International Microbiology (2021) 24:441–453
https://doi.org/10.1007/s10123-021-00182-0

ORIGINAL ARTICLE

Prospective bioremediation of toxic heavy metals in water by surfactant exopolysaccharide of *Ochrobactrum pseudintermedium* using cost-effective substrate

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Received: 30 January 2021 / Revised: 17 April 2021 / Accepted: 30 April 2021 / Published online: 13 May 2021

Abstract

Globally, the underlying peril of cumulative toxicity of heavy metals in water bodies contaminated by industrial effluents is a matter of great concern to the environmentalists. Heavy metals like lead, cadmium, and nickel are particularly liable for this. Such toxic water is not only hazardous to human health but also harmful to aquatic animals. Remedial measures are being taken by physico-chemical techniques, but most of them are neither eco-friendly nor cost-effective. Biological means like bioaccumulation of heavy metals by viable bacteria are often tedious. In the present study, biosorption of heavy metals is successfully expedited by surfactant exopolysaccharide (SEPS) of *Ochrobactrum pseudintermedium* C1 as a simple, safe, and economically sustainable option utilizing an easily available and cost-effective substrate like molasses extract. Its efficacy in bioremediation of toxic heavy metals like cadmium, nickel, and lead have been studied by UV–Vis spectrophotometry and verified by inductively coupled plasma–atomic emission spectroscopy (ICP-AES). FTIR and zeta potential studies have also been carried out to explore this novel biosorption potential. Results are conclusive and promising. Moreover, this particular SEPS alone can remediate all these three toxic heavy metals in water. For futuristic applications, it might be a prospective and cost-effective resource for bioremediation of toxic heavy metals in aqueous environment.

Keywords Bioremediation · Heavy metal · Surfactant exopolysaccharide (SEPS) · Biosorption

Introduction

Metals having high atomic weight and density, at least five times greater than water, are regarded as heavy metals (Tchounwou et al. 2012). They are often toxic to human and sub-human animals (Singh et al. 2011). History reminds us about the disastrous consequence of lead toxicity in ancient Rome where leaden water pipes and containers used by Roman citizens happened to be a leading cause of the fall of Roman Empire (Riva et al. 2012). Today, due to widespread industrialization, various toxic heavy metals are found in industrial effluents disturbing natural ecosystem. Petroleum, chemical, and textile industries emit toxic heavy metals as by-products which eventually drain into water bodies. Alarmingly, 42 rivers in India have already surpassed permissible toxicity limits of 10 µg/l, 20 µg/l, and 3 µg/l for lead, nickel, and cadmium respectively (Pandey et al. 2018; Kaushik et al. 2009). Even some non-alcoholic beverages are reported with toxic metal content exceeding permissible limit (Abdel-Rahman et al. 2019). Cumulative toxicities of heavy metals like nickel and cadmium exhibit dangerous physical, immunological, and genetic consequences. Prolonged exposure to nickel leads to respiratory disorders, asthma, bronchitis, pulmonary, and nasal cancer (Brera and Nicolini 2005). Cadmium affects cell proliferation, differentiation, and DNA repair by inducing reactive oxygen species (ROS). It causes chromosomal aberration and mutations, interferes with apoptosis (Skipper et al. 2016), and binds with mitochondria to inhibit cellular respiration and oxidative phosphorylation (Belyaeva et al. 2012).

Globally, an efficient and cost-effective solution for remediation of toxic heavy metals in water bodies has become a burning need. Researches are being carried out involving various physico-chemical and biological means. Reverse osmosis (RO) has long been implemented for

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water-softening, but it requires considerable amount of pressure and energy. Considering biological options, several bacterial strains and their metabolites were explored as they are non-toxic or less toxic (Kapahi and Sachdeva 2019). For instance, a cocktail of four bacterial strains in synergy successfully remediates lead and cadmium up to 98.3% and 85.4% respectively (Kang et al. 2016). Bioremediation may be accomplished either involving bioaccumulation by viable bacterial cells or biosorption by dead bacterial cells or their metabolites. Bioaccumulation is an active process (Diep et al. 2018) involving cellular gated channels. But biosorption is a simple, safe, fast, and mostly reversible process, and so it is preferred to bioaccumulation (Velásquez and Dussan-G 2009). Bioaccumulation of toxic heavy metals has several practical limitations. Sometimes even the bacteria might pose serious side effects (Belsito et al. 2002; Deguchi et al. 2015). Also, other factors like requirement of ideal nutrient media, definitive physicochemical parameters, stringent temperature or pH obligation, large-scale operators, and judicious effluent treatment often make such processes tedious and cumbersome (Wang 2016). Biosorption, on the contrary, is a simple process of adsorption by bacterial metabolites which does not require stringent measures. Production of bacterial metabolites is usually convenient (Osemwegie et al. 2020). Several bacterial species produce appreciable amounts of secondary metabolites like exopolysaccharides (EPS) by utilizing easily available, low-cost, or discarded substances like industrial, household, or agro-wastes as potential substrates (Amao et al. 2019; Ventorino et al. 2019; Vaishnav et al. 2019, 2020). For effective biosorption of toxic heavy metals in water bodies, a surfactant EPS is required because amphiphilic architecture of a surfactant comprises hydrophilic heads and hydrophobic tails oriented in such a manner that its basic structural moiety remains undisturbed in aqueous solution (Santos et al. 2016).

Ochrobactrum pseudintermedium is capable of producing two contrasting types of EPS, a surfactant EPS (SEPS) and an emulsifier EPS (EEPS), by utilizing different hydrocarbon substrates (Sengupta et al. 2019). The EEPS has already proved its worth in bioremediation of waste lubricating and crude oil (Bhattacharya et al. 2014). Here, SEPS has been explored to find out whether it can remediate toxic heavy metals like cadmium, nickel, and lead in aqueous solution at different concentrations. Results are affirmative and conclusive. Moreover, this particular SEPS can singly remediate these three toxic heavy metals. Simple method like UV–Vis spectrophotometry was primarily carried out and was verified by sophisticated one like inductively coupled plasma-atomic emission spectroscopy (ICP-AES) to estimate metal concentrations in aqueous solution before and after such biological treatment. Inherent charge of SEPS was also analyzed by zeta potential analyzer to demonstrate its interaction with metal ions. FTIR spectroscopy of fresh and metal bound SEPS helped identify its functional groups involved in biosorption. Due to convenience of its production by utilizing easily available and cost-effective sugar-based substrate like molasses extract, it appears to be a potent and economically sustainable tool in bioremediation of toxic heavy metals in water bodies.

Materials and methods

Screening of bacterial strain

Several bacterial strains, like *Bacillus cereus* K1 (GenBank accession no. KJ922989), *Bacillus stratosphericus* A15 (GenBank accession no. KU644139), *Ochrobactrum pseudintermedium* C1 (GenBank accession no. KJ094035), and *Pseudomonas aeruginosa* C2 (GenBank accession no. KU291381), readily available at the laboratory in the Department of Chemical Technology, University of Calcutta, were considered for their propensity to produce EPS. Corresponding yield of EPS was monitored and the particular bacterial strain showing the highest yield was selected.

Maintenance of selected bacterial strain

Consequent upon its highest yield of EPS, *Ochrobactrum pseudintermedium* C1, having accession number KJ094035 following 16S rRNA sequence in GenBank database and selected for the present study, was serially sub-cultured in sterile nutrient agar at an interval of 14 days, regularly maintained under 4 °C refrigeration and Gram stained for identification and verification before further studies.

Selection of suitable substrate

Several easily available and cost-effective substrates were primarily chosen. Among them different sugar-based substrates, like sugarcane extract (bagasse), molasses, fruit pulp, and rice starch were considered, because they were readily utilizable by the bacteria and were capable of producing adequate SEPS (Sengupta et al. 2019).

Bacterial incubation and extraction of bacterial SEPS

Consequent upon its ability for highest yield of SEPS, molasses extract, an easily available byproduct of confectionery, was chosen. *Ochrobactrum* sp. was cultured in Bushnell Haas media (BHM) supplemented with 4% molasses extract after microwave treatment for 2 min. The strain was incubated in Scigenics Biotech Orbitek LJE incubator at 34 °C for 8 days under optimum shaking condition for maximum production

of SEPS. After incubation, bacterial cell mass was removed by centrifugation in Remi C24 Plus Centrifuge at 6000 rpm and the collected supernatant was treated overnight with double volumes of chilled ethanol (v/v). Precipitate comprising of crude SEPS was collected and purified by adding equal volume of ethanol (w/v) and centrifuged at 10,000 rpm for 15 min, thereafter discarding the supernatant and collecting the SEPS precipitate. The process was carried out thrice for ensuring purification. Finally, purified SEPS was stored at 4 °C in sterile Tarson tubes sealed with Parafilm tapes.

