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Maximizing EPS production from *Pseudomonas aeruginosa* and its application in Cr and Ni sequestration

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ABSTRACT

Heavy metal contamination of water bodies has been a cause of grave concern around the globe. Analysis of various industrial effluents has revealed a perilous level of Cr (VI) and Ni (II). *Pseudomonas aeruginosa* is an extracellular polymeric substances (EPSs) producing bacterium. EPS has a great potential in the sequestration of heavy metal ions. In the present study efforts have been made to understand the effect of time, pH, and temperature on production of EPS by *P. aeruginosa* (MTCC 1688). The extracted EPS has been applied for removal of Ni (II) and Cr (VI) ions from aqueous system. The results revealed that highest EPS yield (26 mg/50 mL) can be obtained after 96 h of incubation at pH 6 and 32 °C temperature in 50 mL of culture. Treatment of 10 mg/L Cr (VI) and Ni (II) with 30 mg/L EPS resulted in the removal of 26% and 9% of Cr (VI) and Ni (II), respectively. Fourier-transform infrared spectral analysis revealed the involvement of –OH, –NH, C–O, diketone, and ester functional groups of EPS in the attachment of Cr (VI) ion while involvement of amide and –C═O groups in Ni (II) binding with EPS. Scaling-up the production of EPS using bioreactor may further help in developing an efficient process for treatment of water polluted with Cr and Ni.

1. Introduction

Extracellular polymeric substances (EPSs) have been defined as macromolecules/biosynthetic polymers secreted by microorganisms growing in natural or artificial habitat [1]. These are high-molecular-weight complex macromolecules whose composition depends on the type of microorganism and environmental/operational conditions [2]. The biochemical analysis of EPS derived from various sources revealed the presence of proteins, carbohydrates, lipids, nucleic acids, humic substances, and their derivatives [3,4]. Numerous studies have highlighted the role of EPS as a biosorbent for heavy metals which is attributed to various biopolymers of EPS matrix containing different functional groups such as hydroxyl, carboxyl, amide, phosphate, and amin [5–10].

Leather, steel, electroplating, wood, and pulp-processing industries along with other metal-using factories, discharge a large volume of effluents containing nickel and chromium [11]. The International Agency for Research on Cancer (IARC) has classified metallic Ni (II) and Cr (VI) as group 2B and group 1 carcinogen, respectively [12]. Nickel has been found to be embryotoxic and teratogenic [13]. Cr (III) and Cr (VI) occur in stable-state and have acquired environmental importance, out of nine oxidation states varying from −2 to +6 [14]. Acute- and chronic-exposure to Cr (VI) may lead to various respiratory, gastrointestinal, renal, and genetic disorders [12]. The environment (protection) rules (1986) have recommended the maximum permissible limit of discharge for Cr (VI) and Ni (II) in a range of 0.1–2 mg/L and 3–5 mg/L, respectively, in various water bodies, namely, inland surface water, sewerage water, and marine coastal areas [15].

Biosorption is an eco-friendly and sustainable approach for treatment of wastewater containing heavy metals. Application of EPS extracted from single or consortium of microorganisms as biosorbent is considered advantageous due to its nontoxic and biodegradable nature. Multiple binding sites confer heavy metal removal potential in EPS. An increase in rhamnolipid (component of EPS) production was observed when *Pseudomonas aeruginosa* 78 and *P. aeruginosa* 99 were exposed to 10 mg/L of Cr (VI) indicating the involvement of rhamnolipids in Cr (VI) removal from the aqueous system and providing conducive growth environment [16]. In the above context, *Pseudomonas* spp. (EPS-producing bacteria) have been used to sequester heavy metals such as Cd (II), Cu (II), Cr (VI), and Ni (II) from aqueous solution [5,16–19]. Besides, EPS derived from *Bacillus subtilis* and *P. aeruginosa* has been found to be ≥ 45% efficient in removal of Cr (VI) from the aqueous system [16,

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20]. *Bacillus subtilis* MAI3 has been used in the reduction of Cr (VI) to Cr (III) which is less soluble and consequently less toxic [21]. In another study, the efficiency of nickel removal using *P. aeruginosa* ASU 6a was found to be 71% [17]. The efficiency of EPS and EPS-producing bacteria for sequestration of Cr (VI) and Ni (II) have been reported in a range of 26–100% in a few reports (Table 1).

