168. It has been reported that the purified EPS of *B. subtilis* composed of (mg/g): 340 (organic carbon, (EPS-OC), 80 (nitrogen, EPS-N) and 35 (phosphorus, (EPS-P) [16], [17]. Visualization of untreated *B. subtilis* 168 Cd2 revealed the EPS aggregates with cells of *B. subtilis* 168 Cd2 (Figure.3).

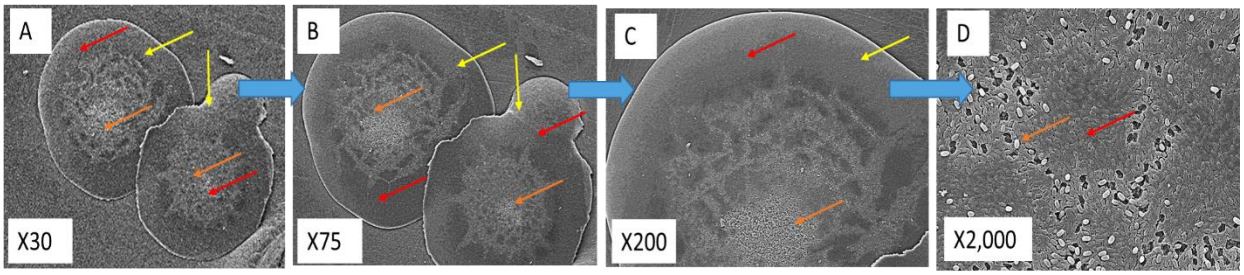

Figure (3). SEM micrographs of untreated *B. subtilis* 168 Cd2. Yellow rows: colony, orange arrows: EPS, and red arrows: bacterial cell.

3.2. Cadmium Adsorption Experiments

The percentage adsorption of cadmium on untreated *B. subtilis* 168 Cd2 and EPS-free *B. subtilis* 168 Cd2 were shown in Figure (4). The maximum percentage adsorption of cadmium on untreated *B. subtilis* 168 Cd2 (70 %) was more than that on EPS-free *B. subtilis* 168 Cd2 (65 %), indicating that bacteria removal from EPS significantly diminutions cadmium adsorption level on bacterial EPS. As compared with control beads, cadmium adsorption diminished by 46 %, indicating that the lack of *B. subtilis* 168 Cd2 or EPS may obviously reduce cadmium adsorption mechanisms. Understanding the role of EPS-free *B. subtilis* 168 Cd2 is critical to

investigate their work in the bioremediation process of cadmium. The adsorption of cadmium showed that the untreated *B. subtilis* 168 Cd2 increased the adsorption percentages. This funding was similar to previous studies of [16].

Carboxyl, amino, and phosphate groups are the main functional groups were extensively reported for cadmium adsorption on the bacterial surface or isolated bacterial EPS [15]. Therefore, the role of surface interaction can be proposed for Ca-alginate beads, bacterial cells, and EPS, showing functional groups similar to the available literature [19], [20]. As was well known, the conceivable cadmium compulsory locations on the efficient groups in Ca-alginate beads are carboxyl and hydroxyl groups. Wei *et al* [16], found that the EPS-free *B. subtilis* have 36.6-62.2% fewer carboxyl and phosphate groups than the untreated *B. subtilis*. Therefore, the cell wall of untreated *B. subtilis* 168 Cd2, consists of carboxyl, hydroxyl, and amide functional groups, whereas, in contrast, the EPS-free *B. subtilis* 168 Cd2 could have fewer groups.