Characterization of bacterial SEPS

Total sugar content of purified SEPS was estimated by phenol–sulfuric acid method using glucose as the standard (DuBois et al. 1956) followed by UV–Vis spectrophotometry (UV-1800, Shimadzu Corp., Japan).

SEPS was subjected to percentage analysis of respective carbon, hydrogen, and nitrogen concentrations by CHN analyzer (CHNS 932, Leco Corp., USA) to analyze the intrinsic constituent of the biosorbent. Acetophenone was considered as reference.

For understanding the intrinsic functional groups FTIR spectroscopic analysis of EPS at mid-spectral range of 4000 to 400 cm^{-1} was performed with KBr as standard (Mathivanan et al. 2021; Kumar et al. 2019) and the process was repeated thrice using Perkin Elmer Spectrum Version 10.5.1, Germany.

Scanning electron microscopy of bacterial SEPS

Bacterial sample and the purified SEPS were separately fixed in 3% glutaraldehyde solution for 2 h followed by sequential dehydration with 20%, 40%, 60%, 80%, and 90% v/v ethanol. Then, the samples were sputter-coated with platinum and images were recorded at different magnifications under SEM (Zeiss Evo 18 Special Edition, Zeiss, Germany at 15 kV).

Preparation of simulated aqueous environment for toxic heavy metals

Water soluble salts of toxic heavy metals like cadmium, nickel, and lead were considered which included cadmium sulfate [$3CdSO_4.8H_2O$], nickel sulfate [$NiSO_4.6H_2O$], and lead nitrate [$Pb(NO_3)_2$] respectively. All these salts were prepared in concentrations ranging from 5 to 100 ppm in deionized water for standardization.

Spectrophotometric estimation of heavy metal concentrations

Cadmium ion concentration was estimated by Alizarin Red S (1,2-dihydroxy anthraquinone-3-sulfonic acid sodium salt) followed by subsequent standardization using UV–Vis spectrophotometer (Ullah and Haque 2011). Similarly, nickel was estimated by dimethyl glyoxime (DMG) (Mitchell and Mellon 2002). Standardization of lead nitrate solution was directly carried out by atomic absorption spectroscopy (Shimadzu AA-6200 Atomic absorption spectrophotometer, Japan).

Optimization of SEPS concentration, biosorption time, and others

Purified SEPS concentrations from 100 to 1000 mg/l were aseptically added to separate Erlenmeyer flasks containing 100 ppm of respective heavy metal solutions under shaking and non-shaking conditions. Decrement in heavy metal concentration with respect to each SEPS concentration was recorded by spectrophotometric analysis. Temperature (15 °C to 45 °C) and shaking speed (0 to 200 rpm) were separately considered for optimized adsorption. Temperature and shaking speed were regulated in incubator and pH was also varied by Systronics Digital pH Meter 335. Time-dependent optimization of biosorption was carried out up to 18 h. For each study, the concentration of heavy metal in solution before and after adsorption was determined and compared by UV–Vis spectrophotometry. For understanding the effect of initial metal concentration in presence of biosorbent and for interpretation of biosorption capacity, the initial concentrations up to 100 ppm were considered. To each particular concentration of metal solution, SEPS at optimized concentration was added.

Biosorption capacity *(Q)* was calculated as follows:

$$Q = \left(C_i - C_f\right)V/m$$

where Q = biosorption capacity, C_i = initial metal concentration, C_f = final metal concentration, V = volume of metal solution, and m = mass of adsorbent (He et al. 2018). A relationship between metal adsorbed and the concentration of SEPS was determined graphically (Lakzian 2008).

Assessment of foaming and surfactant nature

Foaming tendency was assessed with respect to increase in concentration of SEPS in culture media. Based on such findings a probable correlation was established between the two parameters. Surface tension along with critical micelle concentrations (CMC) of SEPS, before and after biosorption was determined by digital tensiometer (Dataphysics DCAT 11, Germany) with the surface tension of deionized water at air–water interface being 71 mN/m (Lunkenheimer and Wantke 1981).

FTIR of metal bound SEPS

After studying FTIR spectra with purified SEPS, the same was carried out with metal bound SEPS for a comparative analysis. Here, SEPS following adsorption of cadmium, nickel, and lead separately, at optimized parameters, were vacuum desiccated and analyzed at spectral range of 4000 to 400 cm^{-1} with KBr as standard for understanding the qualitative changes in functional groups.

Estimation of charge of SEPS

Zeta potential of purified SEPS and SEPS after metal adsorption was determined by dynamic light scattering (DLS) (Zetasizer Nano ZS, Malvern Instruments Ltd., UK) and compared accordingly. The system temperature was set at 25 °C. Both studies were carried out with 4 mW He–Ne laser beam at 633 nm with 173° back scattering angle. The procedures were repeated twice for verification.

Estimation of bioremediation efficiency of SEPS

Maintaining standardized parameters for optimization of heavy metals, aqueous solutions of cadmium, nickel, and lead at concentrations from 200 ppm (high toxicity) to 6.25 ppm (low toxicity) were prepared by serial dilution. Purified SEPS was added to each solution and kept under optimum shaking condition. After SEPS treatment, the resultant decrements in metal concentration in each case were recorded by ICP-AES (Arcos, Simultaneous ICP Spectrometer, Spectro Analytical Instruments GmbH, Germany). A second round of treatment of the bioremediated metal solution was carried out with fresh sample of SEPS and verified again by ICP-AES.

Results

Selection of bacterial strain

Among the available bacterial strains stated above and considered for their capability to produce SEPS, *Ochrobactrum pseudintermedium* C1 was selected as it showed the highest yield of EPS (~ 1800 mg/l) when molasses extract was utilized as sugar-based substrate in BH media. Optical microscopy after Gram staining revealed its Gram-negative, rod-shaped morphology (Fig. 1a).

Production of bacterial SEPS

Optimized incubation for 8 days resulted in formation of a thick (approximately 6 mm) layer of SEPS matrix on the surface of the culture media in Erlenmeyer flask (Fig. 1b). Such an architectural orientation of the bacterial biofilm is often due to cellular oxygen demand following depletion of the dissolved oxygen in culture media (Ahn and Burne 2007).

Characterization of bacterial SEPS

Phenol–sulfuric acid test of SEPS resulted in formation of reddish-brown complex characteristic of furfural derivative which exhibited maximum absorbance at a wavelength of 490 nm (Nowotny 1979). The percentages of elemental carbon, hydrogen, and nitrogen concentrations of SEPS obtained after CHN analysis was C 30.22%, H 4.75%, and N 5.88% respectively which typically suggested a glycoprotein nature of the biomaterial. FTIR spectra of purified SEPS displayed functional groups correlated to bacterial EPS which on comparing with that of metal bound SEPS showed certain subtle changes as mentioned later.

SEM findings of SEPS

SEM findings displayed the potency of *Ochrobactrum* sp. to produce SEPS biofilm (Fig. 2a). Further detailed SEM analysis of SEPS exhibited a characteristic scaffold like matrix with intermittent pores which was somewhat similar to cellulose fibers (Krishnamachari et al. 2011) but unlike fungal hyphae they did not follow any definite architectural pattern. The overall network of fibers was less complex and considerably thicker which offered a wider spanning of the overall surface area that might facilitate biosorption of toxic substances (Fig. 2b).

Detection and standardization of dissolved heavy metal concentration

Dissolved cadmium and nickel were detected from their characteristic greenish yellow and rose red colors with maximum absorbances at 422 nm and 445 nm respectively. Upon standardization, a linear curve was obtained from metal concentrations of 5 ppm to 100 ppm at their characteristic wavelengths respectively (Fig. 3a). A linear curve was also obtained upon standardization of lead by atomic absorption spectroscopy.