Looking at the potential applications of EPS in heavy metal sequestration [16–20,22] and its production from *P. aeruginosa*, experiments have been carried out to optimize the pH, temperature, and incubation period for maximum EPS yield. Studies have also been conducted to look into the efficiency of extracted EPS in the removal of Ni (II) and Cr (VI) from aqueous systems. To best of our knowledge, this is the first report, where the effect of different operational conditions on EPS production of *P. aeruginosa* MTCC 1688 has been evaluated and its efficiency in removal of Ni (II) and Cr (VI) has been reported.

2. Materials and methods

2.1. Microorganism and culture media

P. aeruginosa (MTCC 1688), an aerobic and gram-negative bacterium was procured from Microbial Type Culture Collection (MTCC), Chandigarh, India. The culture was revived using sterile nutrient broth medium (Hi-Media M002) at pH 7 and temperature 37 °C and used in further experiments.

2.2. Extraction of EPS

EPS was separated and purified according to the method described by Lin et al. [23] with minor modifications. Bacterial cells were removed after centrifugation (10,000 rpm for 30 min) of *P. aeruginosa* culture. The supernatant was mixed with 95% ethanol in 1:3 ratio (v/v). The mixture was incubated at 4 °C overnight to precipitate EPS. The EPS was separated by centrifuging the mixture at 12,000 rpm for 20 min. This EPS was re-suspended in double-distilled water and passed through 0.45 μm cellulose acetate membrane to remove traces of bacterial cell, if any present. The EPS was lyophilized and weighed to get the yield. EPS was extracted from 50 mL of bacterial culture hence the yield was given as EPS mg/50 mL.

2.3. Effect of incubation period on EPS production

The sterile production (nutrient broth) medium was inoculated with 24h-old inoculum. The samples were recovered at definite time intervals (24, 48, 72, 96, and 120 h) to study the effect of time duration of cultures on production of EPS, maintaining the cultures at 37 °C temperature and pH 7 in shaking incubator. Bacterial growth was assessed by recording the absorbance at 540 nm using the Uv–Vis spectrophotometer (Thermo Scientific, USA). Concurrently, bacterial biomass was also determined by estimating the dry weight of bacterial cells.

2.4. Optimization of pH and temperature

The influence of pH on the EPS yield of *P. aeruginosa* was investigated at various pH levels like 4.0, 5.0, 6.0, 7.0, and 8.0. The pH of the media was adjusted with 0.1 N HCl or 0.1 N NaOH. The cultures were maintained in shaking incubators at 37 °C for 96 h. To study the effect of temperature on EPS yield, the cultures were incubated at different temperatures (25 °C, 32 °C, 37 °C, and 42 °C) at pH 6 (optimum pH) for 96 h in a temperature-controlled rotary shaker.

2.5. Nickel and chromium removal efficiency of EPS

Separate stock solutions of Cr (VI) and Ni (II) were prepared by dissolving $K_2Cr_2O_7$ and $NiSO_4{\cdot}7H_2O$, respectively, in double-distilled water. In 100 mL solution of 10 mg/L metal ion (pH 7), 30 mg/L EPS was mixed and kept at room temperature; the aliquots from this mixture were withdrawn after 24 h, 48 h and 72 h intervals. These aliquots were then subjected to centrifugation at 12,000 rpm for 20 min for separation of EPS and the supernatant was analyzed for estimating concentrations of Ni (II) and Cr (VI) using atomic absorption spectrophotometer (Thermo Scientific, USA). The metal removal efficiency was calculated as under

$$\text{Percentage metal removal efficiency} = \frac{(C1 - C2)X100}{C1}$$

Where C1 and C2 are concentration of Cr (VI) and Ni (II) in aqueous solution before and after treatment with EPS respectively.

2.6. Fourier-transform infrared spectroscopy

EPS with metal and without metal was used for Fourier-transform infrared (FTIR) spectral studies to understand the involvement of functional groups in interaction with metal ions. EPS and KBr were mixed in ratio of 1:100, and then transmittance was recorded in a range of 4000–400 cm^{-1} using GX FTIR system (Shimadzu, Japan) at a resolution of 4 cm^{-1}. Atmospheric air was the background for the FTIR spectra.

2.7. Statistical analysis of data

Data related to effect of pH on growth of bacteria in terms of biomass (mg) and absorbance at 540 nm (OD) and yield of EPS (mg), obtained from various experiments, were subjected to Levene's test of normality (Table S1). The data was found to be not-significantly different from normality based on the Skewness z value, Kurtosis z value and Kolmogorov-Smirnov (Lilliefors Significance Correction) coefficient. To look into the effect of pH on bacterial growth and EPS yield data were subjected to one-way analysis of variance (ANOVA) using SPSS version 8.0 at the significance level of $p \leq 0.05$. Similarly, data analysis was carried out to look into the effect of temperature on bacterial growth and EPS yield.

