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Biosorption of nickel, chromium and zinc by MerP-expressing recombinant *Escherichia coli*

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Abstract

Escherichia coli hosts able to over-express metal-binding proteins (MerP) originating from Gram-positive (*Bacillus cereus* RC607) and Gram-negative (*Pseudomonas* sp. K-62) bacterial strains were used to adsorb Ni^{2+}, Zn^{2+} and Cr^{3+} in aqueous solutions. The initial adsorption rate and adsorption capacity were determined to evaluate the performance of the biosorbents. With the expression of MerP protein, the metal adsorption capacity of the recombinant strains for Ni^{2+}, Zn^{2+} and Cr^{3+} significantly improved. The cells carrying Gram-positive *merP* gene (GB) adsorbed Zn^{2+} and Cr^{3+} at a capacity of 22.3 and 0.98 mmol/g biomass, which is 121% and 72% higher, respectively, over that of the MerP-free host cells. Adsorption capacity of the cells carrying Gram-negative *merP* gene (GP) also increased 144% and 126% for Zn^{2+} and Cr^{3+}, respectively. Both recombinant strains also exhibited 24% and 5% enhancement in adsorption of Ni^{2+} for GB and GP, respectively. The initial adsorption rate of the recombinant biosorbents was also higher than that of the MerP-free host, suggesting an increased metal-binding affinity with MerP expression. Severe cell damage on GB biosorbent was observed after Cr^{3+} adsorption, probably due to the metal toxicity effect on the cells.
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Keywords: Biosorption; Metal-binding protein; MerP; Chromium; Nickel; Zinc

1. Introduction

The removal of toxic heavy metals from wastewaters is of great interest and importance in the field of water pollution control [1–9]. The conventional process used to remove heavy metal ions from the contaminated environments include solvent extraction, chemical precipitation, ion exchange, activated carbon adsorption, chelation, reverse osmosis, coagulation–precipitation, electrochemical operation, and filtration were adopted [5–17]. However, those physicochemical technologies possess significant drawbacks of being expensive and environmentally disruptive, requiring input of external chemical additives or energy, as well as generating concentrated toxic sludge or other wastes that must be disposed [5–17]. Those disadvantages are especially apparent at low metal concentrations often encountered in wastewaters. Therefore, it is urgent to develop efficient and environmentally compatible means able to remove or detoxify heavy metals in an economical way [2]. Biosorption, often referred to passive uptake and physicochemical binding of chemical species or ions to biomass or biopolymers, is one of the promising alternative approaches to the conventional wastewater treatments and plays an important role in pollution control of heavy-metal contaminated water [5,11,13,16,18–23]. The biosorption process offers the advantages of low operating costs, reduced amount of chemical and/or biological sludge to be disposed of, and high efficiency in decontaminating effluents with dilute metal ion content [1,2,4,6,11,13,16,18,22]. A variety of organisms (e.g. bacteria, fungi, alga, yeast, molds and higher plants) have been used as adsorbents for efficient removal or accumulation of heavy metals from the polluted environments [2,6–10,13,16,18,20,21]. Volesky and Holan [24] summarized the type and performance of biosorbents as well as biosorption treatment processes reported in the literature.

Feasible approaches leading to improvement of heavy-metal biosorption efficiency include development of more powerful biosorbents and the design of more efficient biosorption

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processes. Biosorbent development could be achieved by either isolating natural organisms with high capacity or high specificity to heavy metals or by tailoring genetically modified organisms abundant of high-affinity metal binding proteins or polypeptides [2,25–29]. Recent work showed that expressing *mer* operon genes encoding for cysteine-containing mercuric ion transport proteins (such as periplasmic protein MerP or inner membrane protein MerT) [2,30,31] on *Escherichia coli* hosts could make them very effective biosorbents for heavy metals removal. In addition, several other metal-binding proteins, such as metallothioneins (MTs) [32,33], phytochelatins (PCs) [34], and metal-binding peptides [2] were also expressed on *E. coli* to create powerful biosorbents. Although MerP protein is a target for the development of genetically engineered biosorbents [35], there is little information on utilizing MerP-expressing biosorbents to remove heavy metals (especially for metals other than mercury) [2]. Therefore, the aim of this study was to investigate the potential of using recombinant *E. coli* strains capable of over-expressing MerP proteins originating from Gram-positive and Gram-negative bacteria for the removal of heavy metals (Ni^{2+}, Zn^{2+} and Cr^{3+}) that are commonly found in the industrial effluents in Taiwan.