SEPS concentration and other parameters for optimum biosorption

Optimum concentration of approximately 800 mg/l of SEPS resulted in significant adsorption of cadmium and nickel ions in solution (Fig. 3b). Optimum shaking speed between 90 and 110 rpm was preferred for favorable adsorption of metal ions in solution by SEPS. It was observed that shaking speeds below 85 rpm did not result in considerable remediation of metal ions within the optimized time. This might be

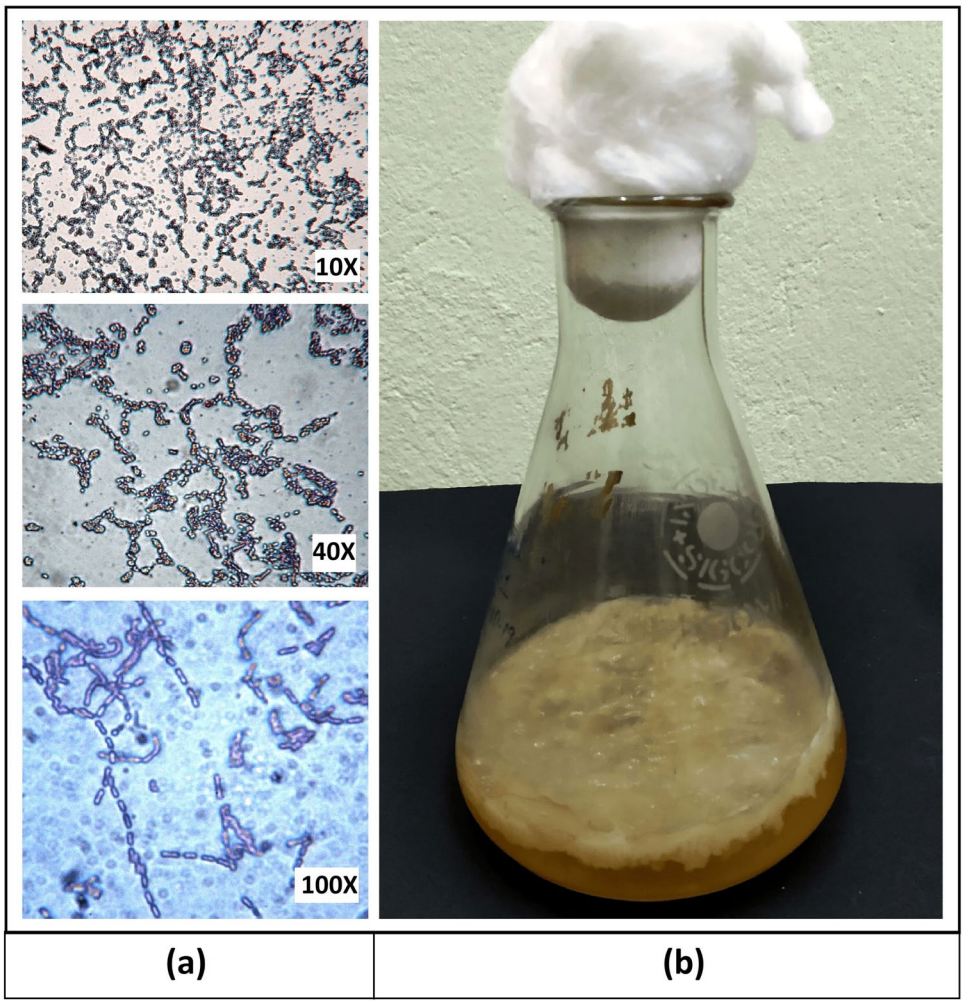

Fig. 1 **a** *Ochrobactrum pseudintermedium* C1 after Gram staining at different magnifications; **b** SEPS produced by *Ochrobactrum pseudintermedium* C1 on the surface of culture media

due to the fact that such rates of agitation were insufficient for maximum binding of metal ions to functional groups present in SEPS surface (Raza et al. 2015). Again, speeds above 120 rpm did not attribute to quantitatively enhanced biosorption (Rezaei 2016). Also, a temperature of 36 (±2) °C and a pH between 6.25 and 7.63 effectively favored biosorption. Lower pH values did not favor metal binding to SEPS. Acidic pH of SEPS surface might predominately be masked by hydrogen ions, thereby restricting the binding of metal ions (Wei et al. 2016). UV–Vis spectral findings for determining time dependent optimization revealed an optimum time of about 8 h for maximum biosorption of cadmium (Fig. 4a) and nickel by SEPS (Fig. 4b). Prolongation of the process did not significantly reduce metal ion concentration in solutions. Maintaining such standardized parameters, the graphical representation of adsorbed

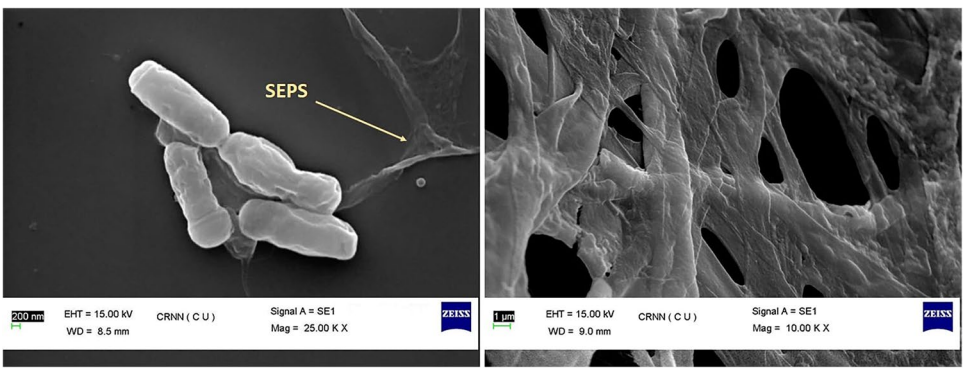

Fig. 2 SEM images showing **a** SEPS produced by *Ochrobactrum pseudintermedium* C1 (left); **b** detailed architecture of SEPS (right)

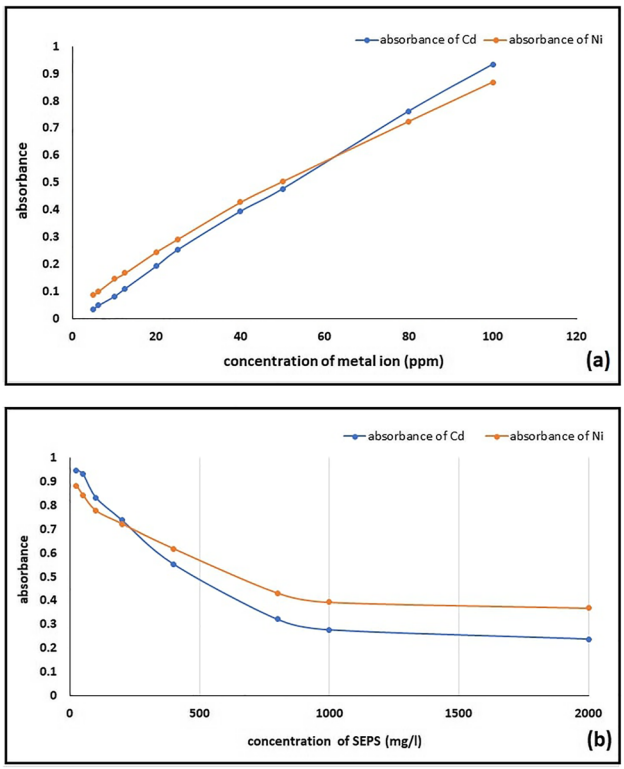

Fig. 3 **a** Standardization of cadmium and nickel at different concentrations; **b** absorbances of dissolved cadmium and nickel following biosorption by SEPS at different concentrations

metal concentration with respect to biosorbent showed that at optimum SEPS concentration of 800 mg/l the bisorption capacity (Q) to cadmium, nickel, and lead were 87.6, 77.7, 71.6 mg/g respectively (Fig. 4c).

Determination of foaming and surfactant character

Under shaking conditions, SEPS production was also associated with foaming on the surface of culture media (Fig. 5a). Such phenomenon is often a character exhibited by several microbial exopolysaccharides (Deng et al. 2016). Gradual increase in SEPS production was associated with increased production of foam. However, such a tendency was limited up to a certain concentration of SEPS. On the other hand, the interaction of SEPS with metal ions in aqueous solution resulted in reduction of such foaming tendency (Fig. 5b). This might be due to interaction between metal cations and SEPS matrix leading to formation of a transient complex (Mohite et al. 2017; Sibikina et al. 2009).

Surfactant property of purified SEPS and that following metal adsorption showed contrasting differences. Purified SEPS exhibited a surface tension as low as 28.27 mN/m, which accounted for about 60% reduction of surface tension. Such finding is comparable with other bacterial surfactant exopolysaccharides (Liang et al. 2014). However, this nature was not that pronounced in SEPS after metal adsorption and the lowest surface tension recorded was 33.62mN/m (Fig. 5c). Critical micelle concentrations (CMC) were found to vary accordingly (Behera et al. 2014). CMC of purified SEPS was found to be as low as 5 g/l, whereas that after metal adsorption was found to be as high as 18–20 g/l.