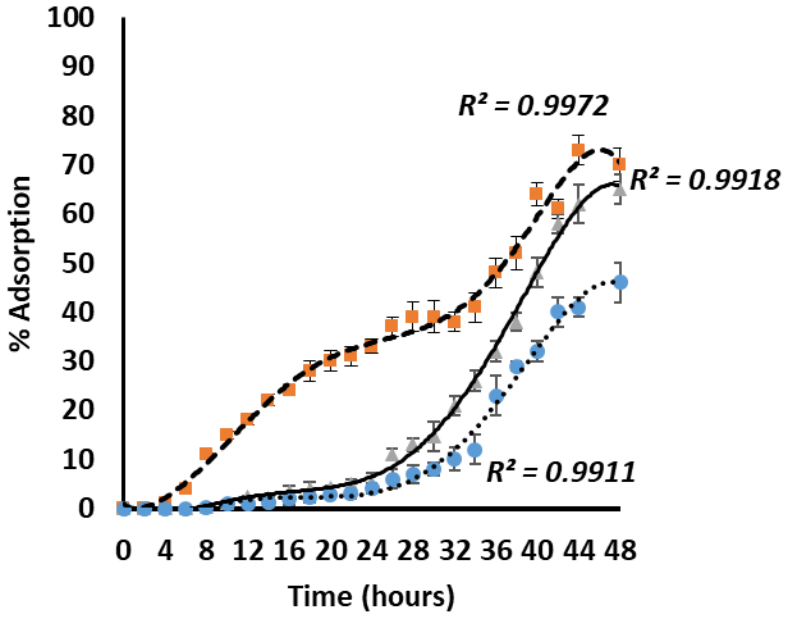

Figure (4). Percentage adsorption of cadmium from solution at pH 7.00 with insignificant concentrations of 1.8 mM Cd in laboratory bench-scale column reactors. (■) untreated *B. subtilis* 168 Cd2, (Δ) EPS-free *B. subtilis* 168 Cd2, and (●) control Ca-alginate beads.

3.3. Effect of pH on Cd Adsorption

The pH of a solution is a critical parameter affecting the adsorption method. The adsorption of Cd reduced with the increasing of pH and was found to be maximum at pH 4.00 for both, untreated *B. subtilis* 168 Cd2, and free *B. subtilis* 168 Cd2, and for control, Ca-alginate beads (Figure 5). The percentage of cadmium adsorption by untreated *B. subtilis* 168 Cd2 was decreased from 89% to 80%, when the pH was increased from 4.00 to 7.50 (at pH 4.00, 7.00, and 7.50). Similarly, at the same pH range, and in case of using EPS-free *B. subtilis* 168 Cd2, the percentage of cadmium adsorption was decreased from 77 % to 66 %, and lese for control Ca-alginate beads (55% to 51%). Under the different pH experimental conditions, untreated *B. subtilis* 168 Cd2 and its EPS negative-charge rises with pH due to protonation of carboxyl (pH 2.0–6.0), phospholipid (pH 2.4–7.2), and phosphodiester (pH 3.2–3.5) groups. This raised negative-charge attract to the positively charged Cd^{2+}.This free ion of cadmium (Cd^{2+}) is well known the dominate at pH 4.00 [21]. While, it appears that opposition from the protonation due to cumulative H^{+} attentions at low pH was not inhibiting adsorption of cadmium. The species of $CdCl^{+}$, $CdHPO_4$ that could have occurred at pH 7.00 and 7.50 hindered the cadmium adsorption process. On the other hand, as it is mentioned above that EPS have less of these groups, therefore, less adsorption observed with EPS-free *B. subtilis* 168 Cd2.

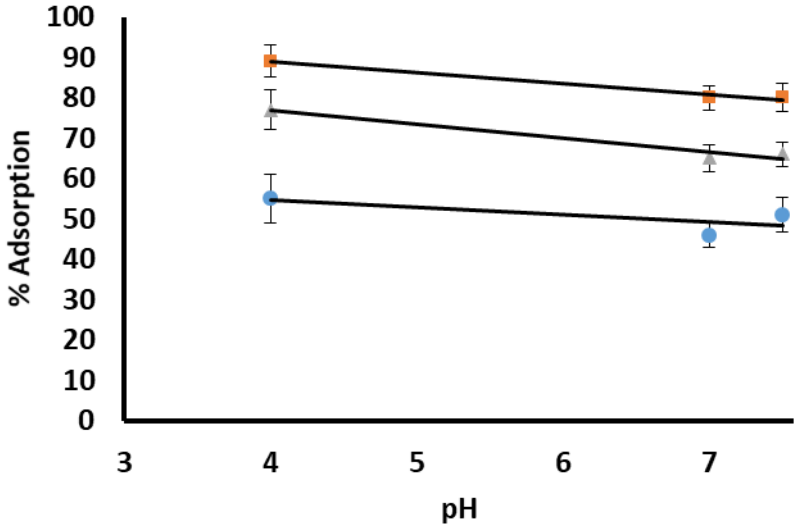

Figure (5). Effect of pH on the percentage of cadmium adsorption from solution at pH 4.00, 7.00, and 7.50 with nominal concentrations of 1.8 mM Cd in laboratory bench-scale column reactors. (■) untreated *B. subtilis* 168 Cd2, (Δ) EPS-free *B. subtilis* 168 Cd2, and (●) control Ca-alginate beads.