2. Materials and methods

2.1. Plasmids and host strain

The recombinant *E. coli* strains harboring *merP* genes were constructed as described in our previous work [2]. The *merP* genes came from a Gram-positive bacterium, *Bacillus cereus* RC607, and a Gram-negative bacterium, *Pseudomonas* sp. K-62. The recombinant plasmids harboring *merP* genes from *B. cereus* RC607 and *Pseudomonas* sp. K-62 were designated as pETBmerP and pETPmerP, respectively. The constructed plasmids were then transformed into *E. coli* BL21(DE3)pLysS (denotes as BL21) for gene expression.

2.2. Expression of MerP proteins in recombinant E. coli

The recombinant strains, namely *E. coli* BL21(DE3)pLysS/pETBmerP (denoted as GB) and *E. coli* BL21(DE3)pLysS/pETPmerP (denoted as GP) were grown at 37 °C with 220 rpm agitation in Luria–Bertani (LB) broth (Difco) amended by 100 mg/mL of ampicillin until the optical density (at 600 nm) of the culture reached 0.6. The culture was subsequently induced with 0.25 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for 6 h. The control strains *E. coli* BL21(DE3)pLysS (denotes as BL21) was cultivated and treated in the same way, except that the medium did not contain antibiotics.

2.3. Preparation of the bacterial biosorbents

Cells with over-expressed MerP proteins (GB and GP) and control strain (BL21) were harvested by centrifugation (6000 rpm, 10 min) and rinsed twice with 0.1 M Tris–HCl buffer (pH 7.0; Sigma) and were concentrated to 300 g cell (dry weight)/L in the same Tris–HCl buffer. The concentrated cells were re-suspended in designated heavy metal solutions to reach an appropriate cell concentration (ca. 3 g dry weight/L) for the biosorption experiments. All the glassware used in the biosorption operations was treated with concentrated HNO_3 solution prior to each experiment to avoid possible adsorption of heavy metals on the surface of glass containers [2,4,32].

2.4. Measurement of heavy metals

The heavy metal adsorbates used in this study were $NiCl_2$, $ZnCl_2$, and $Cr(NO_3)_2{\cdot}9H_2O$, which were obtained from Merck (Germany). Heavy metals in solutions were measured by a Polarized Zeeman Atomic Absorption Spectrometer (AAS; Model Z-6100, Hitachi Co., Japan). Prior to AAS measurement, the heavy metal solutions were properly diluted to ensure that the metal concentration in the sample was linearly dependent on the detected absorbance.

2.5. Determination of initial biosorption rate

The concentrated biosorbents (GB, GP, and BL21) were added to glass tubes containing Ni^{2+}, Zn^{2+} and Cr^{3+} at an initial concentration of 8.52, 250, and 9.62 mM, respectively (at those metal concentrations, the biosorption of each metal nearly reached saturation). After well mixing, samples were taken from the solution at designated time intervals (within 30 s of reaction time) and the heavy metal concentration in the supernatants was measured by AAS. The initial adsorption rate was determined according to Eq. (1):

$$r_0 = -\left(\frac{1}{X}\right)\left(\frac{\mathrm{d}C_i}{\mathrm{d}t}\right)\Bigg|_{t=0} \quad (1)$$

where r_0 represents initial adsorption rate (mmol/s g dry cell), X the initial cell concentration (g/L), C_i the residual concentration of heavy metal component i (mM), and t denotes incubation time (s).

