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Correlation between cell surface physicochemical properties of bacterial strains and their chromium removal potential

Meryem Asri[a] , Alae Elabed[a,c], Nabil Tirry[a] , Aziza Kouchou[a],
Saad Ibnsouda Koraichi[a,b], Naïma El Ghachtouli[a] and Soumya Elabed[a,b]

[a]Laboratoire de Biotechnologie Microbienne, Faculté des Sciences et Techniques, Université Sidi Mohamed Ben Abdellah, Fès, Morocco; [b]Centre Universitaire Régional d'Interface, Université Sidi Mohamed Ben Abdellah, Fès, Morocco; [c]Laboratoire de Génie Chimique, CNRS – Université de Toulouse (INPT), Toulouse, France

ABSTRACT

Physicochemical characterization of microbes has gained recently a great interest by scientific community. It is proved of extreme importance in several fields of science and technology applications such as bioremediation. In this work, we investigated the establishment of a possible correlation between chromium removal capacity of seven bacterial strains isolated from contaminated sites with industrial wastes including tanning processing and their cell surface physicochemical properties. Thus, hydrophobicity and donor/acceptor electrons character were obtained using contact angle measurements. Statistical analysis showed a high significant positive correlation between hexavalent chromium (Cr(VI)) removal by the strains and their acceptor electron character γ^+ (r = 0.90). While significant negative correlation between the Cr(VI) removal potential and the $\Delta Giwi$ value (r = −0.844) and also with their donor electron character γ^- (r = −0.746) were observed. These results may contribute to determine a selectrion criteria of bacteria that can be operated in bioremediation applications.

Introduction

Hexavalent chromium (Cr(VI)) is a highly dangerous heavy metal that has been classified as one of the top 16th toxic pollutants [1]. The extensive use of Cr(VI) by a large number of chromium related industries such as steel industries, metal finishing and tannery leads to the production of massive amounts of chromium containing effluents [2]. These effluents directly discharged into municipal sewers and environment without adequate treatment may cause a serious threat to human beings, animals and plants [3,4]. It is recommended that the permissible limit concentration of Cr(VI) in aqueous media should be 0.1 mg L^{-1} [5]. Therefore, it is essential to intensify reaserches for the removal of chromium from industrial effluents and aqueous environmental systems.

Microbial biosorption is a low cost, eco-friendly and effective method, that receives considerable attention for the elimination of Cr(VI) [6,7]. It seems to be the most promising alternative to convential methods including adsorption, electro-chemical precipitation, reverse osmosis, etc. [8].

Enourmous investigations aimed to understand biosorption mechanisms and factors influencing biosorption potential. In fact, Göksungur et al. (2003) reported that the biosorption of heavy metal ions by microorganisms is influenced by several parameters. These factors include the specific surface properties of the microorganism (biosorbent), physicochemical parameters of the solution such as temperature, pH, initial metal ion concentration, the amount of biomass and the existence of other ions [1,9]. Unlike the other parameters extensively studied [6,10], the effect of microbial surface properties has not been rigorously investigated yet.

Indeed, the relation between cell surface physicochemical properties of bacterial strains and their chromium biosorption potential was not specifically identified. Furthermore, a scrutiny of literature indicates a paucity in depth investigations on important physicochemical parameters such as the cell surface acid-basic component.

Thus, in this work, we investigated for the first time bacterial cells surface characteristics (hydrophobicity, acid-basic component), using contact angle measurement (CAM), in relation with their chromium removal capacity. This work would contribute to understand the mechanisms of the chromium biosorption and to establish a possible correlation between the adsorption potential and the surface cell properties and to determine the most suitable cell surface characteristics for chromium adsorption.

Materials and methods

Bacterial isolation, identification and growth conditions

Seven bacterial strains were isolated from soil, sediment and wastewater samples contaminated with chemical industrial wastes including tanning process in Fez, Morocco. The pH of sampling sites presented values of 7.5–8.2.