Table 1
Removal of Cr (VI) and Ni (II) by extracellular polymeric substance-producing bacteria.

Metal	EPS-producing bacteria	Operating conditions			Initial metal concentration (ppm)	Percentage metal removal	References
		pH	Temp (°C)	Time (h)			
Cr (VI)	*Klebsiella* sp.	1	30, 35, 40, 45	1.33	20	99.2	[37]
	Arthrobacter sp.	–	25	12.00	50	100	[34]
	Arthrobacter viscosus	7	–	24.00	20	96.4	[38]
	Pseudomonas aeruginosa	7	20	96.00	50	94.3	[16]
	Micrococcus sp.	7	20	72.00	100	35.5	[16]
	Ochrobactrum	8	30	48.00	150	20.8	[16]
	Bacillus subtilis	7	RT	24.00	10	48	[23]
	Azotobacter beijerinckii	7	RT	24.00	10	26	[23]
Ni (II)	*Pseudomonas* sp.	–	32	–	125	28	[22]
	Cloacibacterium normanense.	–	–	2.00	48	85	[26]

Note: "–" indicates no information.

3. Results and discussion

3.1. Effect of incubation period on EPS production

To evaluate the most favorable conditions for maximum production of EPS from *P. aeruginosa*, the effect of time, pH, and temperature have been studied. The EPS production has been found to be influenced by growth phase (time) (Fig. 1). Highest EPS yield (22 mg/50 mL) has been recorded after 96 h at pH 7 which further remains same (non-significant difference) up to 120 h. Nouha et al. [24] have reported similar findings where maximum (21.3 g/L) EPS production in *Cloacibacterium normanense* was observed in late stationary phase after 72 h. To look into relationship between biomass of bacteria and EPS yield correlation coefficient was calculated between biomass of bacterial cells and EPS. It was found to be 0.971. It indicates that EPS yield is positively correlated with bacterial biomass (Fig. 1). Bacterial biomass and EPS yield enhanced in the logarithmic phase, while the maximum level was attained at the stationary phase.

In addition, growth in the form of absorbance and bacterial biomass has also been recorded. The bacterial biomass was found to be positively correlated with EPS yield (Fig. 1). The amount of biomass and EPS enhanced up to the logarithmic phase and the maximum amount was recorded at the stationary phase.

3.2. Optimization of pH for EPS production

After standardizing the optimum time of 96 h, the effect of pH on EPS yield has been studied by varying pH from 4 to 8 (Fig. 2). The observations revealed that pH 6 is optimal for the highest EPS production (23.3 mg/50 mL). EPS yield increases with increase in pH from 4 to 6, and then decreases. The decrease in the EPS yield with increasing pH beyond 6 is also statistically significant. These findings are in accordance with earlier reports for *P. aeruginosa* and *B. subtilis* [14,20]

3.3. Optimization of temperature for EPS production

Cultures were incubated at various temperatures ranging from 25 °C to 42 °C to optimize temperature for the highest EPS yield from *P. aeruginosa* at pH 6 after 96 h of incubation period. Results revealed that 32 °C is most suitable temperature to get the highest EPS yield (Fig. 3). However, it may be noticed that optimum temperature for growth of this bacterium is 37 °C. Further increase in the temperature leads to decreased growth and EPS yield.

These results are in conformity with earlier reports for EPS production in *Pseudomonas* sp. GP32 [25]. In contrast, Kılıç and Dönmez [14] and Zhou et al. [26] have reported 20 °C temperature to be optimum for EPS production from *P. aeruginosa* isolated from tannery effluent and *P. aeruginosa* CICC 23618, respectively. More et al. [4] have reviewed that the best possible temperature for growth of microorganisms and EPS production. Lower incubation temperature facilitates EPS synthesis by reducing growth and increasing the availability of precursor for EPS synthesis [27]. The genotype, media composition, and culture conditions such as pH, temperature, and incubation time influence the yield and composition of EPS.