2.6. Determination of adsorption isotherms

The biosorbents were suspended in aqueous solutions containing Ni^{2+}, Zn^{2+} and Cr^{3+} at different initial concentrations, ranging from 0.17 to 17.04, 0.08 to 306.30, and 0.10 to 19.23 mM, respectively. The cell concentration in the solution was approximately 3 g/L (dry weight). The adsorption were carried out under the condition of 28 ± 2 °C and 125 rpm shaking. After equilibrium adsorption (about 2 h of incubation), samples were taken from the solutions, and the metal concentration in the supernatant was measured by AAS. The equilibrium metal adsorption capacity (q; mmol/g cell) was plotted versus the corresponding equilibrium metal concentration in aqueous phase (C_e, mM) to establish the adsorption isotherm curves. The equilibrium adsorption capacity (q) was calculated according to Eq. (2):

$$q = \frac{C_0 - C_e}{X} \quad (2)$$

where, C_0 denotes the initial metal concentration (mM) and X denotes the initial cell concentration (g/L).

2.7. Elemental analysis (EA)

The element content of cells of BL21, GB, and GP (with and without IPTG induction) was measured by elemental analyzer (EA; Elementar vario EL III, CHNOS Rapid F002, Heraeus, Germany) to examine if sulfur content increased with MerP protein production. An appropriate amount of cells was centrifuged and dried in a Refrigerated Vapor Trap (RVT, model 400, Thermo Fisher Scientific, MA, USA). The dried cells were ground into powder for elemental analysis (EA).

2.8. Analysis by the variable vacuum scanning electron microscopy (VVSEM)

Cell morphology of the biosorbents was examined before and after heavy metal adsorption using variable vacuum scanning electron microscopy (VVSEM; Model S3000, Hitachi Co., Japan). After the adsorption experiments, the cells were filtrated through the Whatman filter papers, and then immersed in the solution of 2.5% glutaraldehyde for 3 h to immobilize the cells. The cells were then soaked in acetone solution under the concentrations ranging from 50 to 95% (v/v) for 30 min at each concentration. After that, the cells were subjected to air drying, and then examined by VVSEM.

2.9. Analysis by the energy dispersive spectrometry (EDS)

In addition to AAS analysis for heavy metal measurement, energy dispersive spectrometry (EDS; Horiba, UK) was also used to detect heavy metals adsorbed on the cells with and without MerP proteins expression. Like the procedures used for VVSEM analysis, the metal-loaded cells were collected by centrifugation, and dried in Refrigerated Vapor Trap. Afterwards, an appropriate amount of 95% ethanol was added to the dried sample to increase the dispersive ability. The treated sample was dried in the hood prior to use for EDS analysis.

3. Results and discussion

3.1. Effect of MerP expression on initial adsorption rate

Most metal biosorption studies presented adsorption capacity of metal adsorbates, while very few reports provided information regarding adsorption rate, which is usually a critical factor for process design [18,23]. In this work, the initial adsorption rate of Ni^{2+}, Zn^{2+} and Cr^{3+} was measured for BL21, GB and GP cells within 30 s of reaction time to determine whether expression of metal-binding protein MerP would facilitate biosorption of the tested heavy metals. As indicated in Table 1 and Fig. 1, the initial biosorption rate (r_0) of the MerP-carrying biosorbents markedly increased when compared to the MerP-free host strain (BL21). The r_0 of the strain expressing Gram-positive *merP* gene (GB) increased 72, 74 and 33% over BL21 (control biosorbent) for adsorption of Ni^{2+}, Zn^{2+} and Cr^{3+}, respectively. The r_0 of the strain carrying Gram-negative *merP* gene (GP) also increased 16%, 90% and 131% for Ni^{2+}, Zn^{2+} and Cr^{3+}, respectively. The foregoing results suggest that the presence of MerP protein may enhance metal-biosorbent affinity, thereby resulting in better adsorption rates. However, the extent of enhancement effect could be dependent on the type of MerP protein and the type of metal adsorbates, as indicated in Fig. 1 and Table 1.