The isolation of the bacterial strains was carried out by enrichment culture technique in Luria broth (LB) medium (peptone 10 g L^{-1}, NaCl 10 g L^{-1} and yeast extract 5 g L^{-1} in 1000 mL distilled water, pH 7.0) which was amended with 100–300 mg L^{-1} of a sterile solution of Cr(VI) as K$_2$Cr$_2$O$_4$ and 2 g of soil or 1 mL of wastewater. The medium was incubated at 30 °C, under aerobic conditions, on a shaker incubator at 150 rpm. After 6 days, enriched bacterial strains were isolated by plating on LB agar plate amended with 100–300 mg L^{-1} of Cr(VI). The bacterial strains that showed high tolerance were purified and stored at −4 °C for further uses.

The isolates were streaked on LB agar plates in order to get a single pure culture and were used for molecular identification based on the rDNA 16S sequence analysis. Polymerase chain reaction amplification was performed by using FD1 (5'AGAGTTTGATCCTGGCTCAG3') and RS16 (5'TACGGCTACCTTGTTACGACTT3') primers. The DNA sequencing was performed by using ABI 3130 (Applied Biosystems, France) according to the manufacturer's instructions and the GenBank BLAST (Basic Local Alignment Search) tools served for sequence analysis.

Biosorption experiments

The bacterial growth was obtained at 30 °C, using LB medium at pH 8 where 0.1 M NaOH solutions were used for pH adjustment if needed, on a rotary shaker at 150 rpm.

After 24 h of incubation, bacterial cells were harvested by centrifugation at 7000 g for 10 min, at 4 °C.

In order to study the chromium biosorption potential of bacterial strains, batch experiments were realized. It was carried out by suspending a loopful of biomass in LB medium prepared in sterile distilled water, containing an initial concentration of metal ions of 100 mg L^{-1} of Cr(VI) as K$_2$Cr$_2$O$_7$. The cell concentration was adjusted to an optical density of 0.7–0.8 at 660 nm (A_0) (approximately 10^8 CFU mL^{-1} cell density). Experiments were conducted at 30 °C under agitation system with speed of 150 rpm. Aliquots of 1 mL were taken every 24 h, centrifuged at 6000 g for 10 min at 4 °C and the residual Cr(VI) concentration was determined using diphenylcarbazide method [11]. The assay of Cr(VI) concentration in a medium was carried out using hexavalent chromium specific colorimetric reagent S-diphenyl carbazide (DPC). The reaction mixture was set up in 10 mL volumetric flask as follows: 200 μL of supernatant followed by the addition of 330 μL of 6 M H$_2$SO$_4$ and 400 μL of DPC (0.25% (w/v) prepared in acetone), the final volume was made to 10 mL using distilled water. The concentrations of Cr(VI) in the aqueous solutions were measured immediately at a wavelength of 540 nm using a UV–vis spectrophotometer.

The biosorption capacity was calculated as follows: $Q_t = V(C_i - C_t)/m_B$ [12]. Where Q_t is the amount of metal adsorbed per unit mass of biosorbent at a given time (mg g^{-1} or mmol g^{-1}), V is the volume of solution (L), C_i and C_t are respectively the initial concentrations and the residual concentrations of metal ions at a given time (mg L^{-1}), m_B is the dry weight of the biosorbent applied (g). The difference between the initial and the residual concentrations of the metal ion in the solution at the sampling time allows the determination of biosorption capacity.

The removal percentage was calculated as: Removal (%) = $((C_i - C_t)/C_i) \times 100$.

Biosorption experiments were realized in triplicate to assess reproducibility. To eliminate the abiotic reduction of Cr(VI), an abiotic control set was prepared throughout the course of the study without bacterial cell.

Contact angle measurements (CAM)

Bacterial lawn preparation

Allong with biosorption experiments, CAM were conducted. These measurments were assessed on lawns of organisms deposited on membrane filters [13]. In order to prepare bacterial lawns, a loopful of bacterial strains was inoculated in LB medium and incubated at 30 °C for 24 h under a shaker system where the log phase is attained. The exponential phase was determined following the evolution of OD$_{600}$ with time where OD$_{600}$ is the optical density of the culture neasured at 600 nm. Bacterial cells were then obtained by centrifugation at 8000 g for 15 min, at 4 °C. The cell pellet was washed twice using sterile KNO$_3$ (0.1 M) and resuspended in the same buffer. The cell concentration was adjusted to an optical density of 0.7 - 0.8 at 660 nm (A$_0$) (approximately 10^8 CFU.mL^{-1} cell density) in 0.1 M KNO$_3$. The microbial cell suspensions were then deposited on a cellulose acetate membrane filter (0.45 μm) using negative pressure. Cells lawns were placed to air dry for 30–60 min to be stabilizated [13,14]. The bacterial lawns for the CAM were realized in triplicate with separately cultured bacterial strains.