3.4. Characterization and Analysis of Calcium Alginate Beads

The SEM was used to observe the morphology of adsorbent surface (calcium alginate beads, before and after the adsorption). Figure (6 A–C) demonstrations the SEM micrographs of the adsorbent samples, calcium alginate beads containing untreated *B. subtilis* 168 Cd2, EPS-free *B. subtilis* 168 Cd2, and control Ca-alginate beads, before and after cadmium adsorption. After adsorption (Figure 6 column B), significant changes in the morphology of all types of beads were apparent, and the surfaces of beads seemed wrinkled.

XRF analysis of the beads after cadmium adsorption determined concentrations of cadmium, as shown in Figure (7). The concentrations were confirmed by the adsorbed concentrations of cadmium on beads, where calcium alginate beads containing untreated *B. subtilis* 168 Cd2 recorded the highest concentrations.

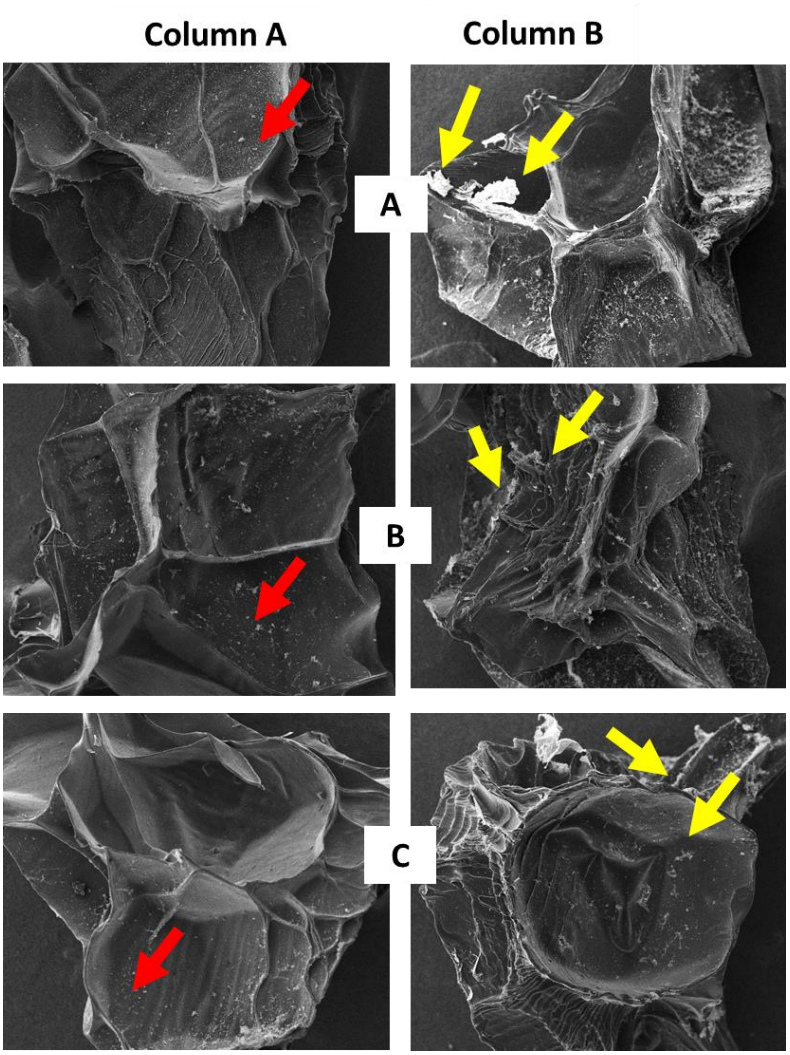

Figure (6). SEM micrographs of calcium alginate beads containing **(A)** untreated *B. subtilis* 168 Cd2 **(B)** EPS-free *B. subtilis* 168 Cd2, and **(C)** control Ca-alginate beads. Column **(A)** before and column **(B)** after cadmium adsorption. Yellow arrows: precipitation and adsorption complexes of cadmium. Red arrows: impurities.

