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Studies on Surface Properties of Yeast Cells with chromium removal potential

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Abstract

Biosorption of hexavalent chromium by microbial cells is a promising technology for its removal. Physicochemical characterization of microorganisms has gained particular attention in bioremediation. In this work, the correlation between the Cr(VI) removal efficiency of yeast strains and their cell surface physicochemical properties was investigated. Thus, the contact angle measurement served to obtain hydrophobicity and donor/acceptor electrons characters of the yeast strains. Statistical analysis showed that hexavalent chromium (Cr(VI)) removal by the yeast strains presented a strong significant positive correlation with their hydrophobicity (R^2 = 0.815) and their donor electron character γ^- (R^2 = 0.911). The biosorption of chromium anions resulted in the modifications of the surface physicochemical properties of yeast strains. The changes in the physiochemical properties, namely surface hydrophobicity and electron donor/acceptor characters, differed depending on the yeast species. These results offer new data on the biosorption mechanisms and selection criteria of yeast with high chromium removal potential.

1. Introduction

Hexavalent chromium (Cr(VI)) is an important heavy metal widely used in various chrome-based industries [1]. As a result, Cr-contaminated effluents are increasingly discharged into environment and Cr contamination of the environment is extensive [2]. This heavy metal is deadly toxic to the environment, plants and animals [3]. Different processes have been adopted for the removal of Cr(VI) [4]. However, the remediation method based on chromium bioremoval using microorganisms, remains an environmental friendly, easy, economical and feasible approach [5], [6]. Thus, it seems to offer the most promising alternative to conventional methods including adsorption, electro-chemical precipitation, reverse osmosis, etc. [7]. The mechanism of this process is undoubtedly complicated and requires further elucidation. The Cr(VI) ions binding onto the biomass surface may occur by complexation, ion exchange, adsorption, coordination, microprecipitation or a combination of these processes [8]. Factors related to the type of biomass and the environmental conditions are known to highly influence the metal biosorption mechanism and hence the metal removal potential, namely the

affinity and the specifity. In order to understand biosorption mechanisms and influencing factors, many works have been conducted. Numerous parameters were reported to govern this process, including specific surface properties of the biosorbent (microorganism), physicochemical parameters of the solution such as pH, temperature, metal concentration and the existence of other ions [9]–[11].

It is noticeable that the biosorption efficiency of heavy metals by microbial cells, is to a large extent governed by the microbial cell surface structure and the content of functional groups in microbial cellular surface [1]. This structure is playing a prominent role in the metal-microorganism interactions [11], [12].

It is well known that depending on the microbial strain, the cell wall may have different overall composition. This affects directly the metal affinity, specificity and the adsorption efficiency. Indeed, the main driving force favoring the heavy metals biosorption is the chemical affinity and the key step of this process is surface adsorption to the different biosorbent surfaces [13], [14]. It is mainly based on ion exchange involving the functional groups on the cell surface. The adsorption onto the biomass cell walls is the main mechanism of heavy metals bioremoval [9].

Physicochemical characterization of microbes using contact angle measurement (CAM) has gained recently increasing importance in several fields of science and technology applications such as bioremediation [15].

Despite the fact that the physicochemical properties play an extremely important role in the biosorption efficiency, limited data studying their effect on this process have been published [15], [16].

Thus, the aim of this work is to investigate the yeast cells surface characteristics (hydrophobicity, acid-basic component), using contact angle measurement (CAM), in relation with their chromium removal potential.

2. Methodology

1.1 Yeast strains and growing conditions

Five yeast strains *Cyberlindnera fabianii*, *Wickerhamomyces anomalus*, *Candida tropicalis*, *Pichia fermentans*, and *Galactomyces geotrichum* were used in this study. These strains were isolated from soil and wastewater samples heavily contaminated with chemical industrial effluents in Fez and were selected on the basis of their chromium resistance as reported by [17]. All studied yeast strains showed a high ability of removing chromium and were considered as excellent biosorbents. *C. fabianii*, *W. anomalus*, and *C. tropicalis* strains were reported to have a high chromium biosorption capacity [11]. It is also the case for *P. fermentans* and *G. geotrichum* (unpublished data). Yeast strains were seeded on yeast medium agar (1% peptone, 1% yeast extract, 2% glucose, and 1.5% agar) plates and incubated for 48 h at 30°C.