Table 1
The initial adsorption rate (r_0) of the host (BL21) and recombinant strains (GB and GP) during biosorption of Ni^{2+}, Zn^{2+}, and Cr^{3+} ions

Metal	Initial adsorption rate (r_0) (mmol metal/s g cell)		
	BL21	GB	GP
Ni^{2+}	0.0105	0.0181	0.0122
Zn^{2+}	0.0245	0.0426	0.0465
Cr^{3+}	0.0090	0.0120	0.0207

Initial metal concentrations: Ni^{2+}, 8.52 mM; Zn^{2+}, 250 mM; Cr^{3+}, 9.62 mM.

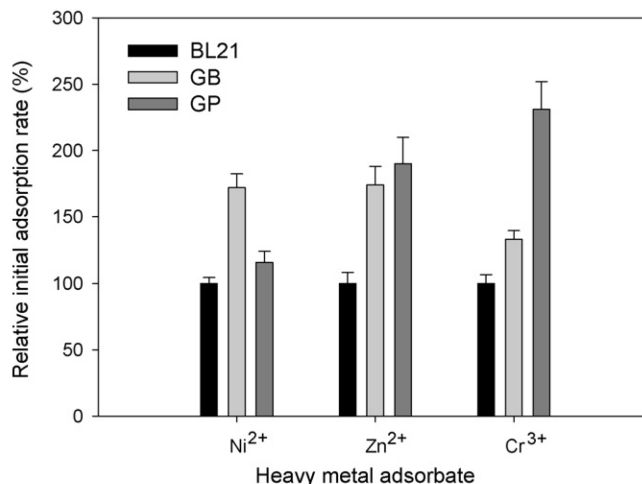

Fig. 1. Comparison of the relative initial adsorption rate (normalized by the results obtained for BL21 as the standard for each metal) of the host (BL21) and recombinant strains (GB and GP) during biosorption of Ni^{2+}, Zn^{2+}, and Cr^{3+}. The initial metal concentration used was 8.52, 250, and 9.62 mM for Ni^{2+}, Zn^{2+}, and Cr^{3+}, respectively.

3.2. Effect of MerP expression on adsorption isotherm

Adsorption isotherm is the most important performance index representing the ability and feasibility of a biosorbent. Thus, the adsorption isotherm was determined for MerP-expressing (i.e., GB and GP) and MerP-free (i.e., BL21) biosorbents. The isotherm results are shown in Fig. 2a–c for adsorption of Ni^{2+}, Zn^{2+}, and Cr^{3+} ions, respectively. Langmuir isotherm (Eq. (3)) was used to describe the adsorption equilibrium data:

$$q = \frac{q_{max} C_e}{K_d + C_e} \quad (3)$$

where q_{max} represents the maximum adsorption capacity (mg/g cell) and K_d is the dissociation constant (mg/L). The isotherm data were simulated according to Eq. (3) and the optimal q_{max} and K_d values estimated from the model are given in Table 2. Over-expression of MerP proteins in the recom-