Contact angles measurements and calculation of the physicochemical characteristics

The measurement of bacterial cell surface hydrophobicity was carried out using a goniometer (GBX Instruments, France) by the sessile drop method [14]. By using three different liquids with known surface parameters values γ_l^{LW}, γ_l^+, and γ_l^-, two polar liquids (water and formamide) and one non-polar liquid (diiodomethane) (Table 1), the unknown surface tension components of microbial surface (the Lifshitz–van der Waals component (γ^{LW}), the electron donor or Lewis base (γ^-) and the electron acceptor or Lewis acid (γ^+)) was calculated using the young equation (1). An average of the contact angles and the standard deviation were calculated and reported ((S) and (L) denote solid surface and liquid phases respectively):

$$\gamma_L(\cos\theta + 1) = (\gamma_S^{LW}\gamma_L^{LW})^{1/2} + (\gamma_S^+\gamma_L^-)^{1/2} + (\gamma_S^-\gamma_L^+)^{1/2} \tag{1}$$

The Lewis acid-base component was obtained by:

$$\gamma_S^{AB} = 2(\gamma_S^-\gamma_S^+)^{1/2}$$

The cell surface hydrophobicity was also estimated using Van Oss approach [15,16], where the degree of hydrophobicity of a given surface (i) is expressed as the free energy of interaction between two entities of that material immersed in water (w). The materiel is hydrophobic when $\Delta Giwi$ is negative and conversely when $\Delta Giwi$ is positive the material is considered as being hydrophilic. $\Delta Giwi$ is calculated according to the following equation:

$$\Delta Giwi = -2\gamma_{iw}$$
$$= -2\left[((\gamma_i^{LW})^{1/2} - (\gamma_w^{LW})^{1/2}) + 2((\gamma_i^+\gamma_i^-)^{1/2} + (\gamma_w^+\gamma_w^-)^{1/2} - (\gamma_i^+\gamma_w^-)^{1/2} - (\gamma_w^+\gamma_i^-)^{1/2})\right]$$

where γ_i^{LW} is Lifshitz-van der Waals component, γ_w^{LW} is Lifshitz-van der Waals component of water, γ_i^+ electron acceptor of a given material (i), γ_i^- electron donor of a given material (i), γ_w^+ and γ_i^- are respectively electron acceptor and electron donor of water.

Statistical analysis

The results were subjected to statistical calculations for means comparison using XLSTAT software. One-way analysis of variance (ANOVA) was used to test the significance of the results followed by *post hoc* tests (TUKEY HSD) [17].

Table 1. Energy characteristics (Lifshitz–van der Waals (γ^{LW}), electron-donor (γ^-) and electron-acceptor (γ^+) parameters (mJ m^{-2})) of pure liquids used to measure contact angles.

Liquids	Surface energy parameters (mJ m^{-2})		
	γ^{LW}	γ^+	γ^-
Water (H_2O)	21.8	25.5	25.5
Formamide (CH_3NO)	39	2.3	39.6
Diiodomethane (CH_2I_2)	50.5	0	0

Results and discussions

Biosorption of Cr(VI) by bacterial cells

Under identical conditions, the seven bacterial strains showed, after 48 h incubation, different percentages of Cr(VI) removal, ranging from 5.32 to 99.87%, (Figure 1). Statistical analysis showed that Cr(VI) removal potential of tested bacterial stains are distributed within four independant groups with significant difference ($p = 0.05$). Strains *Cellulosimicrobium* sp. and *Enterococcus mundtii* offered an excellent potential of Cr(VI) removal, with a removal percentage of 79.87 and 81.39% respectively, grouped within the same group (a). *Leucobacter* sp. and *Enterococcus faecium* presented a good Cr(VI) removal potential with a percentage of 46.71 and 56.85% respectively (group (b)). *Enterococcus* sp. and *Bacillus cereus* presented a lower chromium removal capacity (24.99 and 19.12%, respectively) (group (c)). *Bacillus subtilis* presented the weakest chromium removal potential with a percentage of 5.32% (group (d)).