From the current experimental findings, it may be concluded that bacteria produce EPS to protect the microbial cells from harsh external environmental conditions/stress including extremes of pH and temperature. Therefore, arrival of stressful conditions leads to increased EPS production augmenting the endurance potential of bacterial cells. Further, it has also been observed here that EPS yield was high at the logarithmic phase, as at this time the maximum number of active bacterial populations were contributing in EPS production. Future, studies may be focussed on manipulating environmental conditions to enhance EPS production and develop better scientific understanding regarding bacterial EPS production process.

3.4. Nickel and chromium removal efficiency of EPS

Owing to the presence of multiple binding sites, EPS plays a crucial role as a biosorbent. In the present study, 30 mg/L EPS derived from *P. aeruginosa* removed maximum of 26% and 9% of Cr (VI) and Ni (II), respectively, from aqueous solution when the initial concentration of each metal was 10 mg/L in separate batches (Fig. 4). This difference may be due to difference in their ionization energies (Cr VI- 8744.9 KJ/Mol and Ni (II) 1753 KJ/Mol). This energy governs the interaction of elements with compounds.

Cr (VI) and Ni (II) removal using EPS extracted from *B. subtilis*, *Klebsiella* sp. H-207, *Arthrobacter* sp B4, *Pseudomonas* EJ01, and *C. normanense* have been reported earlier (Table 1). Biosorption of Ni using biomass derived from *P. aeruginosa* and *Pseudomonas fluorescens* 4F39 has also been reported [17,28]. Incubation time required for maximum removal of Ni (II) and Cr (VI) was found to be 24 and 48 h, respectively (Fig. 4). Variation in the time taken for metal ions to get adsorbed on EPS surface is dependent on quality and quantity of EPS [29]. Further, it is also influenced by nature of metal ions and operational conditions.

3.5. Interaction of functional groups of EPS with Cr (VI) and Ni (II)

FTIR spectral analysis of EPS (with and without metal ion(s)), revealed the presence of few functional groups and their interaction at certain frequencies (Table 2). Polysaccharides, proteins, and/or peptides

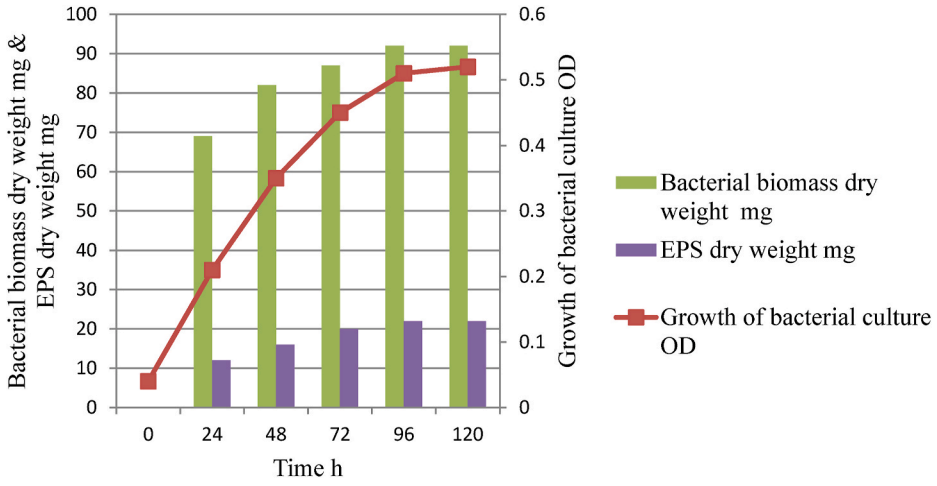

Fig. 1. Effect of time on bacterial growth (OD), biomass production and EPS yield by *Pseudomonas aeruginosa* MTCC 1688 at pH 7 and 37 °C.

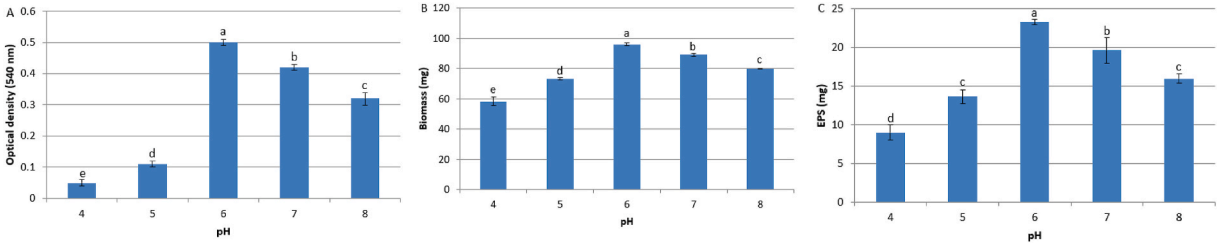

Fig. 2. Effect of pH on (A) Optical density (growth) of bacterial suspension culture; (B) biomass production and (C) EPS yield/production by *Pseudomonas aeruginosa* in 50 mL culture media after 96 h.