1.2 Cells preparation

Preparation of yeast strains suspension for cell surface contact angle measurements (CAM) was carried out following the protocol of Mohd-Al-Faisal et al. (2013) with slight modifications: 2 g of yeast strains was inoculated in yeast medium (YPG) (1% peptone, 1% yeast extract, and 2% glucose) and incubated at 30°C for 48 h where the log phase was attained. Then, cells were harvested by centrifugation at 8,000 x g. Cell pellets were washed twice with KNO_3 (0.1M) and resuspended in the same solution. At 550 nm, the cell density was adjusted at an absorbance of 0.450 that is equivalent to $1x10^7$ cells/mL [18]. Microscopic examination at log phase allowed the visualization of cells presenting characteristics of yeast.

1.3 Contact angle measurements

1.3.1. Hydrophobicity

To prepare microbial lawns suitable for CAM, microbial cells suspended in KNO_3 (0.1M) sterile solution were deposited on a cellulose acetate membrane filter (0.45 µm) by filtration of the suspension using negative pressure. Filters containing microorganisms (yeast cells 1 x 10^7 cell/mm^2) were placed to air dry for 30–60 min to obtain stable lawns for contact angles measurements. The contact angles measurements were performed in triplicate with separately cultured microbes [19]. According to Vogler's approach [20], the value of water contact angle θ_w indicates the hydrophobicity of a surface qualitatively. A surface exhibiting a θ_w value higher than 65° is considered as hydrophobic, while a θ_w value below 65° permits to classify a surface as hydrophilic. Van Oss' approach allows the determination of the absolute degree of a hydrophobicity of a surface. This parameter is expressed as the free energy of interaction between two identical surfaces immersed in water (ΔGiwi). A negative value of ΔGiwi indicates that the interaction between two surfaces is stronger that the interaction between each surface with water, the surface is considered as hydrophobic. Conversely, a positive value of ΔGiwi allows to classify the surface as hydrophilic. It is calculated through the surface tension components of the interacting entities, using the following formula [21].

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

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^+ electron acceptor of water, and γ_i^- electron donor of water.

1.3.2 Surface tension components

The measurement of contact angles allows the determination of the Lifshitz-van der Waals (γ^{LW}) and acid- base (γ^{AB}) surface tension components by the application of the Young- Dupré equation to each probe liquid (Van Oss, 1996). By using water, formamide, and diiodomethane with known surface parameter values γ_l^{LW}, γ_l^+, and γ_l^-, the unknown surface tension components of a solid surface (γ_s^{LW}, γ_s^+, and γ_s^-,) or microbial surface (γ^{LW}, γ^+, and γ^-) can be estimated using the following equation:

$$\gamma_L(\cos\theta + 1) = 2\left[\left(\gamma_s^{LW}\gamma_L^{LW}\right)^{1/2} + \left(\gamma_s^+\gamma_L^-\right)^{1/2} + \left(\gamma_s^-\gamma_L^+\right)^{1/2}\right]$$

where θ refers to the measured contact angle and the subscripts (S) and (L) are solid surface and liquid phases, respectively.

γ^{LW} is the Lifshitz-van der Waals component of the surface free energy, γ^+ and γ^- are the electron acceptor and electron donor parameters, respectively, of the Lewis acid-base component (γ^{AB}).

The surface free energy is expressed as $\gamma_S = \gamma_S^{LW} + \gamma_S^{AB}$

where $\gamma_S^{AB} = 2(\gamma_S^-\gamma_S^+)^{1/2}$ is the acid-base free energy component.

1.4 Biosorption experiments

Microbial growth was obtained at 30 °C, using YPG medium with aeration at 30 °C under continuous shaking (150 rpmn).