Biosorption is an energy independent binding of metal ions to the cell wall of microorganisms [7,18]. Thus, we hypothesized that the cell surface physicochemical properties of the seven isolated bacterial strains would be correlated with their different chromium uptake potentials. Indeed, Several studies aiming to determinate the factors affecting the bioremediation mechanisms revealed that hydrophobicity and surface properties play a key role [19], mainly in the adsorption of charged molecules such as heavy metals.

Physicochemical characteristics of bacterial cell surfaces

The physicochemical characterization of microbial cell surface properties can be realized by several techniques, such as microbial adhesion to solvent (MATH) [20] and CAMs [14].
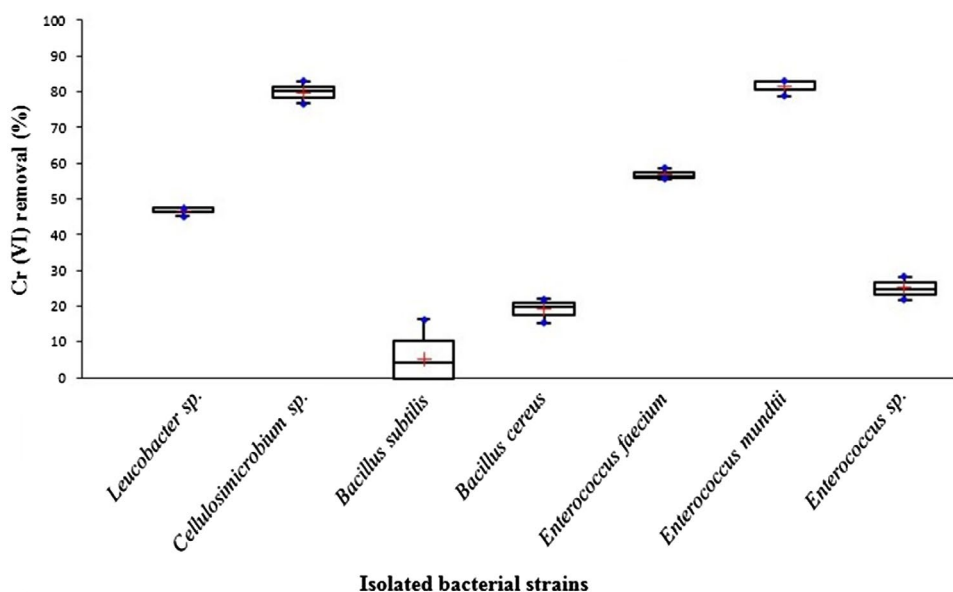

Figure 1. Chromium removal percentage by isolated bacterial strains in LB medium, after 48 h incubation at 30 °C, 150 rpm.

In this work, contact angle technic by the sessile drop served as a prompt and a simple way, to obtain precise and valuable surface energy values. It yields precise estimations of cell surface hydrophobicity and electron donor/acceptor character [13,21].

According to Vogler (1998), a given surface is considered as hydrophobic when the value of θ_w exceeds 65° and it is characterized as hydropholic when θ_w is less than 65° [22]. Moreover, according to Van Oss approach (1988, 1996), a positive value of the free energy surface ($\Delta Giwi$) indicates that the surface is hydrophilic and a negative value means that it is hydrophobic [15,16].

The results of hydrophobicity obtained are expressed qualitatively (θ_w) and quantitatively (in terms of $\Delta Giwi$) (Table 2). The seven isolated bacterial strains had positive values of the free energy of interaction $\Delta Giwi$ and thus were classified as hydrophilic.