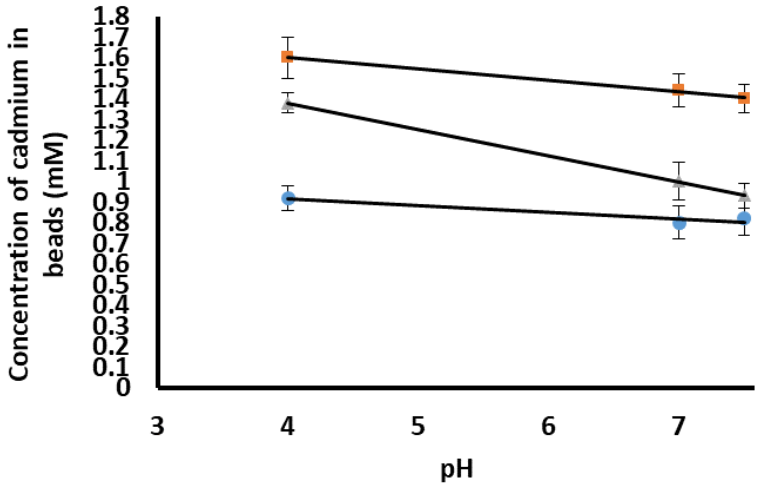

Figure (7). Cumulative concentrations of cadmium in the beads after the adsorption process of cadmium from solution at pH 4.00, 7.00, and 7.50 with nominal concentrations of 1.8 mM Cd in laboratory bench-scale column reactors. (■) untreated *B. subtilis* 168 Cd2, (Δ) EPS-free *B. subtilis* 168 Cd2, and (●) control Ca-alginate beads.

4. Conclusions

EPS in untreated *B. subtilis* 168 Cd2 significantly enhanced cadmium adsorption percentages. The fewer carboxyl and phosphate groups in EPS-free *B. subtilis* 168 Cd2 decreased cadmium adsorption. The free ions of cadmium (Cd^{2+}) increased cadmium adsorption, and the availability of $CdCl^{+}$, and $CdHPO_4$ at higher pH decreased the adsorption.

Conflict of Interest: The authors declare that there are no conflicts of interest associated with this research project. We have no financial or personal relationships that could potentially bias our work or influence the interpretation of the results.

References

[1] T. A. Ismaeel, "Designing a Decentralized Sewage Treatment Plant. Iraqi Journal of Industrial Research", vol. 8, no. 1, pp. 16–28, 2021.

[2] N. M. T. Jebril, "*In Vitro* Bioremediation: A Development Process of Cadmium and Mercury Removal by Environmental Biotechnologies of UV-Mutated *Escherichia coli* K12 and *Bacillus subtilis* 168", *Journal of Baghdad Sci*, vol.17 (1 (Suppl.)), pp. 0244-0244, 2020.

[3] A. Maftouh, O. El Fatni, S. El Hajjaji, M.W. Jawish, and M. Sillanpää, "Comparative review of different adsorption techniques used in heavy metals removal in water", *Biointerface Res Appl Chem*, vol. 13, pp.397-406, 2023.

[4] P. JEYAKUMAR, C. DEBNATH, R. Vijayaraghavan, and M. MUTHURAJ. "Trends in Bioremediation of Heavy Metal Contaminations," *Environmental Engineering Research*, vol. 28, no. 4, 2023.

[5] I. Sultan and Q.M.d R. Hag. "Bacterial Mechanisms for Remediation, "*In Bioremediation of Toxic Metal (loid)* s, pp. 115-136. CRC Press, 2023.

[6] N. Jebril, R. Boden, and C. Braungardt. "Cadmium resistant bacteria mediated cadmium removal: a systematic review on resistance, mechanism and bioremediation approaches," *IOP Conference Series: Earth and Environmental Science*. vol. 1002, no. 1. pp. 012006, IOP Publishing, 2022.

[7] N. M. T. Jebril, "Systems biology design of the microbe toward the bioremediation of cadmium: a review," *International Journal of Psychosocial Rehabilitation*, vol. 24, no. 8, 2020.