After 24 h of incubation, yeast cells were harvested by centrifugation at 7000 g for 10 min, at 4 °C. Batch experiments were realized in order to study the chromium biosorption potential of yeast. It was carried out by suspending a loopful of biomass in YPG-modified medium prepared in sterile distilled water, containing an initial concentration of metal ions of 100 $mg.L^{-1}$ of Cr(VI) as $K_2Cr_2O_7$ [17]. The cell concentration was adjusted at 550 to an absorbance of 0.450 (approximately 10^7 $CFU.mL^{-1}$) [18]. Experiments were maintained at 30 °C under agitation system (150 rpm).

Samples 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 immediately using diphenylcarbazide method at a wavelength of 540 nm using a UV–vis spectrophotometer [22].

The removal percentage was calculated as: Removal (%) = $((C_i - C_t)/C_i) \times 100$. C_i and C_t are respectively the initial concentrations and the residual concentrations of metal ions at a given time ($mg.L^{-1}$). To eliminate the abiotic reduction of Cr(VI), an abiotic control set was prepared throughout the course of the study without yeast cell. Biosorption experiments were realized in triplicate to assess reproducibility.

1.5 Statistical analysis

The results were subjected to statistical calculations for means comparison using XLSTAT soſtware. Linear regression was used to test the significance of the correlation between variables [23].

3. Results and discussion

3.1 Cr(VI) biosorption by yeast cells

Under identical conditions, the five yeast strains showed, aſter 48 h incubation, different percentages of Cr(VI) removal. Indeed, *W. anomalus* allowed the highest efficiency with a removal percentage of 90.15%. While *G. geotrichum, C. tropicalis, C. fabianii and P. fermentes* allowed a Cr(VI) removal percentage of 77.82%, 67.49%, 60.15% and 59.7% respectively.

It is well known that biosorption is an energy independent binding of metal ions to the cell wall of microorganisms [24]. Among the main factors affecting the bioremediation mechanisms, hydrophobicity and surface properties have been revealed to play a key role in this process [25]. Thus, the cell surface physicochemical properties of the studied yeast strains might be correlated with their different chromium uptake potentials. In this regard, this work is aiming to determinate the effect of these properties on the Cr(VI) biosorption.

3.2 Cell surface physicochemical properties and biosorption capacity of yeast strains

For the effective use of microorganism cells in different biotechnological processes, physicochemical characters, in particular, hydrophobicity and acid/basic Lewis character have been proved of extreme importance [26]. However, an insufficient attention was so far paid to the characterization of the yeast strains involved in heavy metals biosorption [27]. The employed yeast strains have showed differences in their surface hydrophobicity and acid/base character as shown in Figure 1.