Statical analysis showed that cell surface hydrophobicity of the bacterial stains tested are grouped within five independant groups with significante difference. Thus, group (a) includes $\Delta Giwi$ values of 42.17 mJ m^{-2} (*B. subtilis*) and 38.79 mJ m^{-2} (*B. cereus*). Group (b) is formed by $\Delta Giwi$ values of 38.79 mJ m^{-2} (*B. cereus*) and 36.07 mJ m^{-2} (*Enterococcus* sp.). *Enterococcus* sp. and *Leucobacter* sp. exibits a lower hydrophobic character with $\Delta Giwi$ values 36.07 and 34.93 within group (c). Group (d) includes *Leucobacter* sp. and *Enterococcus faecium* with $\Delta Giwi$ values of 34.93 and 32.21 respectively and group (e) regroups the lowest $\Delta Giwi$ values 26.48 (*Cellulosimicrobium* sp.) and 26.13 (*E. mundtii*).

These findings agree with the previous results of Elabed et al. (2011), which reported that different bacterial species exihibit different $\Delta Giwi$ values [14]. Many studies have aimed to understand the cell surface properties. A linear correlation between surface properties and the chemical composition of cell surface has been found [21]. It is known that the most hydrophilic microbial surfaces tends to have lower protein/carbohydrate ratios [14].

The results concerning electron donor/acceptor character are presented in Table 2. All isolated strains are predominantly electron donors with values of γ^- ranging from 49.73 ± 0.364 to 59.69 ± 1.396 mJ m^{-2} and presents a weak electron acceptor character with values of γ^+ 0.13 ± 0.053 to 0.69 ± 0.025 mJ m^{-2}.

Table 2. Contact angle values (θ_w, θ_F, θ_D), Lifshitz–van der Waals (γ^{LW}), electron-donor (γ^-) and electron-acceptor (γ^+) parameters and surface energies ($\Delta Giwi$) of bacterial cells.

Bacterial strains	Contact angles (°)			Surface tension: components and parameters (mJ m^{-2})			Surface energies
	θ_w	θ_F	θ_D	γ^{LW}	γ^+	γ^-	$\Delta Giwi$ (mJ m^{-2})
Leucobacter sp.	16.10 ± 0.95	19.80 ± 1.28	28.13 ± 0.72	44.88 ± 0.28	0.30 ± 0.06 (c)	55.49 ± 1.24 (b,c)	34.93 ± 2.00 (c,d)
Cellulosimicrobium sp.	20.90 ± 0.26	15.13 ± 0.40	27.03 ± 0.50	45.3 ± 0.19	0.52 ± 0.01 (b)	49.73 ± 0.36 (d)	26.48 ± 0.65 (e)
Bacillus subtilis	14.23 ± 0.55	24.73 ± 1.83	29.23 ± 0.50	44.44 ± 0.20	0.13 ± 0.05 (d)	59.69 ± 1.39 (a)	42.17 ± 2.56 (a)
Bacillus cereus	18.93 ± 0.59	25.83 ± 2.56	30.77 ± 1.12	43.81 ± 0.47	0.18 ± 0.09 (c)	57.02 ± 1.83 (b)	38.79 ± 3.44 (a,b)
Enterococcus faecium	12.87 ± 0.61	13.13 ± 1.10	27.73 ± 0.21	45.04 ± 0.08	0.49 ± 0.03 (b)	54.53 ± 0.29 (c)	32.21 ± 0.02 (d)
Enterococcus mundtii	17.20 ± 0.60	8.10 ± 1.22	27.47 ± 0.15	45.14 ± 0.06	0.69 ± 0.03 (a)	50.43 ± 0.49 (d)	26.13 ± 0.73 (e)
Enterococcus sp.	10.90 ± 0.62	15.83 ± 1.86	21.00 ± 0.62	47.38 ± 0.14	0.19 ± 0.05 (c)	56.85 ± 0.98 (b)	36.07 ± 1.76 (b,c)

Note: Letters indicates independent groups with significant difference ($p = 0.05$) according to ANOVA test.

The Statistical analysis showed that the electron acceptor character of tested bacterial stains are grouped within four independant groups with significate difference ($p = 0.05$). Thus, group (a) included *E. mundtii* ($\gamma^+ = 0.025$ mJ m^{-2}), group (b) formed by *Cellulosimicrobium* sp. and *E. faecium* with γ^+ values of 0.52 and 0.49 mJ m^{-2}, group (c) with γ^+ values of 0.18 mJ m^{-2} (*B. cereus*) and 0.19 (*Enterococcus* sp.), group (d) regroups *B. subtilis* with γ^+ value of 0.13 mJ m^{-2}.

