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RESEARCH ARTICLE

Ecuadorian yeast species as microbial particles for Cr(VI) biosorption

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Abstract

Pollution caused by heavy metals is a prime concern due to its impact on human health, animals, and ecosystems. Cr(VI), generated in a range of different industries as a liquid effluent, is one of the most frequent contaminants. In the work presented herein, the adsorption efficiency of three species of native yeasts from Ecuador (*Kazachstania yasuniensis*, *Kodamaea transpacifica*, and *Saturnispora quitensis*) for Cr(VI) removal from simulated wastewater was assessed, taking *Saccharomyces cerevisiae* as a reference. After disruption of the flocs of yeast with a cationic surfactant, adsorption capacity, kinetics, and biosorption isotherms were studied. *K. transpacifica* isolate was found to feature the highest efficiency among the four yeasts tested, as a result of its advantageous combination of surface charge, individual cell size (4.04 μm), and surface area (1588.27 m^2/L). The performance of *S. quitensis* was only slightly lower. The remarkable biosorption capacities of these two isolates (476.19 and 416.67 mg of Cr(VI)/g of yeast, respectively) evidence the potential of non-conventional yeast species as sorption microbial particles for polluted water remediation.

Keywords Biosorption · Cationic surfactant · Cr(VI) · Microbial particles · Yeasts · Specific surface area

Introduction

Urbanization and industrialization have led to a large production of toxic effluents, most of which end up in rivers, lakes, and seas. The most frequent wastes in water are heavy metals (viz., Cr, Fe, Co, Cu, Zn, Mo, Hg, Cd, Ni, Pb, Sb, Bi), which are poisonous even at low concentrations (Tchounwou et al. 2014). Heavy metals are accumulated in vegetal and animal tissues, and the repeated ingestion of small amounts of these contaminants ends up producing high concentrations of metals in the cells (Adams and Zhitkovich 2011; Jaishankar et al. 2014).

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Chromium in water may be found in two forms, either as Cr(III), less mobile and less hazardous to health, or as Cr(VI), more mobile and much more toxic (by a factor of 10) (Adams and Zhitkovich 2011). According to the US Environmental Protection Agency and the World Health Organization, the maximum allowable limits for Cr(VI) in drinking water are 0.10 mg/L and 0.05 mg/L, respectively (Hawley and Jacobs 2016).

Although nowadays most factories have wastewater treatment plants, those that produce effluents containing chromium and other heavy metals are still in need of innovative and efficient removal systems to avoid exceeding the permitted concentrations. Conventional chemical processes (e.g., chemical precipitation, electrochemical treatment, activated carbon, membrane technology) require reagents, equipment, and technologies that are expensive and not very efficient at low metal concentrations in aqueous solutions (Meena and Busi 2016; Bankar and Nagaraja 2018; Kumar and Gunasundari 2018; Kumari et al. 2018).

Alternative approaches, based on more environmentally friendly technologies, may be based on adsorption or on biosorption. The former is a phenomenon in which a substance (adsorbate) adheres to solid matrices (adsorbent) (Fomina and Gadd 2014). Depending on the nature of the process, we may distinguish between physical and chemical adsorption, depending on whether it occurs due to Van del

Waals forces or due to chemical interaction between the adsorbate and the adsorbent, respectively. In both cases, the process is spontaneous and exothermic (De Rossi et al. 2018).

As regards biosorption, it involves using a biomaterial as the adsorbent surface or matrix (Farhan and Khadom 2015; Zeraatkar et al. 2016). In this mechanism, the displacement of metal ions from the solution to the outer layer of the biosorbent matrix is followed by external diffusion and then by intraparticular diffusion. Biosorption enables the recovery of metals of commercial importance through their desorption by regeneration methods (Mahmoud et al. 2009; Fernández et al. 2013). The biomaterials used in wastewater treatments generally come from residues from industrial processes, either from plants (e.g., bagasse, husks, seeds, cellulose), from animals (chitin, crustacean shells), or from microorganisms (fungi, bacteria, microalgae, and yeasts) (Wang and Chen 2006).

In the biosorption of heavy metals using microorganisms, there are two forms of metal capture: the so-called passive mode, in which the metal is adsorbed by dead or inactive biomass, and the active mode, in which the metal is adsorbed by living biomass (Zeraatkar et al. 2016). The passive mode is independent of the energy and acts mainly through the chemical functional groups of the biomaterial (yeast cell wall), whereas the active mode is dependent on metabolism and is related to the transport and accumulation of metals within the cell (although passive adsorption may also occur when the cell is metabolically active) (Vendruscolo et al. 2017).