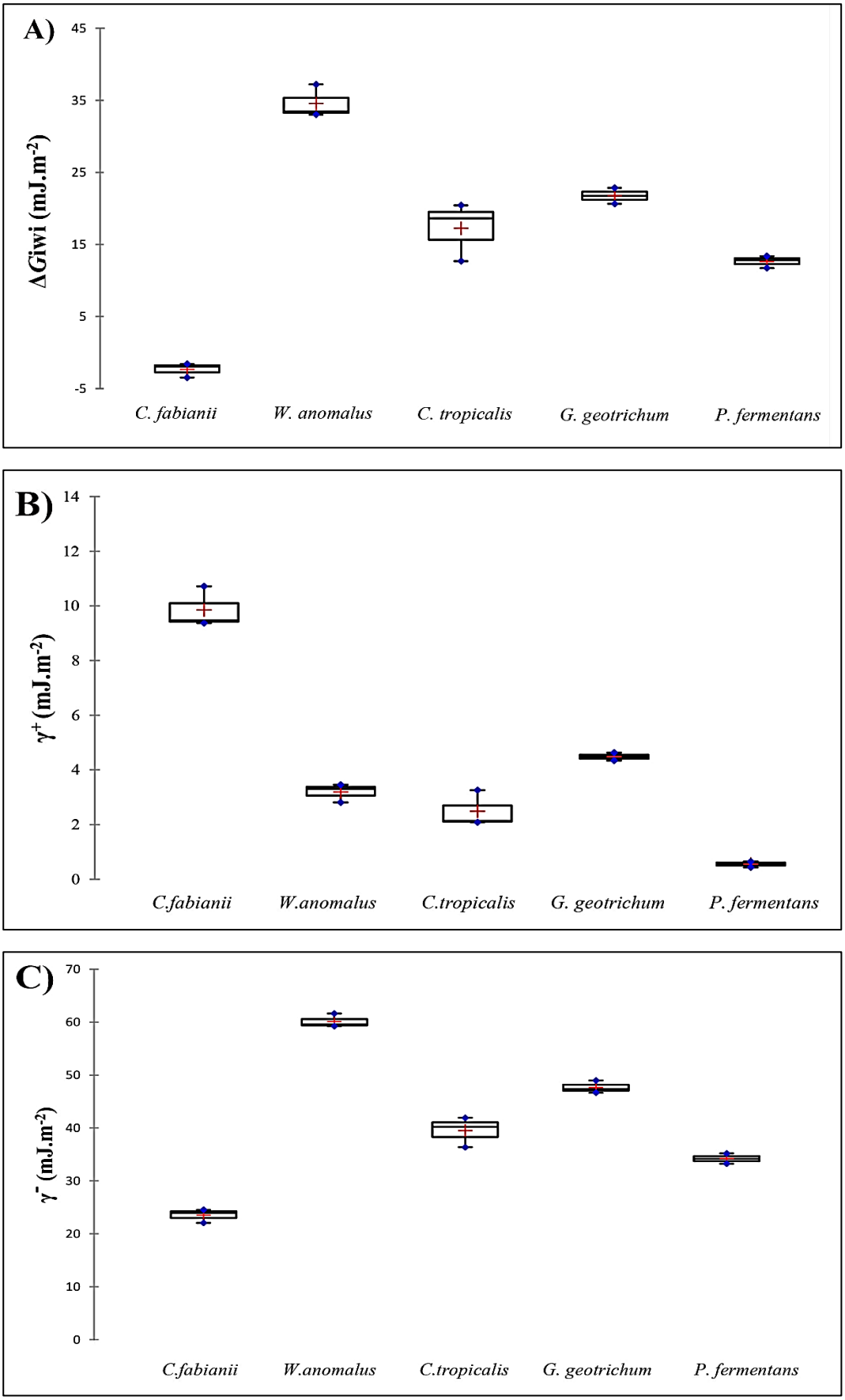

Figure 1. (A) Surface energies (ΔGiwi), (B) electron-acceptor (γ^+) and (C) electron-donor (γ^-) of yeast cells.

Indeed, the highest hydrophobicity was observed for *C. fabianii* with a negative surface energy value ΔGiwi of -2.33 ± 1.05 mJ.m^{-2} and the lowest was obtained for *W. anomalus* exhibiting a hydrophilic character with a positive surface energy value ΔGiwi of 34.58 ± 2.23 mJ.m^{-2}.

It has been reported that cell wall phosphates and carboxyl groups is the major determinant of yeast cell surface composition [27]. The hydrophobicity degree of yeast gives an indication mainly on the presence of hydrophilic or hydrophobic groups situated on the cell walls. Hydrophilic molecules are generally polar or charged while hydrophobic are non-polar [28]. The hydrophilic degree of the studies yeasts surface may be related to the presence of polar groups such as carboxyls, mannosyphosphates at the yeast cell surface [27]. It is commonly known that the availability of negative and/or polar sites at the yeast surface results in the higher number of active sites for heavy metal ions fixation [28].

The CAM also allowed the obtaining of the donor electron character γ−. the surfaces of all studied yeast strain behave predominantly as electron donors with high values of γ− ranging from 34.58 ± 1.29 mJ.m^{-2} obtained for *C. fabianii* to 60.14 ± 1,30 mJ.m^{-2} obtained for *W. anomalus*.

This is also in agreement with a previous study showing that microbial cell surfaces are mainly electron-donating [29]. This was particularly in agreement with results previously reported, showing that yeast cells are exhibiting a dominant Lewis basic character [30], [31].

The electron donor character of yeast strains is mainly in relation with the nature of chemical groups on their surface. The predominance of their electron-donor character can be attributed to the presence of the chemical groups negatively charged or neutral exposed on the surface mainly carboxylate groups, amino groups, phosphate groups, phospholipids and lipo-polysacharrides [32]–[34].