Our findings are in agreements with a previous study reported by Van der Mei et al. (1998) showing that microbial cell surfaces are electron-donating, while seldom electron-accepting cell surfaces can be found [13]. The surface of microorganisms is essentially composed of polysaccharides, proteins and lipids. This composition offers a dominant electron donor character due to the presence of many negatively charged functional groups such as carboxylate, hydroxyl, thiol, sulphonate, phosphate, amino and imidazole groups [19,23]. Their presence is involved in polluted aqueous media bioremediation and capacity of binding metal ions [23].

Relation between cell surface physicochemical properties of bacterial strains and their chromium removal potential

Voleskey et al. (1995) had reported that the microbial cell walls are mainly responsible for the metal biosorption behavior. In fact, the first phase of heavy metal biosorption is attributed to surface adsorption, essentially based on anion exchange with the participation of the functional groups on the cell surface [24]. In addition, it was reported that the adsorption onto the biomass surface is the main mechanism of heavy metal uptake [9].

In order to get an insight into the biosorption process, we proposed to examinate the possible correlation between bacterial physicochemical properties and their chromium removal capacity. For this purpose, CAM were employed to determinate both of CSH and microbial acid-base components of surface free energy. Cell wall properties are a factor that can significantly influence Cr(VI) biosorption efficiency, since it was reported that chemical affinity is the most important driving force that favours ions adsorption to the biosorbent surface [5]. Furthermore, the content of complexing functional groups in microorganisms cellular wall may influence their surface properties and hence their biosorption capacity [7].

It is noteworthy that Cr(VI) exists in aqueous media in different anionic forms. It may exist predominately as chromate (CrO_4^{2-}), dichromate ($Cr_2O_7^{2-}$) or hydrogen chromate ($HCrO_4^{-}$) according to aquatic chemistry of chromium [18] depending on environmental pH as follows the Cr(VI) speciation diagram [25].

Our results show a negative significant correlation obtained between bacterial CSH and their chromium removal potential ($r = -0.844$) (Figure 2). Thus, the more hydrophobic bacterial strains have a better chromium removal potential and have a better biosorption capacity of chromium anions. It is generally accepted that the increase of cell surface hydrophobicity is a defense system against stress conditions and several studies showed a good correlation between microorganisms hydrophobicty and their bioremediation potential [20]. Płaza et al. (2005) reported that microorganisms with higher CSH have a better potential in petroleum bioremediation [26]. It was also reported that *Serratia* spp. with higher hydrophobicity could adhere to betacypermethrin, absorb and degrade it more easily [20]. Obuekwe et al. (2009) demonstrated that cell surface hydrophobicity of microbial strains was an important factor in biodegradation of crude oil and the most hydrophobic variants were the best degraders [27].
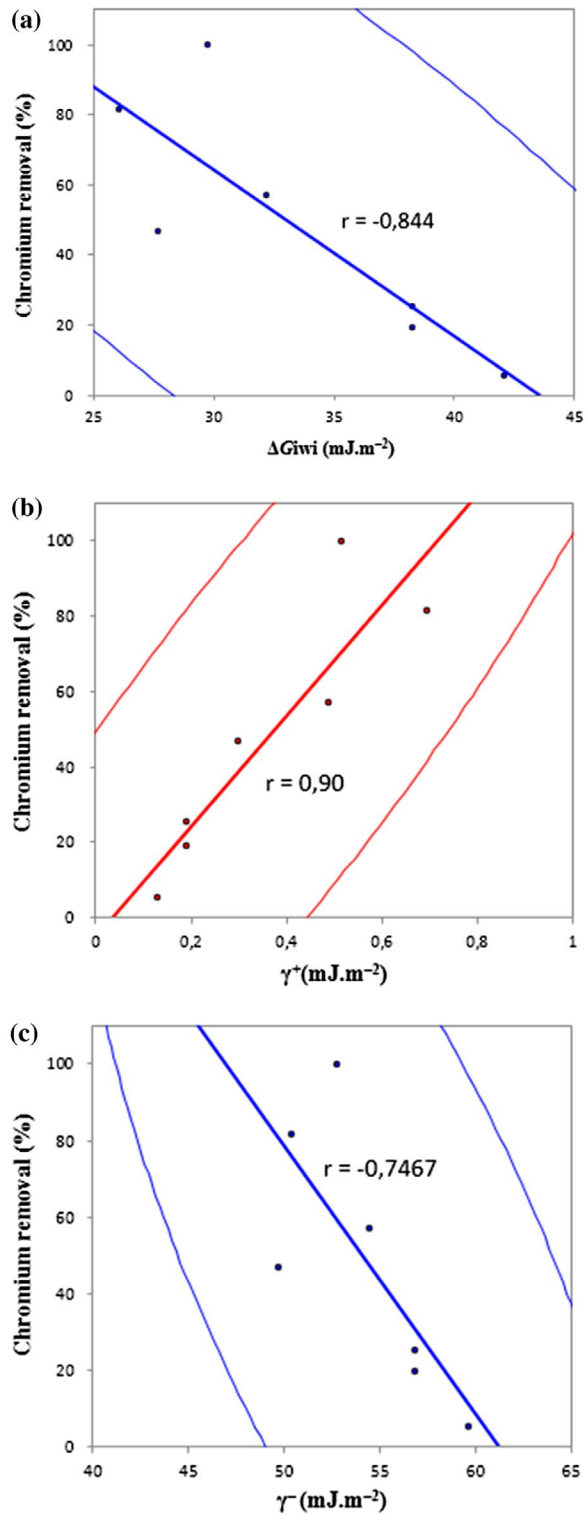
Figure 2. Correlation between chromium removal percentage by isolated bacterial strains and their surface physicochemical properties: (a) cell surface hydrophobicity (CSH), (b) Acceptor electron character γ^+ and (c) donor electron character γ^-.