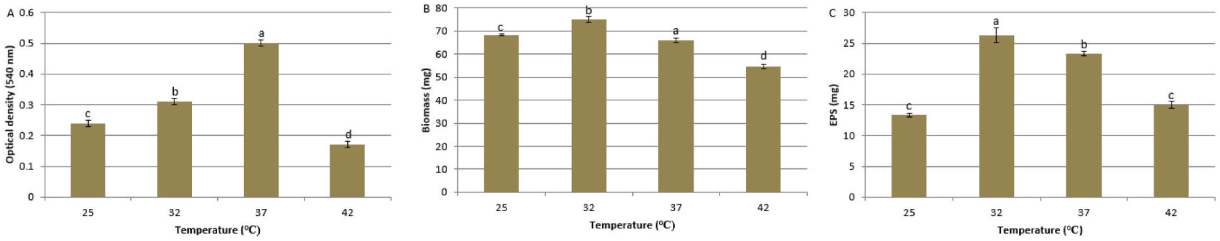

Fig. 3. Effect of temperature on (A) Optical density (growth) of bacterial suspension culture; (B) biomass production and (C) EPS yield/production by *Pseudomonas aeruginosa* in 50 mL culture media after 96 h.

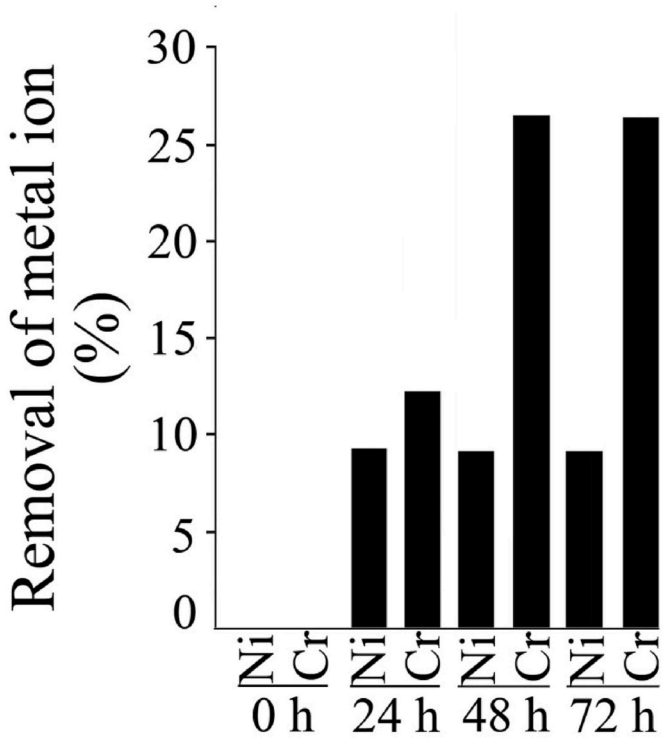

Fig. 4. Removal of Ni (II) and Cr (VI) from aqueous system using EPS of *Pseudomonas aeruginosa* at room temperature (volume of solution: 100 mL, initial concentration of metals: 10 ppm, EPS: 30 ppm).

in the extracted EPS are evident from the appearance of functional groups including –OH, –NH stretch in amines, $–CH_3$ and $–CH_2$ stretch of aliphatic compounds, –C═O bond, $–NH_2$ group, diketone, ester, and the C–N stretch of amide III bonds. Similar findings have been also reported earlier [8,30,31]. These functional groups may interact with metal ions which are an electron-rich moiety [18,32].

When EPS is treated with Cr (VI) and Ni (II), the spectral peaks shift to a new position (Fig. S1 and Table 2) indicating the involvement of certain functional groups in chelating the metal ions. The spectral analysis points out the binding of chromium with EPS on –OH group, –NH (amines), C–O, diketone, and ester functional groups as depicted in Fig. S1 and Table 2 and similar results have been reported earlier as well [5,32,33]. The FTIR spectrum indicates that the amide II band, which appeared at 1540 cm^{-1} in EPS, disappeared after treatment with Ni (II). A new stretch of –C═O appeared at 1659 and 1702 cm^{-1} after interaction of Ni (II) with EPS. Our results are in agreement with earlier reports where binding of Ni (II) with amide and –C═O group has been observed [17].