3.3 Relation between cell surface physicochemical properties of yeast strains and their chromium removal capacity

It is generally agreed that cell surface hydrophobicity may strongly affect biosorption capacity, facilitating hydrophobic bonds. Relevant works have reported the extreme importance of the interaction between microorganisms and the abiotic surfaces in environmental systems [35]. In microbially mediated depollution, these interactions are involved in the microorganisms' migration in geological formation and determines consequently the pollutant removal efficacy [36], [37].

However, the hydrophobic character was mainly investigated in the organic compounds bioremediation [38], [39]. The relation between microorganisms surface properties and the heavy metal removal effectiveness was only studied in few works [15], [27].

In order to study the possible relation between yeast surface properties and the heavy metal removal efficiency, a correlation test by linear correlation at the 95% confidence level was performed using XSLTAT software. The obtained results showed a strong positive significant correlation between yeast CSH and their chromium removal potential (R^2=0.815 and p-value = 0.0095) (Figure 2). Thus, the more hydrophilic yeast strains exhibit a better chromium removal potential. This is in agreement with previous results showing that the decrease of hydrophobicity of activated sludge enhanced the removal efficiency of metal ions by the increase of the availability of fixation sites [28]. However, it is in disagreement with a further works reporting that bacteria with higher CSH exhibits a better efficiency in petroleum bioremediation [40]**.** Another work has reported that *Serratia* spp. with higher hydrophobicity could absorb and degrade beta-cypermethrin more easily [16]. A further work demonstrated that CSH of bacterial strains was an extremely important factor in biodegradation of crude oil, the most hydrophobic variants allowed the best hydrocarbon-degrading ability [38].