The use of microorganisms, such as bacterial biomass, as biosorption matrices has been widely studied in relation to the structure of the cell wall and its composition (i.e., the metal biosorption sites) (Fomina and Gadd 2014). For instance, the major binding sites for metal cations in the walls of Gram-positive bacteria are the carboxyl groups of peptidoglycans, while phosphate groups present in the walls constitute the binding sites in Gram-negative bacteria (Mohan and Pittman 2006). Other bacterial binding sites are made up of polymeric materials that include proteins and polysaccharides on the surface of the cell wall (Saha and Orvig 2010).

In comparative studies, such as the classic work by Wang and Chen (2006), the uptake of heavy metals through biosorption for several types of biomass (including bacteria, yeasts, fungi, and marine algae) has been assessed. Although *S. cerevisiae* would feature an average adsorption capacity, it has associated advantages in terms of ease of cultivation, availability as a by-product, safety, and ease of genetic manipulation, as noted by Wang and Chen (2006). Some of these advantages would be shared by other yeasts, which may feature higher adsorption capacities.

Given the opportunities for new green technologies that arise from microbial biodiversity, the aim of this work has been to evaluate the suitability of three novel yeast species isolated in Ecuadorian natural environments for the remediation of Cr(VI) in simulated wastewater. Through a study of the kinetics and adsorption isotherms, together with an analysis of the relationships among specific surface area, efficiency, and biosorption capacity, an assessment of their potential for the decontamination of polluted waters is reported.

Materials and methods

Preparation of Cr(VI) solutions

A stock solution (1000 mg Cr(VI)/L) was prepared by dissolving 2.829 g of potassium dichromate (CAS 7778-50-9; ≥ 99.0%; Sigma–Aldrich) in 1 L of deionized distilled water. For biosorption experiments, diluted solutions were prepared with concentrations ranging from 10 to 100 mg Cr(VI)/L.

Yeast isolates

The three yeast species from Ecuador used in the study (*Kazachstania yasuniensis* (CLQCA-20-280), *Kodamaea transpacifica* (CLQCA-24i-158), and *Saturnispora quitensis* (CLQCA-10-114) (James et al. 2011, 2015; Freitas et al. 2013) were supplied by the Colección de Levaduras Quito-Católica (CLQCA). These isolates were discovered as part of the CLQCA yeast bio-prospecting program, aimed at cataloguing and characterizing indigenous yeast species found in Ecuador.

Kazachstania yasuniensis (CLQCA-20-280) strain, found in 2013, was isolated from soil samples collected in the Yasuní National Park, as part of a project focused on the isolation of new ethanol-tolerant species (James et al. 2015). *Kodamaea transpacifica* (CLQCA-24i-158) strain was collected from ephemeral flower samples in 2009, in Isabela Island (Galápagos Islands), as part of an investigation on ancient human transpacific contact (Freitas et al. 2013). Apropos of *Saturnispora quitensis* (CLQCA-10-114) strain, it was isolated from the fruit of an unidentified species of bramble (*Rubus* sp.) collected from the Maquipucuna cloud forest reserve in Pichincha. This genus is characterized by teleomorphic species that produce one to four spheroidal ascospores (James et al. 2011).

An industrial strain of *Saccharomyces cerevisiae* was used as the control (NCYC 1529).

The isolates were activated in a yeast malt agar solid medium (0.3% (w/v) yeast extract, 0.3% (w/v) malt extract; 0.5% (w/v) peptone, 1% (w/v) glucose, 2% (w/v) agar). The obtained yeast biomass was inoculated in 50 mL of yeast peptone dextrose broth liquid medium, and incubated at 25 °C at 200 rpm for a period of 18 h in 100 mL glass flasks. The biomass concentration was set at 5×10^6 CFU/mL (colony-forming units per milliliter).

Cationic surfactant pretreatment

Yeast biomass of the four isolates was pretreated using a solution of benzalkonium chloride (BZK; CAS 63449-41-2; ≥ 95.0%; Sigma–Aldrich) cationic surfactant in the concentration and conditions established in Bingol et al. (2004): 1.460 g/L, at 25 °C for 2 h, stirring at 150 rpm, followed by centrifugation at 5000 rpm.