FTIR findings of SEPS and metal bound SEPS

In the FTIR spectra of purified SEPS, broad peak at 3317 cm^{-1} was indicative of either hydroxyl group or intermolecular hydrogen bonding pertaining to its constitutional integrity (Sardari et al. 2016). Similar peaks at 3420 to

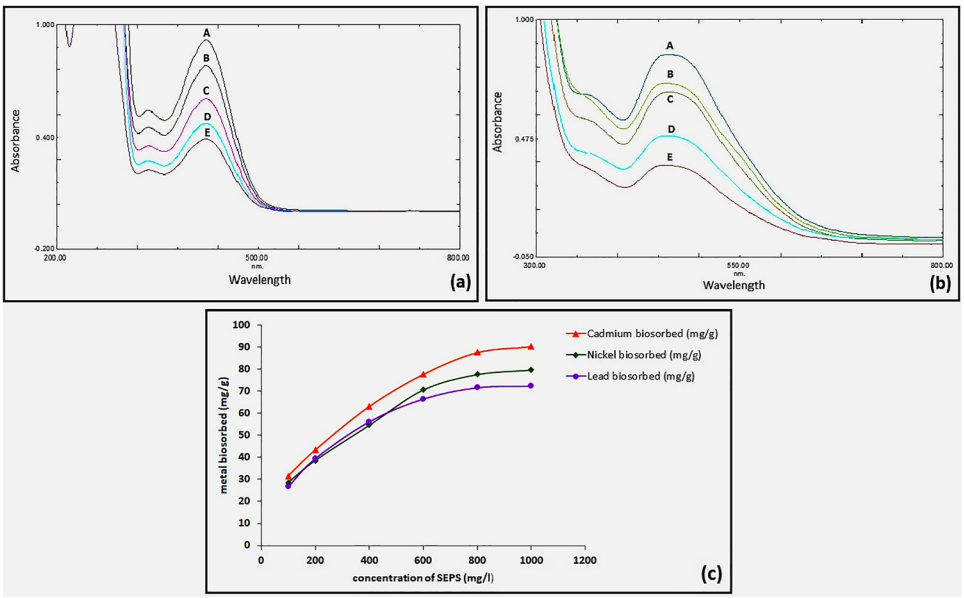

Fig. 4 Time-dependent estimation of SEPS mediated biosorption of **a** cadmium and **b** nickel at 0, 4, 6, 8, 18 h represented by A, B, C, D, E respectively; **c** graphical interpretation of adsorption capacities (Q) of SEPS for cadmium, nickel, and lead

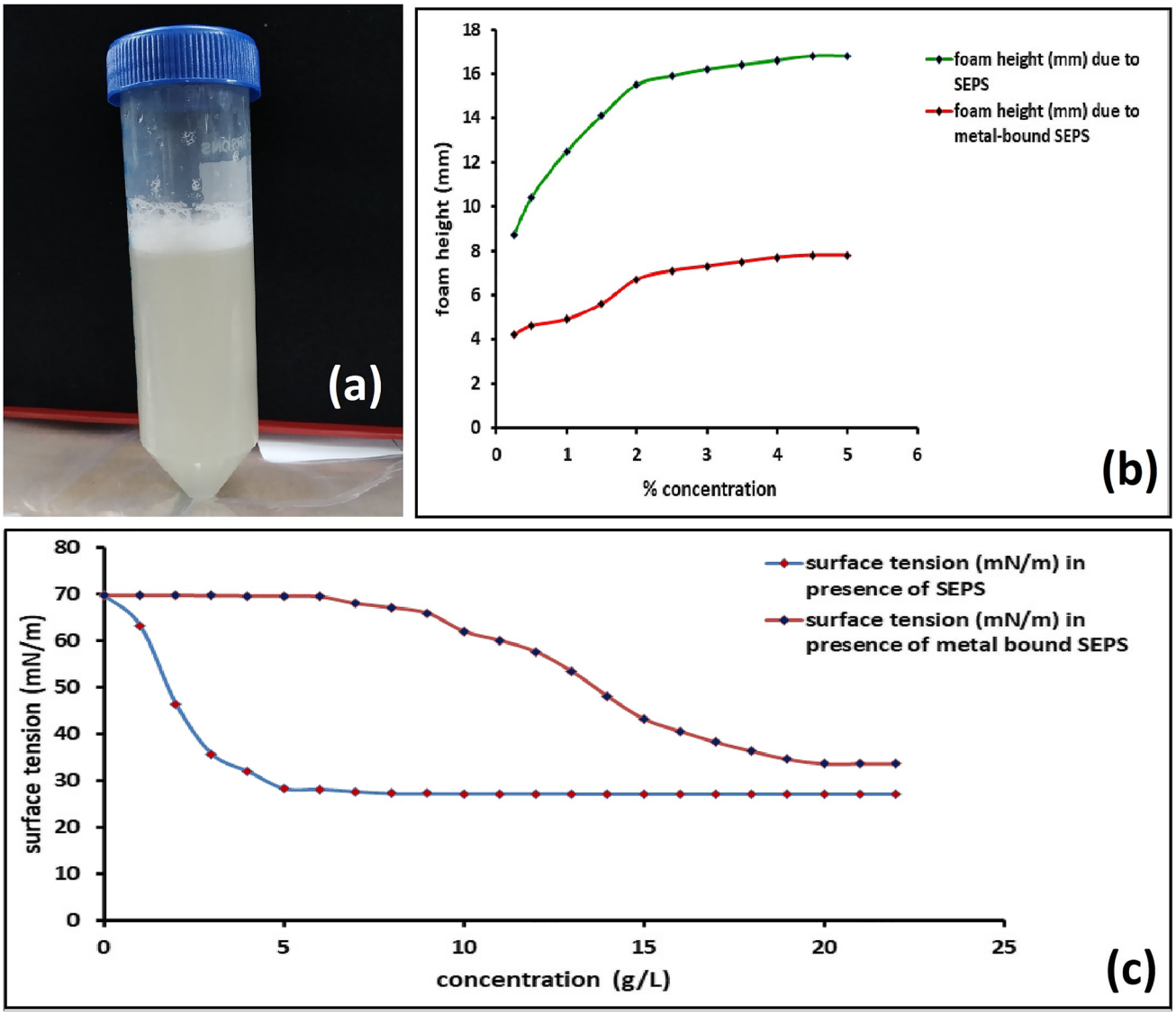

Fig. 5 **a** Production of foam on the surface of SEPS suspension; **b** foaming tendency at different concentrations of SEPS compared with that after metal binding; **c** surface tension at different concentrations of SEPS compared with that after metal binding

3000 cm^{-1} were also present in metal bound SEPS. Purified SEPS as well as metal bound SEPS displayed medium to strong peaks around 2925 cm^{-1} which ascertained presence of constitutional alkane (C-H) groups (Gugliandolo et al. 2015). Presence of N–H group was also indicated by peaks around 1650 to 1660 cm^{-1} in both pure and metal bound SEPS (Upadhyay et al. 2017). This indicated a probable glycoprotein nature of SEPS which was also evident from CHN analysis. Presence of a short yet sharp peak at 1743.3 cm^{-1} in purified SEPS suggested carbonyl (C = O) group (Castellane et al. 2015). However, such a peak was absent or insignificant in case of its metal bound fraction.

Upon detailed investigation and comparison between IR spectra of purified SEPS and that of metal bound SEPS, certain spectral shifts were identified particularly in the fingerprint zone (Fig. 6a). In purified SEPS, a peak characteristic to C–O–C bond stretch due to glycoside linkage of the constitutional carbohydrate units of SEPS moiety was noted at 1035.72 cm^{-1} (Wang et al. 2020). But in case of cadmium, nickel, and lead, the adsorbed SEPS showed a shift to 1080.5 cm^{-1}, 1042.21 cm^{-1}, and 1040.71 cm^{-1} respectively. Even such small shifts in the fingerprint zone of IR spectra of macromolecules often quite assertively render a change in overall electronegativity. Functional groups like C = O, C–O–C, or –OH generally display the potential binding sites of metal biosorption by bacterial EPS (Zhang et al. 2017). Among the three metal ions considered here, cadmium exhibited highest spectral shifts in comparison with nickel and lead which was evident by complete absence of carbonyl stretch and relatively high