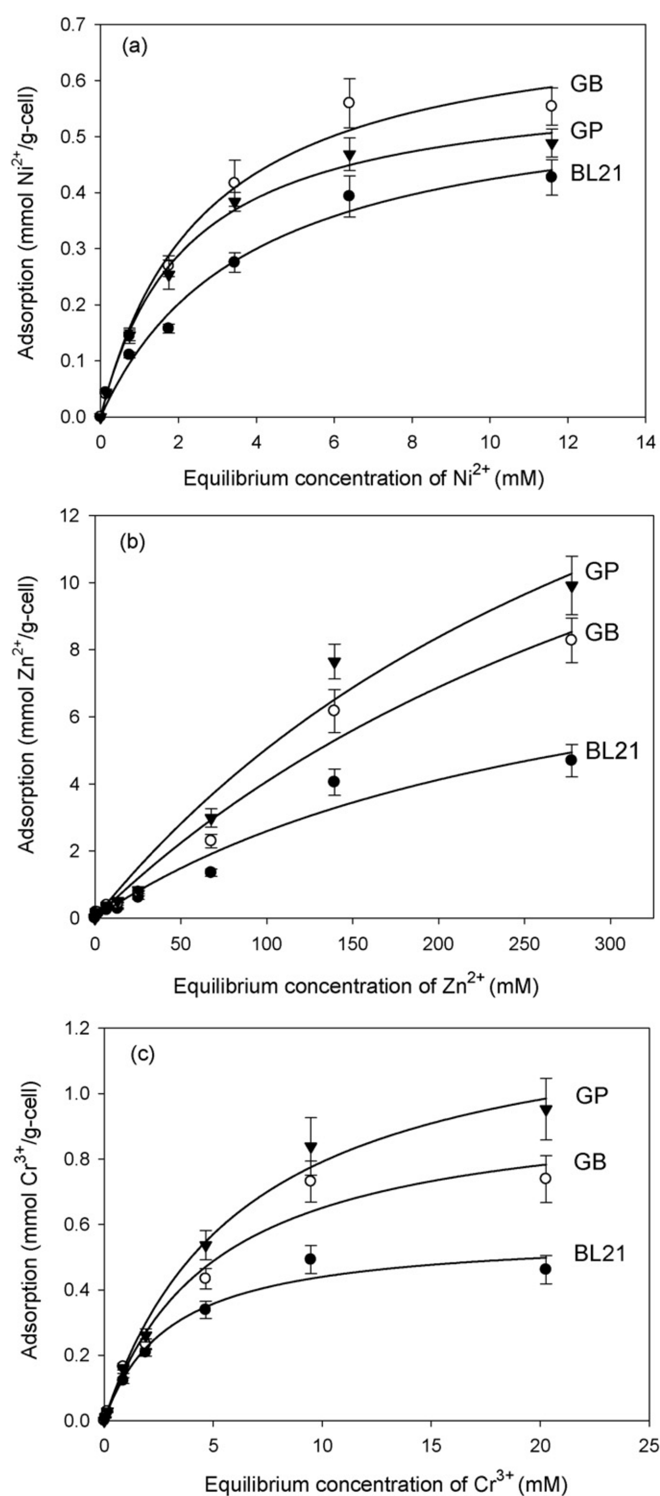

Fig. 2. Adsorption isotherms from biosorption of (a) Ni^{2+}, (b) Zn^{2+}, and (c) Cr^{3+} by recombinant *E. coli* strains expressing MerP proteins (GB and GP) and the MerP-free host strain (BL21).

binant strains (GB and GP) appeared to result in significant enhancement in equilibrium adsorption capacity for Zn^{2+} and Cr^{3+} adsorption over the control strain (BL21), giving a 121% and 72% increase in q_{max}, respectively, for GB cells, and a 144% and 126% increase, respectively, for GP cells (Fig. 2b and c). In contrast, there was only slightly increase in Ni^{2+} adsorption (24% and 5% increase for GB and GP), respectively (Table 2 and Fig. 2a). The adsorption capacity of Zn^{2+} and Cr^{3+} decreased in the order of GP > GB > BL21, while the metal adsorbate preference of the three biosorbents decreased in the order of $Zn^{2+} \gg Cr^{3+} \geq Ni^{2+}$.

It may not be fair if the biosorption ability of the biosorbents was assessed only based on adsorption capacity, as the adsorption rate or affinity should also be taken into account. Hence, a new performance index, namely overall adsorption efficiency (η) defined as q_{max}/K_d ratio, was used for evaluation of the biosorbent performance. The η value considers the effect of both adsorption capacity (represented by q_{max}) and affinity (represented by $1/K_d$), thereby should be a better indicator for biosorption efficiency. As indicated in Table 2, it is evident that the η value of GB and GP was higher than that of BL21 for all the metal adsorbates tested in this study. These results again confirm that the MerP-expressing recombinant strains were better biosorbents over MerP-free host strain regardless of the type of metal ion adsorbed.