It is noticeable that these previous investigations relating cell surface properties to microbial bioremediation capacities mainly focused on cell surface hydrophobicity character (CSH). These works especially dealt with the interaction of microorganisms with organic substrates, where the hydrophobic strains were considered as more efficient strains in environmental applications [27–30]. Our results also suggest that hydrophobic microbial strains may have a great bioremediation capacity in heavy metal removal. However, CSH should not be the only selection criteria for the isolation of performant bacterial strains especially in the case of metal ions bioremediation.

Van Oss (1996) reported that the energy of acid-base interactions can be twice greater than that due to hydrophobic interactions [16], but their role in the phenomenon of bioremediation is rarely studied. For this reason, we suggested to study the role of acid-base properties of the cell surface in the phenomenon of bioremediation of heavy metal ions.

The results show a negative correlation between the bacterial chromium removal potential and the donor electron character γ^- ($r = -0.746$) (Figure 2). Bacterial strains with high donor electron character present weak chromium uptake potential. Thus, the higher is the surface electron donor character, the greater the approach of negatively charged metal anions will be inhibited. This result is consistent with the Gupta et al. (2009) work, which reported a relation between functional groups and chromate ions biosorption. This work showed that the more the functional groups in algal cell surface become negatively charged at high pH values, the more they tend to repulse the negatively charged ions chromate and thus affects the adsorption to the algal wall [1].

Our results showed also a high positive correlation between bacterial electron acceptor character γ^+ and their chromium removal potential ($r = 0.90$) (Figure 2). These findings suggest that surface bacterial donor/acceptor character can significantly influence their chromium biosorption capacity. The acid component of cell surface is related to their capacity of Cr(VI) adsorption, an increase in the acid component promotes the adsorption process of chromium anions.

Conclusion

Under their own optimum removal conditions, the chromium removal capacity of isolated bacterial strains presents a significant positive correlation with their electron acceptor character, while it shows a negative significative correlation with ΔGiwi value and electron donor character. To our knowledge, this is the first report about the correlation between cell surface hydrophobicity, acid-basic component and chromium removal using CAM. This paper confirms the importance of physicohemical characteristics of microbial strains as selection criteria of bacteria for their use in bioremediation projects.

Acknowledgments

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Meryem Asri ⓘ http://orcid.org/0000-0002-0886-9582
Nabil Tirry ⓘ http://orcid.org/0000-0002-6127-2857

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