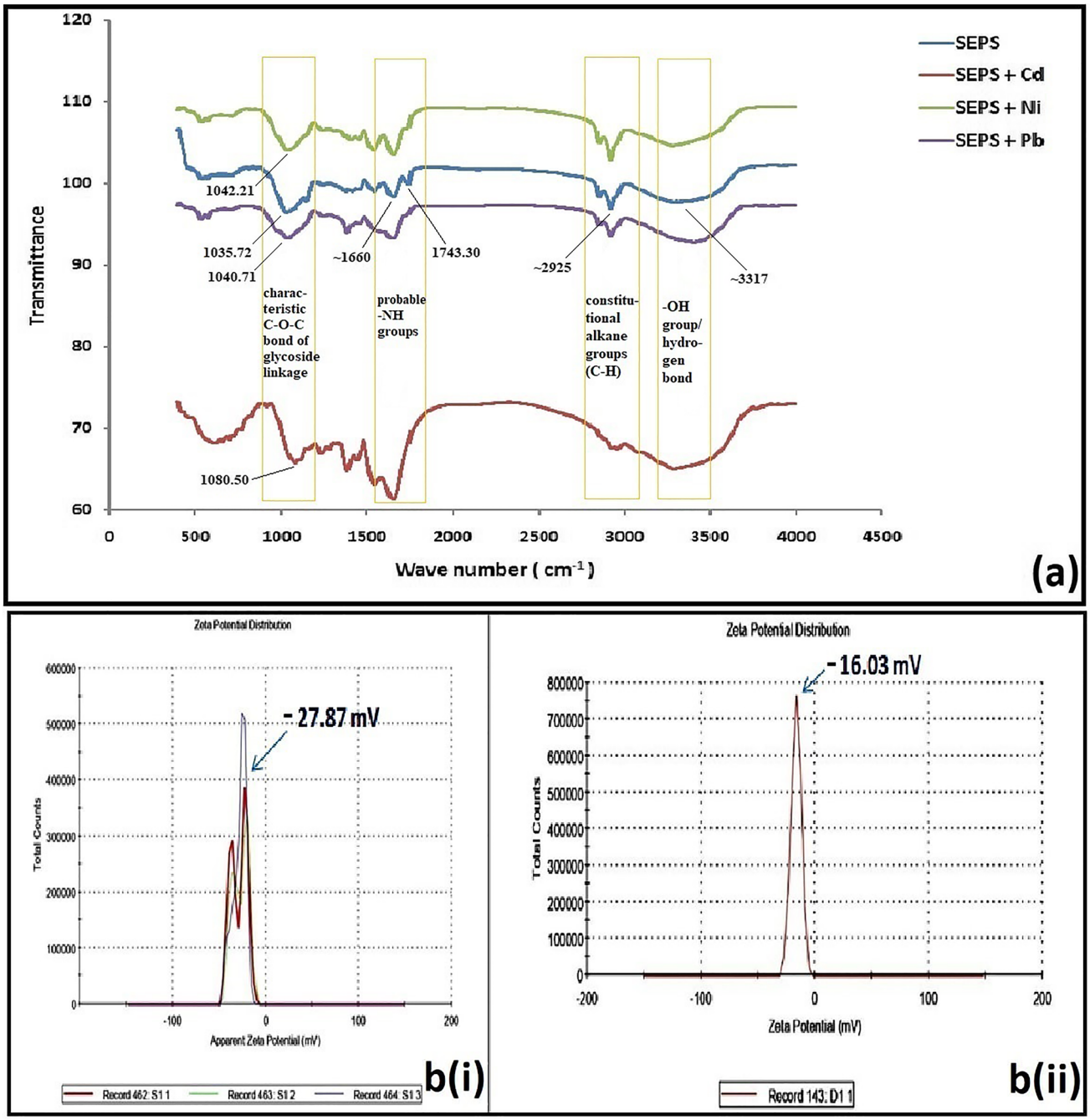

Fig. 6 **a** FTIR spectra of SEPS and different metal bound SEPS; **b** zeta potential of (i) SEPS and (ii) metal bound SEPS

spectral shift at C–O–C stretch of the cadmium bound SEPS.

From a combined analysis of quantitative estimation by UV–Vis spectrophotometry and qualitative assessment by FTIR spectroscopy, it could be assumed that the process of metal biosorption is highly selective and is a conglomerative effect of several factors like molecular state, atomic size and bond energy of the metal ions.

Analysis of contrasting changes in zeta potential

Purified SEPS displayed an intrinsic anionic character with a zeta potential of − 27.87 mV. However, such prominent anionic nature of the biomolecule showed significant change after its interaction with metal cations. A zeta potential of − 16.03 mV was observed after interaction of SEPS with heavy metals (Fig. 6b), particularly in case of cadmium.

Such findings not only verified a successful binding of metal ions with SEPS moiety but also the contrasting change in zeta potential showed resemblance with exopolysaccharide of *Bacillus pumilus* and its interaction with silver nanoparticle (Khan et al. 2011).

Estimation of biosorption percentage

From spectrophotometric analysis, which was further verified by ICP-AES, it was revealed that the SEPS was capable of reducing the dissolved heavy metal load even at a concentration of 200 ppm. It was also clearly noted that there was a further significant reduction in metal concentration if the bioremediation process was repeated for a second round with fresh sample of SEPS. After a first round of treatment, SEPS in a dose of 800 mg/l was capable of reducing a cadmium load from 100 to 29.96 ppm (70.04%), but after a second round it further reduced the concentration to as low as 6.41 ppm (Fig. 7a), thereby reducing the overall cadmium concentration by 93.59%. Similarly, nickel concentration was reduced from an initial load of 100 ppm to 37.88 ppm (62.12%) after first round of treatment and then up to as low as 9.23 ppm after the second round (Fig. 7b), thereby reducing the overall nickel concentration by 90.77%. In case of lead also, it was from 100 to 42.67 ppm (57.33%) after the first round and to as low as 10.97 ppm after the second round (Fig. 7c) showing an overall reduction by 89.03%.

Comparative assessment with other bacterial EPS

Comparative assessment of metal (cadmium, nickel, and lead) adsorption capacities and/or their bioremediation percentages by different bacterial EPS further justified SEPS of *Ochrobactrum* sp. to be an efficient biosorbent (Table 1). Its preferential adsorption capacity was found to be in the order: $Cd^{2+} > Ni^{2+} > Pb^{2+}$. It is also worth mentioning here that this particular SEPS singly could remediate three metals whereas other bacterial EPS usually remediate a single metal.

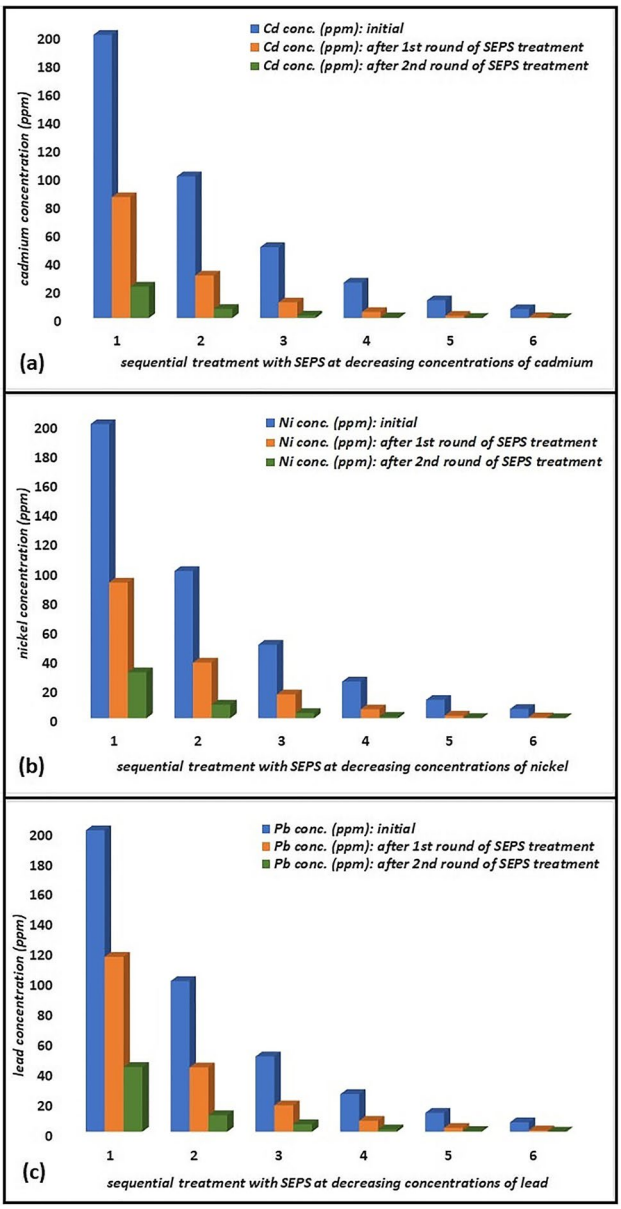

Fig. 7 ICP-AES analyses showing reduced concentrations of **a** cadmium, **b** nickel, and **c** lead in aqueous solution after first and second round of SEPS treatment

Discussion

Even today, potentially hazardous, low-cost, synthetic surfactants, or chelating agents are being used indiscriminately as a readymade measure against industrial effluents containing toxic heavy metals (Di Palma et al. 2015; Sobrino-Figueroa 2018). Consequently, unprecedented accumulation of such surfactants in water bodies pose serious damage by significantly hindering the aquatic life forms (Lechuga et al. 2016; Jardak et al. 2016). Such consequences often involve drastic increase in cell membrane permeability leading to disintegration of exosomes and cellular structures of different aquatic life forms (Yuan et al. 2014). In the circumstances, exploring biosurfactants for their safety profile and intrinsic potentiality to bioremediate toxic heavy metals dissolved in water bodies is being judiciously considered.