The foregoing results show that the adsorption ability and preference are dependent on both biosorbent and adsorbate. From the biosorbent side, the origin of MerP proteins (from Gram-positive or Gram-negative bacteria) may have some effects on the adsorption of the metal ions, as the MerP protein from GB possesses vicinal cysteines, while that from GP often contains two cysteines separated by two residues [2,36]. Our previous results showed that this difference affected the selectivity in heavy-metal binding of Pb, Cu and Cd and provides more direct evidence suggesting that different metal-binding motif in the two heterogeneous MerP proteins would lead to distinct properties in the binding of metals [2]. On the other hand, from the viewpoint of adsorbates, the types and characteristics of adsorbates (such as molecular weight, electron negativity, valence of charge, etc.) may also influence the adsorption preference of the biosorbents [4,37]. For the adsorbates used, the molecular weight of the three metal ions decreased in the order of $Zn^{2+} > Ni^{2+} > Cr^{3+}$, the electron negativity decreased in the order of Ni^{2+} (1.8) > Zn^{2+} (1.6) = Cr^{3+} (1.6), while the valence of charge decreased in

Table 2
Optimal Langmuir isotherm parameters, maximal adsorption capacity (q_{max}) and dissociation constant (K_d), estimated from biosorption of Ni^{2+}, Zn^{2+}, and Cr^{3+} with cells of the recombinant strains (GB and GP) and the host strain (BL21)

Metal	Parameters	Biosorbents		
		BL21	GB	GP
Ni^{2+}	q_{max} (mmol/g cell)	0.585	0.723	0.602
	K_d (mM)	3.805	2.645	2.172
	q_{max}/K_d	0.154	0.273	0.277
Zn^{2+}	q_{max} (mmol/g cell)	10.084	22.266	24.577
	K_d (mM)	289.179	446.935	386.775
	q_{max}/K_d	0.035	0.050	0.064
Cr^{3+}	q_{max} (mmol/g cell)	0.572	0.985	1.293
	K_d (mM)	3.004	4.996	6.340
	q_{max}/K_d	0.191	0.197	0.204

the order of $Cr^{3+} > Zn^{2+} = Ni^{2+}$ [20,38,39]. It is likely that the combined effects of the foregoing characteristics contributed to the adsorption preference of ($Zn^{2+} \gg Cr^{3+} \geq Ni^{2+}$) obtained in this study. This adsorption preference is consistent with that of Jeon et al. (Zn > Ni) [39], Congeevaram et al. (Cr > Ni) [40], and Chergui et al. (Zn > Cr) [41].

3.3. Confirmation of metal adsorption by energy dispersive spectrometry

Although EDS could only estimate the quantify of metal associated with the biomass, it is still useful for verifying the presence of three heavy metal adsorbates on the cell surface of the three bacterial strains in terms of weight fractions. This is valuable information because most biosorption studies determine metal adsorption by just measuring the decrease in residual metal ion concentration in the supernatant before and after adsorption was carried out [1,4,5,8,13]. This type of measurement provides only indirect evidence on metal adsorption and may be misleading if the disappearance of metal ions occurs due to mechanisms other than biosorption; for instance, metal precipitation or adsorption on the surface of the vessel used for the experiments. Hence, it is nice to directly detect the presence of metal on the surface of biosorbents using EDS. Moreover, the weight fraction of metals on the surface of three different cells seemed to display consistent results to those observed in the adsorption isotherm studies (Fig. 2 and Table 2). Compared to the host strain (BL21), the relative weight fractions of Ni^{2+}, Zn^{2+} and Cr^{3+} ions on the surface of MerP-expressing strains (GB and GP) also significantly increased. For GB cells, the increase in adsorption of Ni^{2+}, Zn^{2+} and Cr^{3+} ions was 122%, 16%, and 133%, respectively, while for GP cells, the increase was 100%, 58% and 183%, respectively (Fig. 3). According to the results of EDS analysis, the weight fraction of Ni^{2+} ion on the three biosorbents decreased in the order of GB > GP > BL21, while the weight fraction of Zn^{2+} and Cr^{3+} decreased in the order of GP > GB > BL21 (Fig. 3). These trends appeared to be in agreement with the data indicated in Fig. 2 and Table 2, thereby further supporting the results obtained from adsorption isotherm studies.