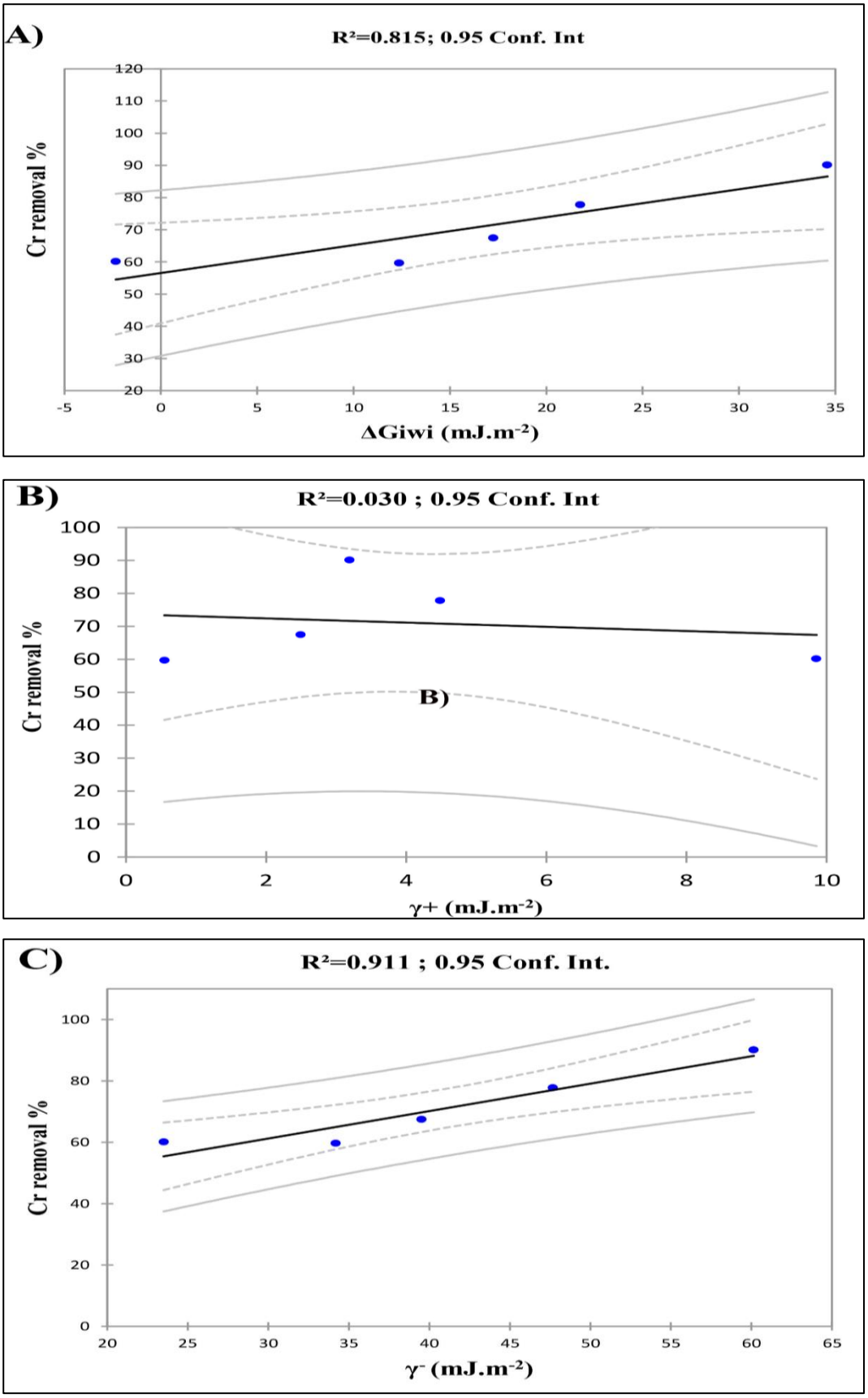

Figure 2. Correlation between chromium removal percentage by isolated yeast strains and their surface physicochemical properties: (A) cell surface hydrophobicity (CSH), (B) Acceptor electron character γ+ and (C) donor electron character γ−.

In our previous work, it has been shown that bacteria isolated from chromium-contaminated sites with high CSH showed the best performance in terms of chromium removal from aqueous media [15]. In a comparative study, a great correlation between acclimated residential biomass hydrophobicity and their adsorption amount of phenol and chlorophenols has been shown [41]. The hydrophobic species have been often cited as the most interesting species in bioremediation applications; nevertheless, our results show that hydrophilic yeast strains could also be potential candidates for heavy metals bioremediation. This confirms the extreme important role that this character could play in heavy metal sorption.

The investigations relating cell surface properties to microbial bioremediation capacities, focused mainly on bacterial species and their cell surface hydrophobicity. Nevertheless, yeast cells are considered as good biosorbents, and showed good efficiency in many environmental applications [6]. Furthermore, the energy of acid-base interactions has been reported to be twice greater than that due to hydrophobic interactions [42], however, their role in the phenomenon of bioremediation is rarely studied. Although, CSH is an extremely important character that influences strongly the biosorption phenomenon, it cannot be the only criteria for the selection of performant microbial strains, particularly for metal ions bioremediation [15]. For this reason, we proposed to study the impact of acid-base properties of the cell surface in the phenomenon of bioremediation of heavy metal ions.

The linear regression results show a strong positive correlation between the yeast chromium removal potential and the donor electron character $\gamma-$ ($R^2 = 0.911$) (Figure 2). Yeast strains with high donor electron character present higher chromium uptake potential**.** Thus, the higher is the surface electron donor character, the greater the approach of chromium anions (chromate (CrO_4^{2-}), dichromate ($Cr_2O_7^{2-}$) or hydrogen chromate ($HCrO^-_4$)) to the yeast surface will be promoted.

These results are in disagreement with our previous results dealing with bacterial strains, where the most performant species presented the lowest electron donor character [15].

This is also in disagreement with a previous work, reporting a relation between functional groups and chromate ions biosorption. In this work, it was shown that functional groups in algal cell surface become negatively charged at high pH values. They tend hence to repulse the negatively charged ions chromate and thus affects the adsorption to the algal wall [10]. Concerning the electron acceptor character $\gamma+$, the obtained results show that this character is not correlated to their chromium removal potential (R^2=0,030). A lack of significant correlation between yeast electron acceptor character $\gamma+$ and their chromium removal potential in our study disagrees with a previous study, where the electron donor properties of bacterial cells determined by CAM and their Cr(VI) removal efficiency correlated with each other [15]. The obtained results in the previous work showed that the bacteria with the higher acid component present a higher affinity to chromium forms existing in the aqueous media. While our data do not suggest any correlation between this character and the chromium uptake efficiency of yeast cells. This may be ascribed to the structural diversity between the cell surfaces of different microorganisms.