For bioremediation of toxic heavy metals, dead and dried biomass of *Ochrobactrum anthropi* was earlier reported to exhibit its potency for adsorption of chromium, cadmium, and copper ions (Ozdemir et al. 2003). In the present study, SEPS produced and isolated from *Ochrobactrum pseudintermedium* C1 has been expedited to elicit its capability to adsorb toxic heavy metals like cadmium,

Table 1 Different bacterial EPS showing their heavy metal (Cd^{2+}, Ni^{2+}, Pb^{2+}) biosorption capacity and/or bioremediation percentage

Heavy metal	EPS producing bacteria	Biosorption capacity	Bioremediation percentage	References
Cd^{2+}	*Enterobacter cloacae*	16 mg g^{-1}	65%	Iyer et al. 2005
	Paenibacillus jamilae	60 mg g^{-1}	-	Morillo et al. 2006,
	Paenibacillus polymyxa,	-	87.12%	de Oliveira et al. 2008
	Serratia marcescens	-	81%	Xing et al. 2020
	Microbacterium sp. MC3B10	97 mg g^{-1}	-	Camacho-Chab et al. 2018
	Ochrobactrum pseudintermedium	87.6 mg g^{-1}	70.04%*, 93.59%**	*Present study*
	Bacillus sp. MC3B22	141 mg g^{-1}	-	Camacho-Chab et al. 2018
Ni^{2+}	*Ensifer meliloti*	54 mg g^{-1}	85%	Lakzian 2008
	Ochrobactrum pseudintermedium	77.7 mg g^{-1}	62.12%*, 90.77%**	*Present study*
Pb^{2+}	*Pseudomonas* sp.	-	65%	Kalita and Joshi 2017
	Paenibacillus peoriae	22.38 mg g^{-1}	89%	Temzi et al. 2018
	Ochrobactrum pseudintermedium	71.6 mg g^{-1}	57.33%* 89.03%**	*Present study*
	Oceanobacillus profundus	-	97%	Mwandira et al. 2020

*Bioremediation percentage after first round of SEPS treatment

**Bioremediation percentage after second round of SEPS treatment

nickel, and lead dissolved in water. After a considerable period of bacterial incubation, this SEPS was found to exhibit a characteristic foaming tendency which happens to be a common indicator (Capodici et al. 2015) for surfactant production. It was verified by showing a decrement in surface tension of culture media to a significant degree which is quite comparable to several potent biosurfactants (Freitas et al. 2016). Physical method like ion-exchange chromatography or reverse osmosis is often employed as a separation technique for heavy metals, but it poses some practical demerits. Low thermal stability of ion-exchange resins restricts its application in processes demanding high temperature (Tulupov and Polyanskii 2007). This bacterial SEPS, like other biopolymers, offers metal selectivity apart from its inherent biosorption efficiency. In this perspective, less popular strains and their metabolites, isolated from remote geographical locations, having biosorption efficacy are being reported. Extracellular polymeric substance of *Parapedobacter* sp. is recently found to selectively adsorb chromium ions (Tyagi et al. 2020). Here, our SEPS of concern exhibits its unique ability to target multiple heavy metals like cadmium, nickel, and lead; although, the degree of their biosorptions vary. When SEPS treatment of the concerned metal was repeated with fresh sample of SEPS, the overall reduction in metal concentration was found to be significant enough. After second round of treatment by SEPS under optimized conditions, there was highest reduction in concentration of dissolved cadmium (~94%) followed by nickel (~91%) and lead (~89%) from an initial load of 100 ppm of the metal in each case. Cadmium biosorption efficiency after the second round of treatment was better than that of EPS of marine metal-chelating bacteria *Enterobacter cloacae* which was reported to reduce cadmium load of 100 ppm by 65% (Iyer et al. 2005). It was noteworthy that such an adsorption process was an expression of interaction between constitutional anionic groups of SEPS moiety and respective metal cations. This was quite evident from the conspicuous increase in zeta potential of the SEPS from a level of − 27.87 mV to − 16.03 mV after a single round of cadmium ion treatment. Such selectivity of SEPS towards metal ions was also predicted by subtle changes in their respective FTIR spectra. The participating anionic groups in biosorption are usually C = O, C–O–C, or –OH of the polysaccharide moiety (Shuhong et al. 2014). However, the predominant metal interaction is generally observed at the C–O–C bond which also indicates vibrations of glycosidic linkage of SEPS moiety (Zhang et al. 2017). Generally, O = groups of polysaccharides form complexes with metal ions during biosorption thus reducing electron cloud density of oxygen-containing functional groups and altering the bond vibration frequency and intensity (Guibaud et al. 2005).

This bioremediation procedure also proves cost-effective because adequate SEPS production can conveniently be done by utilizing an easily available, sugar-based substrate like molasses extract, a common byproduct of confectioneries. So, this bioremediation property of SEPS obtained from *Ochrobactrum pseudintermedium* is likely to welcome further works in future.

Acknowledgement The authors remain grateful to (1) Department of Chemical Technology, University of Calcutta; (2) Department of Science and Technology, Govt. of West Bengal (WB-DST); (3) Promotion of University Research and Scientific Excellence (PURSE), Phase II program, Department of Science and Technology (DST), Govt. of

India; (4) UGC sponsored Special Assistance Program (SAP), University of Calcutta; and (5) Sophisticated Analytical Instrument Facility (SAIF), IIT Bombay for providing academic and technical support.

Authors' contributions DS[1] conceived and designed the research. DS[1], SB[4] and SD[5] conducted the experiments. DS[1] and SD[2] analyzed the data. DS[1] wrote the manuscript and SD[2] supervised the study. SD[2] and DB[3] provided infrastructural support and contributed new reagents or analytical tools. All authors read and approved the manuscript.

Funding Funding of the entire study was provided by the Department of Science and Technology, Government of West Bengal (WB-DST), India, vide Ref. 115 dated 30/01/2017 and Sanction No. 430 (Sanc.)/ST/P/S&T/15G-7/2016 dated 12/03/2019.

Data availability The authors declare that all data generated or analyzed and materials used during the study are mentioned in this article.

Declarations

Ethical approval The study did not involve any animal or human subject.

Consent to participate The authors give their consent to participate in this research work.

Consent to publish The authors give their consent to publish this original article.

Conflict of interest The authors declare that they have no conflict of interest.

References

Abdel-Rahman GN, Ahmed MBM, Sabry BA, Ali SSM (2019) Heavy metals content in some non-alcoholic beverages (carbonated drinks, flavored yogurt drinks, and juice drinks) of the Egyptian markets. Toxicol Rep 6:210–214. https://doi.org/10.1016/j.toxrep.2019.02.010

Ahn SJ, Burne RA (2007) Effects of oxygen on biofilm formation and the AtlA autolysin of *Streptococcus mutans*. J Bacteriol 189(17):6293–6302. https://doi.org/10.1128/JB.00546-07

Amao JA, Omojasola P, Barooah M (2019) Isolation and characterization of some exopolysaccharide producing bacteria from cassava peel heaps. Scientific African 4:e00093

Behera M, Varade S, Ghosh P, Paul P, Negi A (2014) Foaming in micellar solutions: effects of surfactant, salt, and oil concentrations. Ind Eng Chem Res 53:18497–18507. https://doi.org/10.1021/ie503591v

Belsito DV, Fransway AF, Fowler JF Jr, Sherertz EF, Maibach HI, Mark JG Jr, Mathias CG, Rietschel RL, Storrs FJ, Nethercott JR (2002) Allergic contact dermatitis to detergents: a multicenter study to assess prevalence. J Am Acad Dermatol 46(2):200–206. https://doi.org/10.1067/mjd.2002.119665