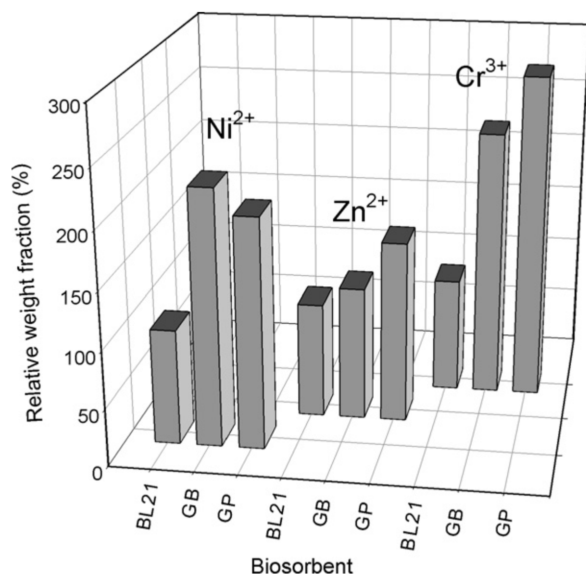

Fig. 3. Relative weight fractions (%) of Ni^{2+}, Zn^{2+}, and Cr^{3+} ions on the surfaces of the recombinant *E. coli* strains expressing MerP proteins (GB and GP) and the host strain (BL21) through EDS analysis. Adsorption capacity of the three biosorbents BL21, GB and GP for Ni^{2+} was 0.39, 0.56 and 0.47 mmol/g cell, respectively; for Zn^{2+} was 4.69, 9.28 and 10.92 mmol/g cell, respectively; for Cr^{3+} was 0.46, 0.74 and 1.09 mmol/g cell, respectively.

3.4. Elemental analysis and morphology observation of biosorbents with and without MerP expression

Since MerP is known as a cysteine-rich protein [2,36,42], the increase in the cell sulfur content could be an indication of over-expression of MerP proteins. This hypothesis is verified by conducting EA on the cells with or without MerP expression. The results shown in Table 3 indicate that after appropriate induction by IPTG, the sulfur content considerably increased by 18% and 63% in GB and GP cells, respectively, when compared to the non-induced control. Although EA is only a semi-quantitative tool for element detection, these results still give an indirect proof of expression of MerP proteins by the recombinant strains with IPTG induction. In contrast, since BL21 cells do not contain *merP* gene, there was no difference in the sulfur content with or without addition of IPTG (Table 3). In addition, the content of the other elements (i.e., C, H, N and O) did not vary significantly with or without IPTG induction.

It has been reported that adsorption of metals may have some impact on cell surface morphology [39,43]. Therefore, the change in surface morphology of the biosorbents was observed with VVSEM before and after metal adsorption. Analysis with VVSEM shows some differences in the surface structure of GB cells after heavy metal biosorption. Compared to the control image (GB cells before metal adsorption) (Fig. 4a), the surface morphology did not change markedly after Ni^{2+} adsorption (b), but clearly altered when Zn^{2+} and Cr^{3+} were adsorbed (c and d). In particular, the adsorption of Cr^{3+} resulted in severe damage on cells as indicated in d. It is likely that Zn^{2+} and Cr^{3+} ions (especially Cr^{3+} ions) are toxic to GB cells, causing damages on cell membrane or leading to cell lysis. This might suggest that adsorption of Zn^{2+} and Cr^{3+} ions on GB cells may be due to metabolism-independent surface adsorption, as the cells may not be viable to trigger energy-consuming intracellular accumulation of the metals.

Table 3
Elemental analysis of the cells with MerP protein expression (GB and GP) and without MerP protein expression (BL21)

Biosorbent	Element content (weight fraction %)				
	O	S	C	H	N
BL21	29.2	0.35	41.3	6.8	11.4
BL21 + IPTG	28.3	0.36	41.9	6.7	11.6
GB	28.6	0.31	41.3	6.9	12.2
GB + IPTG	29.0	0.42	42.0	7.2	11.5
GP	30.5	0.40	41.7	7.2	12.0
GP + IPTG	28.4	0.58	42.4	6.8	11.6