Table 2
FTIR transmittance data for EPS derived from *Pseudomonas aeruginosa* with and without Cr (VI) and Ni (II) (approx. frequency in cm^{-1}).

EPS (Without metal)	EPS with Cr (VI)	EPS with Ni (II)
553.57	567.07	
686.66	675.09	
808.17	844.82	878
		921
1020.34	989.48	964
1236.37	1228.66	1181
	1286.52	1214
	1363.67	
1408.04	1452.40	
	1506.41	
1539.20	1537.27	
1625.09	1624.06	1659
1680.00	1668.43	
	1703.14	1702
		1746
	2331.94	
2353.16	2360.87	
2885.51	2881.65	2746
2996.52		2854
3190.26		
	3496.94	
3545.16	3738.05	
	3890.42	

4. Conclusion

From the present study, it is evident that *P. aeruginosa* MTCC 1688

can produce maximum EPS when inoculated on nutrient broth having pH 6 at 32 °C after 96 h incubation. This EPS has the potential to remove 26% Cr (VI) and 9% Ni (II) respectively from the aqueous system at pH 7 and room temperature. FTIR spectral observations indicate that metal ions interacted with various functional groups present on EPS. Industrial effluent may be treated in batches for the removal of these metals using this EPS. Mutation studies and/or stress may be helpful in enhancing the quantity and quality of EPS, which can make *P. aeruginosa* a better resource in combating Cr (VI) and Ni (II) pollution.

Significance and impact of study

The leather, steel and textile industries release a huge amount of waste water effluent containing Cr and Ni above the permissible limits which poses serious hazardous effect on flora and fauna of aquatic ecosystem. Removal of Cr and Ni using extracellular polymeric substances (EPS) derived from bacteria provides an eco-friendly and sustainable solution to resolve this problem. Use of EPS in removal of Cr and Ni is even a better approach than the use of living bacteria, as later approach has a constrain to maintain the bacteria in living state. Owing to the advantages associated with EPS-mediated heavy metal removal, present work provides an insight on the factors influencing EPS production by *Pseudomonas aeruginosa*. Besides, the potential of the extracted EPS has also been explored to sequester Cr and Ni. Findings of the present study will help in developing an efficient strategy to treat water contaminated with Cr and Ni.

Declaration of competing interest

The authors of this manuscript have no conflict of interest for this manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2021.100972.