Belyaeva EA, Sokolova TV, Emelyanova LV, Zakharova IO (2012) Mitochondrial electron transport chain in heavy metal-induced neurotoxicity: effects of cadmium, mercury, and copper. Sci World J 2012:136063. https://doi.org/10.1100/2012/136063

Bhattacharya M, Biswas D, Sana S, Datta S (2014) Utilization of waste engine oil by *Ochrobactrum pseudintermedium* strain C1 that secretes an exopolysaccharide as a bioemulsifier. Biocatal Agric Biotechnol 3. https://doi.org/10.1016/j.bcab.2014.09.002

Brera S, Nicolini A (2005) Respiratory manifestations due to nickel. Acta otorhinolaryngologica Italica: organo ufficiale della Società italiana di otorinolaringologia e chirurgia cervico-facciale 25:113–115

Camacho-Chab JC, Castañeda-Chávez M, Chan-Bacab MJ, Aguila-Ramírez RN, Galaviz-Villa I, Bartolo-Pérez P, Lango-Reynoso F, Tabasco-Novelo C, Gaylarde C, Ortega-Morales BO (2018) Biosorption of cadmium by non-toxic extracellular polymeric substances (EPS) synthesized by bacteria from marine intertidal biofilms. Int J Environ Res Public Health 15(2):314. https://doi.org/10.3390/ijerph15020314

Capodici M, Di Bella G, Nicosia S, Torregrossa M (2015) Effect of chemical and biological surfactants on activated sludge of MBR system: microscopic analysis and foam test. Bioresour Technol 177:80–86. https://doi.org/10.1016/j.biortech.2014.11.064

Castellane TC, Otoboni A, Lemos E (2015) Characterization of exopolysaccharides produced by rhizobia. Rev Bras Ciênc Solo 39:1566–1575. https://doi.org/10.1590/01000683rbcs20150084

de Oliveira MPS, de Almeida NF, Leite SG (2008) Application of a bacterial extracellular polymeric substance in heavy metal adsorption in a co-contaminated aqueous system. Braz J Microbiol 39(4):780–786. https://doi.org/10.1590/S1517-83822008000400034

Deguchi H, Aoyama R, Takahashi H, Yoshinari I, Tsutsumi Y (2015) Harmful effects of synthetic surface-active detergents against atopic dermatitis. Case Rep Dermatol Med 2015:898262. https://doi.org/10.1155/2015/898262

Deng Q, Li H, Sun H, Sun Y, Li Y (2016) Hyperbranched exopolysaccharide-enhanced foam properties of sodium fatty alcohol polyoxyethylene ether sulfate. Colloid Surface B 141:206–212. https://doi.org/10.1016/j.colsurfb.2016.01.050

Di Palma L, Petrucci E, Pietrangeli B (2015) environmental effects of using chelating agents in polluted sediment remediation. Bull Environ Contam Toxicol 94:340–344. https://doi.org/10.1007/s00128-014-1437-9

Diep P, Mahadevan R, Yakunin AF (2018) Heavy metal removal by bioaccumulation using genetically engineered microorganisms. Front Bioeng Biotechnol 6:157. https://doi.org/10.3389/fbioe.2018.00157

DuBois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28(3):350–356. https://doi.org/10.1021/ac60111a017

Freitas BG, Brito JG, Brasileiro PP, Rufino RD, Luna JM, Santos VA, Sarubbo LA (2016) Formulation of a commercial biosurfactant for application as a dispersant of petroleum and by-products spilled in oceans. Front Microbiol 7:1646. https://doi.org/10.3389/fmicb.2016.01646

Gugliandolo C, Spanò A, Maugeri T, Poli A, Arena A, Nicolaus B (2015) Role of bacterial exopolysaccharides as agents in counteracting immune disorders induced by *herpes* virus. Microorg 464-483. https://doi.org/10.3390/microorganisms3030464

Guibaud G, Comte S, Bordas F, Dupuy S, Baudu M (2005) Comparison of the complexation potential of extracellular polymeric substances (EPS), extracted from activated sludges and produced by pure bacteria strains, for cadmium, lead and nickel. Chemosphere 59:629–638. https://doi.org/10.1016/j.chemosphere.2004.10.028

He J, Sun F, Han F, Gu J, Minrui O, Xu W, Xu X (2018) Preparation of a novel polyacrylic acid and chitosan interpenetrating network hydrogel for removal of U(VI) from aqueous solutions. RSC Adv 8:12684–12691. https://doi.org/10.1039/C7RA13065A

Iyer A, Mody K, Jha B (2005) Biosorption of heavy metals by a marine bacterium. Mar Pollut Bull 50(3):340–343. https://doi.org/10.1016/j.marpolbul.2004.11.012

Jardak K, Drogui P, Daghrir R (2016) Surfactants in aquatic and terrestrial environment: occurrence, behavior, and treatment processes.