[8] N. Jebril, R. Boden, and C. Braungardt. "Cadmium Removal with Mutant *Brevibacillus Agri* C15 Cd r Entrapped in Calcium Alginate Gel: a New Process," *IOP Conference Series. Earth and Environmental Science*, vol. 761, no. 1. pp. 012028, IOP Publishing, 2021.

[9] N.M.T. Jebril, "Novel use of XRF in the adsorption processes for the direct analysis of cadmium and silver in absorbent Na-alginate beads", *Journal of Baghdad Sci*., vol. 17, pp.1139, 2020.

[10] N.M.T. Jebril. "Evaluation of two fixation techniques for direct observation of biofilm formation of *Bacillus subtilis* in situ, on Congo red agar, using scanning electron microscopy," *Veterinary World*, vol. 13, no. 6 , pp. 1133, 2021.

[11] D. Kim, W. Kim, and J. Kim, "New Bacterial Surface Display System Development and Application Based on *Bacillus subtilis* YuaB Biofilm Component as an Anchoring Motif". *Journal of Biotechnology and Bioprocess Engineering*, vol. 26, no.1, pp. 39-46, 2021.

[12] H. Xu, E. He, W. J. Peijnenburg, L. Song, L. Zhao, X. Xu, X. Cao, and H. Qiu, "Contribution of pristine and reduced microbial extracellular polymeric substances to Cu (II) reduction", *Journal of hazardous materials*, pp. 125616, 2021.

[13] L. Fang, X. Wei, P. Cai, Q. Huang, H. Chen, W. Liang, and X. Rong, "Role of extracellular polymeric substances in Cu (II) adsorption on *Bacillus subtilis* and *Pseudomonas putida*", *Bioresource technology*, vol.102 no. 2, pp. 1137-1141, 2011.

[14] L. Fang, S. Yang, Q. Huang, A. Xue, and P. Cai, "Biosorption mechanisms of Cu (II) by extracellular polymeric substances from *Bacillus subtilis*", *Chemical Geology*, vol. 386, pp. 143-151, 2014.

[15] R. Mikutta, U. Zang J. Chorover, L. Haumaier, and K. Kalbitz, "Stabilization of extracellular polymeric substances (*Bacillus subtilis*) by adsorption to and coprecipitation with Al forms", *Geochimica et Cosmochimica Acta*, vol. 75, no.11, pp. 3135-3154, 2011.

[16] X. Wei, L. Fang, P. Cai, Q. Huang, H. Chen, W. Liang and X. Rong, "Influence of extracellular polymeric substances (EPS) on Cd adsorption by bacteria", *Environmental Pollution*, vol. 159, no. 5, pp. 1369-1374, 2011.

[17] A. Omoike, and J. Chorover, "Adsorption to goethite of extracellular polymeric substances from *Bacillus subtilis*", *Geochimica et Cosmochimica Acta*, vol. 70, no. 4, pp. 827-838, 2006.
[18] B. Buszewski, E. Dziubakiewicz, P. Pomastowski, K. Hrynkiewicz, J. Ploszaj-Pyrek, E. Talik, M. Kramer, K. Albert, "Assignment of functional groups in Gram-positive bacteria", *J Anal Bioanal Tech*, vol. 6, pp.1-7, 2015.
[19] A.-M. Zou, M.-L. Chen, Y. Shu, M. Yang, and J.-H. Wang, "Biological cell-sorption for separation/preconcentration of ultra-trace cadmium in a sequential injection system with detection by electrothermal atomic absorption spectrometry", *J Anal At Spectrom*, vol. 22, pp.: 39-45, 2007.
[20] J. Shim, M. Kumar, S. Mukherjee and R. Goswami, "Sustainable removal of pernicious arsenic and cadmium by a novel composite of MnO2 impregnated alginate beads: A cost-effective approach for wastewater treatment", *J Environ Manage*, vol. 234, pp. 8-20, 2019.
[21] N. Jebril, R. Boden, and C. Braungardt. "The effect of pH, calcium, phosphate and humic acid on cadmium availability and speciation in artificial groundwater", *In Journal of Physics Conference Series*, vol. 1879, no. 2, pp. 022020, 2021.