References

[1] O.Y.A. Costa, J.M. Raaijmakers, E.E. Kuramae, Microbial extracellular polymeric substances: ecological function and impact on soil aggregation, Front. Microbiol. 9 (2018) 1636, https://doi.org/10.3389/fmicb.2018.01636.
[2] Y. Yang, A.J. Wikieł, L.T. Dall'Agnol, P. Eloy, M.J. Genet, J.J.G. Moura, W. Sand, C. C. Dupont-Gillain, P.G. Rouxhet, Proteins dominate in the surface layers formed on materials exposed to extracellular polymeric substances from bacterial cultures, Biofouling 32 (2016) 95–108, https://doi.org/10.1080/08927014.2015.1114609.
[3] M. Marvasi, P.T. Visscher, L. Casillas Martinez, Exopolymeric substances (EPS) from Bacillus subtilis : polymers and genes encoding their synthesis, FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Lett. 313 (2010) 1–9, https://doi.org/10.1111/j.1574-6968.2010.02085.x.
[4] T.T. More, J.S.S. Yadav, S. Yan, R.D. Tyagi, R.Y. Surampalli, Extracellular polymeric substances of bacteria and their potential environmental applications, J. Environ. Manag. 144 (2014) 1–25, https://doi.org/10.1016/j.jenvman.2014.05.010.
[5] X. Wei, L. Fang, P. Cai, Q. Huang, H. Chen, W. Liang, X. Rong, Influence of extracellular polymeric substances (EPS) on Cd adsorption by bacteria, Environ. Pollut. 159 (2011) 1369–1374, https://doi.org/10.1016/j.envpol.2011.01.006.
[6] W.W. Li, H.Q. Yu, Insight into the roles of microbial extracellular polymer substances in metal biosorption, Bioresour. Technol. 160 (2014) 15–23, https://doi.org/10.1016/j.biortech.2013.11.074.
[7] B. Volesky, Z.R. Holan+, REVIEW Biosorption of Heavy Metals Quantitative Evaluation of 245 Multimetal Biosorption Systems Possible Effects of the Biosorbent 246 Material Architecture Biosorbents for Sorption Column 246 Applications Conclusions 247, 1995. https://pubs.acs.org/sharingguidelines. (Accessed 8 December 2020).
[8] G. Liu, X. Miao, Switching cultivation for enhancing biomass and lipid production with extracellular polymeric substance as co-products in Heynigia riparia SX01, Bioresour. Technol. 227 (2017) 214–220, https://doi.org/10.1016/j.biortech.2016.12.039.
[9] J. Camacho-Chab, M. Castañeda-Chávez, M. Chan-Bacab, R. Aguila-Ramírez, I. Galaviz-Villa, P. Bartolo-Pérez, F. Lango-Reynoso, C. Tabasco-Novelo, C. Gaylarde, B. Ortega-Morales, Biosorption of cadmium by non-toxic extracellular polymeric substances (EPS) synthesized by bacteria from marine intertidal biofilms, Int. J. Environ. Res. Publ. Health 15 (2018) 314, https://doi.org/10.3390/ijerph15020314.
[10] A. Ayangbenro, O. Babalola, Metal(loid) bioremediation: strategies employed by microbial polymers, Sustainability 10 (2018) 3028, https://doi.org/10.3390/su10093028.
[11] S. Congeevaram, S. Dhanarani, J. Park, M. Dexilin, K. Thamaraiselvi, Biosorption of chromium and nickel by heavy metal resistant fungal and bacterial isolates, J. Hazard Mater. 146 (2007) 270–277, https://doi.org/10.1016/j.jhazmat.2006.12.017.
[12] L. Assem, H. Zhu, Chromium Toxicological Overview, Institute of Environment and Health, Cranfield University, Health Protection Agency, Bedfordshire, UK, 2013. Accessed March. 17 (2007).
[13] C.Y. Chen, T.H. Lin, Nickel toxicity to human term placenta: in vitro study on lipid peroxidation, J. Toxicol. Environ. Health Part A 54 (1998) 37–47, https://doi.org/10.1080/009841098159015.
[14] N.K. Kiliç, G. Dönmez, Environmental conditions affecting exopolysaccharide production by Pseudomonas aeruginosa, Micrococcus sp., and Ochrobactrum sp, J. Hazard Mater. 154 (2008) 1019–1024, https://doi.org/10.1016/j.jhazmat.2007.11.008.
[15] Anonymous, The Environment (Protection) Rules, Ministry OF environment and forests, 1986. https://upload.indiacode.nic.in/showfile?actid=AC_MP_74_308_00003_00003_1543231806694&type=rule&filename=ep_rules_1986.pdf, 1986. (Accessed 8 December 2020).
[16] S. Ozturk, T. Kaya, B. Aslim, S. Tan, Removal and reduction of chromium by Pseudomonas spp. and their correlation to rhamnolipid production, J. Hazard Mater. 231–232 (2012) 64–69, https://doi.org/10.1016/j.jhazmat.2012.06.038.
[17] R.M. Gabr, S.H.A. Hassan, A.A.M. Shoreit, Biosorption of lead and nickel by living and non-living cells of Pseudomonas aeruginosa ASU 6a, Int. Biodeterior. Biodegrad. 62 (2008) 195–203, https://doi.org/10.1016/j.ibiod.2008.01.008.
[18] L. Fang, X. Wei, P. Cai, Q. Huang, H. Chen, W. Liang, X. Rong, Role of extracellular polymeric substances in Cu(II) adsorption on Bacillus subtilis and Pseudomonas putida, Bioresour. Technol. 102 (2011) 1137–1141, https://doi.org/10.1016/j.biortech.2010.09.006.
[19] C.C. Chien, B.C. Lin, C.H. Wu, Biofilm formation and heavy metal resistance by an environmental Pseudomonas sp, Biochem. Eng. J. 78 (2013) 132–137, https://doi.org/10.1016/j.bej.2013.01.014.
[20] R. Chug, V.S. Gour, S. Mathur, S.L. Kothari, Optimization of Extracellular Polymeric Substances production using Azotobacter beijreinckii and Bacillus subtilis and its application in chromium (VI) removal, Bioresour. Technol. 214 (2016) 604–608, https://doi.org/10.1016/j.biortech.2016.05.010.
[21] P.A. Wani, S. Wahid, R. Singh, A.M. Kehinde, Antioxidant and chromium reductase assisted chromium (VI) reduction and Cr (III) immobilization by the rhizospheric Bacillus helps in the remediation of Cr (VI) and growth promotion of soybean crop, Rhizosphere 6 (2018) 23–30, https://doi.org/10.1016/j.rhisph.2018.01.004.
[22] R. Dobrowolski, A. Krzyszczak, J. Dobrzyńska, B. Podkościelna, E. Zięba, M. Czemierska, A. Jarosz-Wilkołazka, E.A. Stefaniak, Extracellular polymeric substances immobilized on microspheres for removal of heavy metals from aqueous environment, Biochem. Eng. J. 143 (2019) 202–211, https://doi.org/10.1016/j.bej.2019.01.004.
[23] M.H. Lin, Y.L. Yang, Y.P. Chen, K.F. Hua, C.P. Lu, F. Sheu, G.H. Lin, S.S. Tsay, S. M. Liang, S.H. Wu, A novel exopolysaccharide from the biofilm of Thermus aquaticus YT-1 induces the immune response through toll-like receptor 2, J. Biol. Chem. 286 (2011) 17736–17745, https://doi.org/10.1074/jbc.M110.200113.
[24] K. Nouha, R.S. Kumar, R.D. Tyagi, Heavy metals removal from wastewater using extracellular polymeric substances produced by Cloacibacterium normanense in wastewater sludge supplemented with crude glycerol and study of extracellular polymeric substances extraction by different methods, Bioresour. Technol. 212 (2016) 120–129, https://doi.org/10.1016/j.biortech.2016.04.021.
[25] M.E. Lee, H.D. Lee, H.-H. Suh, Production and characterization of extracellular polysaccharide produced by Pseudomonas sp, GP32, 생명과학회지. 25 (2015) 1027–1035.
[26] L. Zhou, S. Xia, Z. Zhang, B. Ye, X. Xu, Z. Gu, X. Wang, Effects of pH, Temperature and Salinity on Extracellular Polymeric Substances of Pseudomonasaeruginosa Biofilm with N-(3-Oxooxtanoyl)-L-Homoserine Lactone Addition, 2014.
[27] I.W. Sutherland, Microbial polysaccharides from Gram-negative bacteria, in: International Dairy Journal, Elsevier, 2001, pp. 663–674, https://doi.org/10.1016/S0958-6946(01)00112-1.
[28] A. López, N. Lázaro, J.M. Priego, A.M. Marqués, Effect of pH on the biosorption of nickel and other heavy metals by Pseudomonas fluorescens 4F39, J. Ind. Microbiol. Biotechnol. 24 (2000) 146–151, https://doi.org/10.1038/sj.jim.2900793.
[29] Y. Zheng, X. Fang, Z. Ye, Y. Li, C.A.I. weimin, Biosorption of Cu(II) on extracellular polymers from Bacillus sp. F19, J. Environ. Sci. 20 (2008) 1288–1293, https://doi.org/10.1016/S1001-0742(08)62223-8.