To assess the effect of the cationic surfactant, yeast biomass—either pretreated or not—was suspended in 20 mL of Cr(VI) solution (100 mg/L) at pH 4.5 and was incubated at 25 °C for 4 h under stirring at 200 rpm.

Biosorption kinetics

To evaluate how viable a biosorbent is, sorption kinetics need to be studied, in order to determine the speed in which the biosorption equilibrium is reached and to estimate the time to achieve that equilibrium (Tapia et al. 2003; Zhang and Yi 2017). In this study, the metal adsorption rate was estimated using two models: the pseudo-first order and pseudo-second order model, described below (Michalak et al. 2013; Zhang and Yi 2017).

In the *pseudo-first order model*, Lagergren equation is used:

$$q_t = q_e \cdot \left(1 - e^{-k_1 \cdot t}\right) \tag{1}$$

which may be linearized as:

$$ln(q_e - q_t) = ln(q_e) - k_1 \cdot t \tag{2}$$

where q_e, q_t, and k_1 represent the quantity of biosorbed sorbate in equilibrium (mg/g), the quantity of biosorbed sorbate at any time (mg/g), and the pseudo-first order rate constant (1/min), respectively.

In the *pseudo-second order model*, Eq. (3) or its linearized version (Eq. (4)) are used instead:

$$q_t = \frac{t}{\frac{1}{k_2 \cdot q_e^2} + \frac{t}{q_e}} \tag{3}$$

$$\frac{t}{q_t} = \frac{1}{k_2 \cdot q_e^2} + \frac{t}{q_e} \tag{4}$$

where q_e is the quantity of biosorbed sorbate in equilibrium (mg/g), q_t is the quantity of biosorbed sorbate at any time (mg/g), and k_2 is the pseudo-second order rate constant (g·1/(mg·min)).

Simple linear regression was used instead of non-linear regression to facilitate comparisons with the equilibrium and kinetic parameters reported in the literature, which generally use the former approach. Nonetheless, it is worth noting that studies focused on comparing both linear and non-linear regression methods in adsorption processes concluded that the differences in the calculated adsorption capacity values were not statistically significant (Gautam 2015; Ho 2006; Wang and Chen 2006; Nagy et al. 2013; Kumar et al. 2008; Kumar 2006; Lataye et al. 2008; Parham et al. 2012).

Experiments for biosorption kinetic studies were performed at different Cr(VI) concentrations over time: the yeast biomass (pretreated with cationic surfactant) was suspended in 20 mL of Cr(VI) solution at a concentration of 10, 25, 50, 75, or 100 mg/L at pH 4.5, and was then incubated at 25 °C for 1, 5, 10, 15, 30, 60, 120, 180, and 240 min under stirring at 200 rpm.

Constant values were set for pH, temperature, and biomass concentration parameters, on the basis of preliminary trials and the consulted literature (Kapoor and Viraraghavan 1995; Goyal et al. 2003; Özer and Özer 2003; Zouboulis et al. 2004; Bhattacharya et al. 2008; Bankar et al. 2009).

Biosorption isotherms

For a solid-liquid system, biosorption isotherms can be obtained by relating the amount of solute adsorbed by biosorbent mass (q_e) and the concentration of the solute in equilibrium (C_e) (Levine 2004; Michalak et al. 2013), according to Eq. (5):

$$q_e = \frac{(C_i - C_e) \cdot V_s}{m_B} \tag{5}$$

where q_e is the biosorption capacity (mg/g), C_i is the initial metal concentration (mg/L), C_e is the metal concentration remaining or in equilibrium (mg/L), and V_s is the volume of solution (L).

To predict the mechanisms of the biosorption system, different models were used to fit the experimental data:

Henry's isotherm

Based on Henry's law, it represents the capacity of adsorption at low concentrations of sorbate (Levine 2004):

$$q_e = K \cdot C_e \tag{6}$$

where K is linear adsorption constant (L/g biosorbent).

Langmuir isotherm

This model assumes that the sorbate forms a monolayer on the surface, that the surface of the biosorbent is homogeneous, that each active center of the surface is equal, that there is no interaction between the biosorbed particles, and that the biosorbed particles do not move on the surface (Kikuchi and Tanaka 2012; Michalak et al. 2013; Zhang and Yi 2017).

$$q_e = \frac{q_{max} \cdot b \cdot C_e}{1 + b \cdot C_e} \tag{7}$$

$$\frac{1}{q_e} = \frac{1}{q_{max}} + \frac{1}{q_{max} \cdot b} \cdot \frac{1}{C_e} \quad (8)$$

where q_{max} is the maximum biosorption capacity (mg/g), and b is the Langmuir affinity constant between the biosorbent and sorbate (L/mg), in such a way that the higher b is, the greater the affinity will be.