3.4 Surface properties of yeast cells after chromium biosorption

The changes in the hydrophobicity and the surface Lewis acid/base character before and after Cr(VI) biosorption at 50 $mg.L^{-1}$ are illustrated in Table 1. An increase of the quantitative hydrophobicity after biosorption of chromium was observed for *C. tropicalis* and *P. fermentans*. For the surface hydrophobicity of *W. anomalus* and *C. fabianii* cells, it does not have a statistically significant tendency

to change due to sorption of chromium. While, in the case of *G. geotrichum*, the surface became more hydrophilic after chromium contact. It is generally agreed that the increase of cell surface hydrophobicity is a defense system to face environmental challenges such as heavy metals pollution.

Table 1. Contact angle values using water (θw), formamide (θ_F) and diiodométhane (θ_D), Lifshitz-vander Waals (γ^{LW}), Electron-Donor (γ-) and Electron-Acceptor (γ+) Parameters and Surface Energies (Δ*G*iwi) of Yeast Cells.

		Contact angles (°)			**Surface tension: components and parameters and surface energies ($mJ.m^{-2}$)**				
		θ_w	**θ_F**	**θ_D**	**γ^{LW}**	**γ^+**	**γ^-**	**γ^{AB}**	**ΔGiwi**
P. fermentans	*Initial*	53.0	51.3	58.5	29.38	0.55	34.19	-6.89	12.63
	After Cr(VI) contact	51.7	24.7	72.1	21.66	10.14	17.87	3.07	-6.13
G. geotrichum	*Initial*	32.5	35.3	72.0	21.68	4.49	47.68	-10.88	21.76
	after Cr(VI) contact	33.4	38.6	33.7	42.53	3.47	51.87	-21.60	36.27
C. fabianii	*Initial*	51.0	35.6	81.1	16.87	9.85	23.51	0.75	-2.33
	after Cr(VI) contact	51.7	35.8	61.8	27.48	4.01	23.72	1.09	-2.87
C. tropicalis	*Initial*	48.4	50.4	74.2	20.48	2.49	39.51	-8.64	17.25
	after Cr(VI) contact	49.12	32.5	73.8	20.73	8.09	24.42	0.47	-0.97
W. anomalus	*Initial*	33.5	49.3	82.5	16.18	3.19	60.14	-17.67	34.58
	after Cr(VI) contact	33.0	39.9	51.7	33.25	0.51	52.68	-19.16	35.87

Concerning electron acceptor character, a decrease of its value was observed for *C. fabianii*, *W. anomalus*, *G. geotrichum* while there was an increase in this character with *C. tropicalis* and *P. fermentans.*

An increase of the electron donor character of *G. geotrichum* was observed, while it decreased for *W. anomalus*, *C. tropicalis* and *P. fermentans* and no significant change was observed with *C. fabianii*. The tendency of changes in the hydrophobicity and surface electron donor/acceptor character after biosorption were not the same for all studied yeast and the changes in the relative hydrophobicity of yeast cells and the surface Lewis acid/base characters were not statistically significant. It can result from the difference in the resistance and the biosorption mechanisms and the cell surface composition of each microorganism. Owing to this fact, no conclusion of surface behavior after Cr(VI) biosorption can be concluded. These findings are in agreement with the work of Kordialik-Bogacka (2011), reporting that different changes of the cell surface physic-chemical properties after heavy metals sorption could be obtained and the tendency of changes was dependent on the species [27].

Conclusion

This work aimed the study of the physicochemical properties of yeast strains in relation with their chromium removal potential. The obtained results showed that the chromium biosorption efficiency was highly correlated to the hydrophobicity and the electron donor character of the used biomass. However, no correlation was observed with electron acceptor character. Depending on the yeast species, different changes in the hydrophobicity and the surface Lewis acid/base character occurred after Cr(VI) biosorption.

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Disclosure statement

Conflict of Interest: The authors declare that there are no conflicts of interest.

Compliance with Ethical Standards: This article does not contain any studies involving human or animal subjects.

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