[30] Y. Jiao, G.D. Cody, A.K. Harding, P. Wilmes, M. Schrenk, K.E. Wheeler, J. F. Banfield, M.P. Thelen, Characterization of extracellular polymeric substances from acidophilic microbial biofilms, Appl. Environ. Microbiol. 76 (2010) 2916–2922, https://doi.org/10.1128/AEM.02289-09.

[31] B. Cao, L. Shi, R.N. Brown, Y. Xiong, J.K. Fredrickson, M.F. Romine, M.J. Marshall, M.S. Lipton, H. Beyenal, Extracellular polymeric substances from Shewanella sp. HRCR-1 biofilms: characterization by infrared spectroscopy and proteomics, Environ. Microbiol. 13 (2011) 1018–1031, https://doi.org/10.1111/j.1462-2920.2010.02407.x.

[32] Y. Li, Q. Li, Y. Fengying, J. Bao, Z. Hu, W. Zhu, Y. Zhao, Z. Lin, Q. Dong, Chromium (VI) detoxification by oxidation and flocculation of exopolysaccharides from Arthrobacter sp. B4, Int. J. Biol. Macromol. 81 (2015) 235–240, https://doi.org/10.1016/j.ijbiomac.2015.07.013.

[33] S. Chatterjee, I. Ghosh, K.K. Mukherjea, Uptake and removal of toxic Cr (VI) by Pseudomonas aeruginosa: physico-chemical and biological evaluation, Curr. Sci. (2011) 645–652.