Environ Sci Pollut Res 23:3195–3216. https://doi.org/10.1007/s11356-015-5803-x
Kalita D, Joshi SR (2017) Study on bioremediation of lead by exopolysaccharide producing metallophilic bacteria isolated from extreme habitat. Biotechnol Rep 16. https://doi.org/10.1016/j.btre.2017.11.003
Kang CH, Kwon YJ, So JS (2016) Bioremediation of heavy metals by using bacterial mixtures. Ecol Eng 89:64–69. https://doi.org/10.1016/j.ecoleng.2016.01.023
Kapahi M, Sachdeva S (2019) Bioremediation options for heavy metal pollution. J Health Pollut 9(24):191203. https://doi.org/10.5696/2156-9614-9.24.191203
Kaushik A, Kansal A, Santosh M, Kumari S, Kaushik CP (2009) Heavy metal contamination of river Yamuna, Haryana, India: assessment by metal enrichment factor of the sediments. J Hazard Mater 164(1):265–270. https://doi.org/10.1016/j.jhazmat.2008.08.031
Khan SS, Mukherjee A, Chandrasekaran N (2011) Impact of exopolysaccharides on the stability of silver nanoparticles in water. Water Res 45(16):5184–5190. https://doi.org/10.1016/j.watres.2011.07.024
Krishnamachari P, Hashaikeh R, Tiner M (2011) modified Cellulose Morphologies and its Composites; SEM and TEM analysis. Micron (Oxford, England:1993) 42:751–761. https://doi.org/10.1016/j.micron.2011.05.001
Kumar M, Pandey A, & Thakur I (2019) Genomic analysis of carbon dioxide sequestering bacterium for exopolysaccharides production. Sci Rep 9. https://doi.org/10.1038/s41598-019-41052-0
Lakzian A (2008) Adsorption Capability of lead, nickel and zinc by exopolysaccharide and dried cell of *Ensifer meliloti*. Asian J Chem 20:6075–6080
Lechuga M, Fernández-Serrano M, Jurado E, Núñez-Olea J, Ríos F (2016) Acute toxicity of anionic and non-ionic surfactants to aquatic organisms. Ecotoxicol Environ Saf 125:1–8. https://doi.org/10.1016/j.ecoenv.2015.11.027
Liang TW, Wu CC, Cheng WT, Chen YC, Wang CL, Wang IL, Wang SL (2014) Exopolysaccharides and antimicrobial biosurfactants produced by *Paenibacillus macerans* TKU029. Appl Biochem Biotechnol 172(2):933–950. https://doi.org/10.1007/s12010-013-0568-5
Lunkenheimer K, Wantke K (1981) Determination of the surface tension of surfactant solutions applying the method of Lecomte du Noüy (ring tensiometer). Colloid Polym Sci 259:354–366. https://doi.org/10.1007/BF01524716
Mathivanan K, Chandirika JU, Mathimani T, Rajaram R, Annadurai G, Yin H (2021) Production and functionality of exopolysaccharides in bacteria exposed to a toxic metal environment. Ecotoxicol Environ Saf 208:111567. https://doi.org/10.1016/j.ecoenv.2020.111567
Mitchell AM, Mellon MG (2002) Colorimetric determination of nickel with dimethylglyoxime. Ind Eng Chem Anal Ed 17. https://doi.org/10.1021/i560142a012
Mohite BV, Koli SH, Narkhede CP, Patil SN, Patil SV (2017) Prospective of microbial exopolysaccharide for heavy metal exclusion. Appl Biochem Biotechnol 183:582–600. https://doi.org/10.1007/s12010-017-2591-4
Morillo JA, Aguilera M, Ramos-Cormenzana A, Monteoliva-Sánchez M (2006) Production of a metal-binding exopolysaccharide by *Paenibacillus jamilae* using two-phase olive-mill waste as fermentation substrate. Curr Microbiol 53(3):189–193. https://doi.org/10.1007/s00284-005-0438-7
Mwandira W, Nakashima K, Kawasaki S, Arabelo A, Banda K, Nyambe I, Chirwa M, Ito M, Sato T, Igarashi T, Nakata H, Nakayama S, Ishizuka M (2020) Biosorption of Pb (II) and Zn (II) from aqueous solution by *Oceanobacillus profundus* isolated from an abandoned mine. Sci Rep 10(1):21189. https://doi.org/10.1038/s41598-020-78187-4
Nowotny A (1979) Carbohydrate determination by phenol-sulfuric acid. In: Basic exercises in immunochemistry. Berlin, Heidelberg: Springer. https://doi.org/10.1007/978-3-642-67356-6_52
Osemwegie OO, Adetunji CO, Ayeni EA, Adejobi OI, Arise RO, Nwonuma CO, Oghenekaro AO (2020) Exopolysaccharides from bacteria and fungi: current status and perspectives in Africa. Heliyon 6(6):e04205. https://doi.org/10.1016/j.heliyon.2020.e04205
Ozdemir G, Ozturk T, Ceyhan N, Isler R, Cosar T (2003) Heavy metal biosorption by biomass of *Ochrobactrum anthropi* producing exopolysaccharide in activated sludge. Bioresour Technol 90(1):71–74. https://doi.org/10.1016/s0960-8524(03)00088-9
Pandey K, Sengupta R, Bajpai I (2018) 42 rivers have extremely high concentration of neurotoxic heavy metals. https://www.downtoearth.org.in/news/water/huge-amounts-of-toxic-heavy-metals-swim-in-indian-rivers-60545
Raza MH, Sadiq A, Farooq U, Athar M, Hussain T, Mujahid A, & Salman M (2015) Phragmites karka as a biosorbent for the removal of mercury metal ions from aqueous solution: Effect of modification. J Chem 293054. https://doi.org/10.1155/2015/293054
Rezaei H (2016) Biosorption of chromium by using *Spirulina* sp. Arabian J Chem 9(6):846–853. https://doi.org/10.1016/j.arabjc.2013.11.008
Riva MA, Lafranconi A, D'Orso MI, Cesana G (2012) Lead poisoning: historical aspects of a paradigmatic "occupational and environmental disease." Saf Health Work 3(1):11–16. https://doi.org/10.5491/SHAW.2012.3.1.11
Santos DK, Rufino RD, Luna JM, Santos VA, Sarubbo LA (2016) Biosurfactants: multifunctional biomolecules of the 21st Century. Int J Mol Sci 17(3):401. https://doi.org/10.3390/ijms17030401
Sardari R, Kulcinskaja E, Ron E, Bjornsdottir S, Fridjonsson O, Hreggvidsson G, Karlsson E (2016) Evaluation of the production of exopolysaccharides by two strains of the thermophilic bacterium *Rhodothermus marinus*. Carbohydr Polym 156. https://doi.org/10.1016/j.carbpol.2016.08.062
Sengupta D, Datta S, Biswas D (2019) Exploring two contrasting surface-active exopolysaccharides from a single strain of *Ochrobactrum* utilizing different hydrocarbon substrates. J Basic Microbiol 59(8):820–833. https://doi.org/10.1002/jobm.201900080
Shuhong Y, Meiping Z, Hong Y, Han W, Shan X, Yan L, Jihui W (2014) Biosorption of Cu^{2+}, Pb^{2+} and Cr^{6+} by a novel exopolysaccharide from *Arthrobacter* ps-5. Carbohydr Polym 101:50–56
Sibikina O, Iozep A, Moskvin A (2009) Polysaccharide complexes with metal cations: structure and application (a review). Pharm Chem J 43:341–345. https://doi.org/10.1007/s11094-009-0292-1
Singh R, Gautam N, Mishra A, Gupta R (2011) Heavy metals and living systems: an overview. Indian J Pharmacol 43(3):246–253. https://doi.org/10.4103/0253-7613.81505
Skipper A, Sims JN, Yedjou CG, Tchounwou PB (2016) Cadmium chloride induces DNA damage and apoptosis of human liver carcinoma cells via oxidative stress. Int J Environ Res Public Health 13(1):88. https://doi.org/10.3390/ijerph13010088
Sobrino-Figueroa A (2018) Toxic effect of commercial detergents on organisms from different trophic levels. Environ Sci Pollut Res Int 25(14):13283–13291. https://doi.org/10.1007/s11356-016-7861-0
Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ (2012) Heavy metal toxicity and the environment. Exp Suppl 101:133–164. https://doi.org/10.1007/978-3-7643-8340-4_6
Temzi S, Yalaoui-Guellal D, Rodríguez-Carvajal M, Belhadi D, Madani K, Yahia K (2018) Removal of lead by exopolysaccharides from *Paenibacillus peoriae* strainTS7 isolated from rhizosphere of durum wheat. Biocatal Agric Biotechnol 16. https://doi.org/10.1016/j.bcab.2018.09.016
Tulupov P, Polyanskii N (2007) Thermal stability of anion-exchange resins. Russ Chem Rev 42:754. https://doi.org/10.1070/RC1973v042n09ABEH002730

Tyagi B, Gupta B, Thakur IS (2020) Biosorption of Cr (VI) from aqueous solution by extracellular polymeric substances (EPS) produced by *Parapedobacter* sp. ISTM3 strain isolated from Mawsmai cave, Meghalaya, India. Environ Res 191: 110064. https://doi.org/10.1016/j.envres.2020.110064

Ullah M, Haque M (2011) Spectrophotometric determination of toxic elements (cadmium) in aqueous Media. J Chem Eng 25. https://doi.org/10.3329/jce.v25i0.7233

Upadhyay A, Kochar M, Rajam MV, Srivastava S (2017) Players over the surface: unraveling the role of exopolysaccharides in zinc biosorption by fluorescent *Pseudomonas* strain Psd. Front Microbiol 24(8):284. https://doi.org/10.3389/fmicb.2017.00284

Vaishnav A, Upadhyay K, Tipre D, Dave S (2019) Characterization of potent exopolysaccharide producing bacteria isolated from fruit pulp and potato peels and enhancement in their exopolysaccharide production potential. J Microbiol Biotechnol Food Sci 6(3):874–877

Vaishnav A, Upadhayay K, Tipre D, Dave S (2020) Utilization of mixed fruit waste for exopolysaccharide production by *Bacillus* species SRA4: medium formulation and its optimization. 3 Biotech 10(12):550

Velásquez L, Dussan-G J (2009) Biosorption and bioaccumulation of heavy metals on dead and living biomass of *Bacillus spharicus*. J Hazard Mater 167:713–716. https://doi.org/10.1016/j.jhazmat.2009.01.044

Ventorino V, Nicolaus B, Di-Donato P, Pagliano G, Poli A, Robertiello A, Iavrone V, Pepe O (2019) Bioprospecting of exopolysaccharide-producing bacteria from different natural ecosystems for biopolymer synthesis from vinasse. Chem Biol Technol Ag 6(18). https://doi.org/10.1186/s40538-019-0154-3

Wang WX (2016) Bioaccumulation and biomonitoring. In Marine ecotoxicology current knowledge and future issues, eds: Blasco J, Chapman PM, Campana O, Hampel M (Cambridge: Academic Press), 99–119

Wang S, Li H, Zhang A, Fang F, Chen Y, Yan P, Jinsong G, Ma T, Shen Y (2020) Importance of exopolysaccharide branched chains in determining the aggregation ability of anammox sludge. Sci Total Environ 734. https://doi.org/10.1016/j.scitotenv.2020.139470

Wei W, Wang Q, Li A, Yang J, Ma F, Pi S, Wu D (2016) Biosorption of Pb (II) from aqueous solution by extracellular polymeric substances extracted from *Klebsiella* sp. J1: Adsorption behavior and mechanism assessment. Sci Rep 6. 31575. https://doi.org/10.1038/srep31575

Xing Y, Luo X, Liu S, Wan W, Huang Q, Chen W (2020) Synergistic effect of biofilm growth and cadmium adsorption via compositional changes of extracellular matrix in montmorillonite system. Bioresour Technol 315:123742. https://doi.org/10.1016/j.biortech.2020.123742

Yuan C, Xu ZZ, Fan MX, Liu HY, Xie Y, Zhu T (2014) Study on characteristics and harm of surfactants. J Chem Pharma Res 6:2233–2237

Zhang Z, Cai R, Zhang W, Fu Y, Jiao N (2017) A novel exopolysaccharide with metal adsorption capacity produced by a marine bacterium *Alteromonas* sp. JL2810. Mar Drugs 15(6):175. https://doi.org/10.3390/md15060175