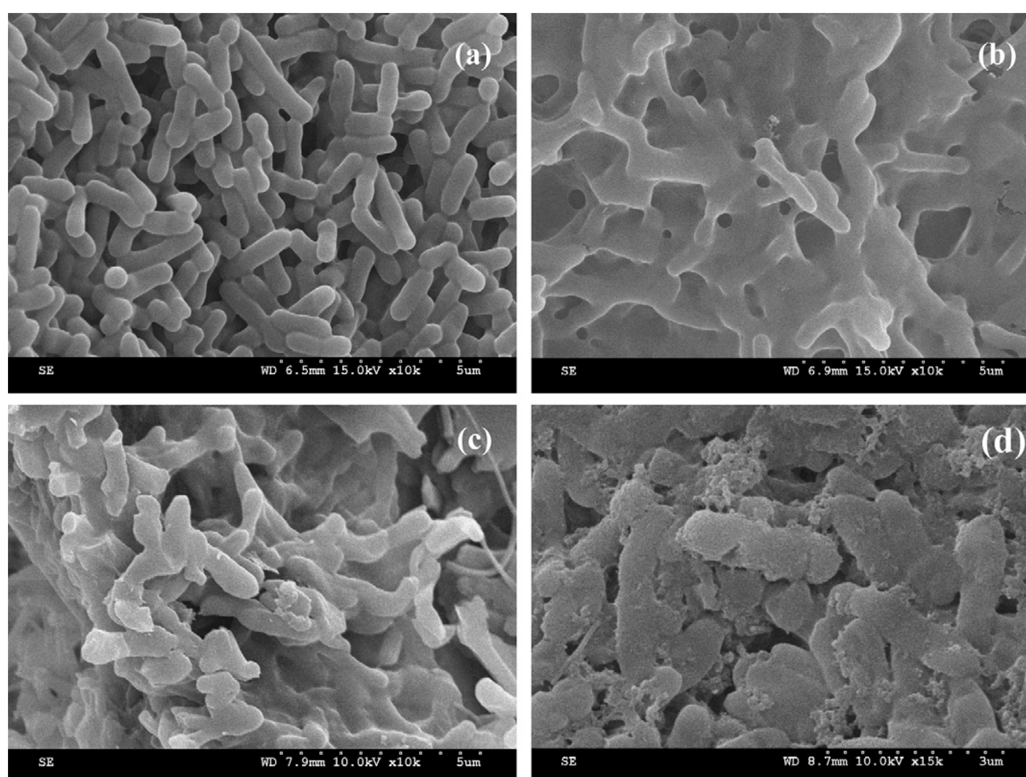

Fig. 4. Cell morphology of the GB the cells detected by VVSEM before and after heavy metal adsorption: (a) pure GB cells without metal adsorption, (b) GB cells after Ni^{2+} adsorption, (c) GB cells after Zn^{2+} adsorption, and (d) GB cells after Cr^{3+} adsorption.

4. Conclusions

The recombinant bacterial biosorbents (GB and GP) with over-expression of MerP proteins gave rise to a marked improvement in both adsorption capacity (5–144% increase) and adsorption rate (16–131% increase) of the metal adsorbates examined (especially for Zn^{2+} and Cr^{3+}) when compared to the control host strain (BL21). This validates the concept that expressing metal-binding proteins on genetically engineered *E. coli* hosts could be a feasible strategy for developing better heavy-metal biosorbents. The type of MerP proteins expressed also affected the performance of the recombinant biosorbents. Biosorbents containing Gram-positive MerP proteins (i.e., GB cells) had better adsorption for Ni^{2+}, while GP cells with Gram-negative MerP displayed higher adsorption capacity for Zn^{2+} and Cr^{3+} ions. Using both of the adsorption capacity and rate as the performance index (i.e., the ratio of q_{max}/K_d), the GP cells appeared to be better metal biosorbent for the three metal adsorbates examined. The q_{max}/K_d ratio of GP cells was similar to that of GB cells for Ni^{2+} adsorption but was significantly higher for adsorption of Zn^{2+} and Cr^{3+}.

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