Freundlich isotherm

This model differs from the one proposed by Langmuir in that it recognizes the possibility of interaction between the molecules adsorbed in the different active centers and that it can be applied to multilayer adsorption. This isotherm model is used in heterogeneous systems (Michalak et al. 2013; Fomina and Gadd 2014; Sathvika et al. 2015).

$$q_e = k_F \cdot C_e^{\frac{1}{n_F}} \quad (9)$$

$$ln(q_e) = ln(k_F) + \frac{1}{n_F} ln(C_e) \quad (10)$$

where k_F is the equilibrium constant and n_F is the affinity constant between sorbate and biosorbent. If $n < 1$, the interaction is weak; if $n > 1$, the interaction is strong; and if $n = 1$, it would be a Langmuir-type isotherm.

For the adsorption isotherm assays, the yeast biomass (pretreated with cationic surfactant) was suspended in 20 mL of Cr(VI) solution (at a concentration of 10, 25, 50, 75, or 100 mg/L) at pH 4.5, and was then incubated at 25 °C for 4 h under stirring at 200 rpm.

Assessment of the effect of specific surface area on biosorption

To evaluate the effect of yeast cell particle size on Cr(VI) biosorption, a specific surface area analysis of the four yeast isolates was performed, assuming that the shape of the yeast cells was spherical and spheroidal (since the cell's shape along the yeasts life cycle goes from spherical to spheroidal, an average value of the area of both shapes was considered). For the calculation of the specific surface area of each isolate, the average diameter was determined using a phase-contrast microscope (Olympus BX51, Model U-LH100-3, Tokyo, Japan).

All biosorption assays were performed in triplicate, using a 100 mg/L Cr(VI) solution as the control to measure its concentration after 4 h.

Analytical method

Quantification of Cr(VI) in the aqueous samples was carried out according to the standard colorimetric method for the determination of Cr(VI) (Clesceri et al. 1999), i.e., by complexation of Cr(VI) with 1,5-diphenylcarbazide (CAS 140-22-7; ≥ 98.0%; Sigma–Aldrich). An HELIOS β spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) was used for measurements at λ = 540 nm.

Statistical analysis

The experimental results were analyzed using INFOSTAT (Córdoba, Argentina) statistical software. The applied design method was a CRD (completely randomized design). ANOVA tests were run to verify significant differences between the means of each yeast isolate. Tukey's HSD test was used to compare multiple interactions between the means of the yeast isolates and the studied variables. Linear regressions were used for the study of the kinetics and biosorption isotherms, comparing the coefficients of determination (R^2).

Results and discussion

Effect of cationic surfactant pretreatment

The biosorption efficiencies of the four yeasts under study, with and without BZK pretreatment, are shown in Fig. 1. The yeast cell wall pretreatment with the cationic surfactant significantly improved all yeasts' Cr(VI) sorption efficiencies (by 124%, 140%, 111%, and 101%, for *K. yasuniensis*, *K. transpacifica*, *S. quitensis*, and *S. cerevisiae*, respectively). Significant differences ($p < 0.0001$) were found for the isolate and surfactant variables, and for isolate × surfactant interaction. The pretreated samples of the three novel yeasts under study (*K. yasuniensis*, *K. transpacifica*, and *S. quitensis*) performed better than the control (*S. cerevisiae*), but efficiencies above 75% were attained in all cases.

The biosorption efficiency enhancement may be ascribed to an increase in the available surface area for sorption, provided that the positive charge contributes to the breakage of yeast flocs. Moreover, upon pretreatment with the cationic surfactant, an increase in the electrostatic interaction between the surface of the yeast biomass and Cr(VI) may be expected: Saha and Orvig (2010) suggested that the non-polar portion of the cationic surfactant molecule would interact with the cell wall, while the polar portion (functional groups) would interact with the Cr(VI) ions. In a study that used another cationic surfactant (cetrimonium bromide, CTAB) to modify *S. cerevisiae* biomass, also aimed at Cr(VI) biosorption, Bingol et al. (2004) found that the efficiencies for unmodified yeasts at pH > 2 were lower than 20%, while for the modified cells efficiencies higher than 80% were attained, reaching an efficiency as high as 99.5% at pH 5.5.

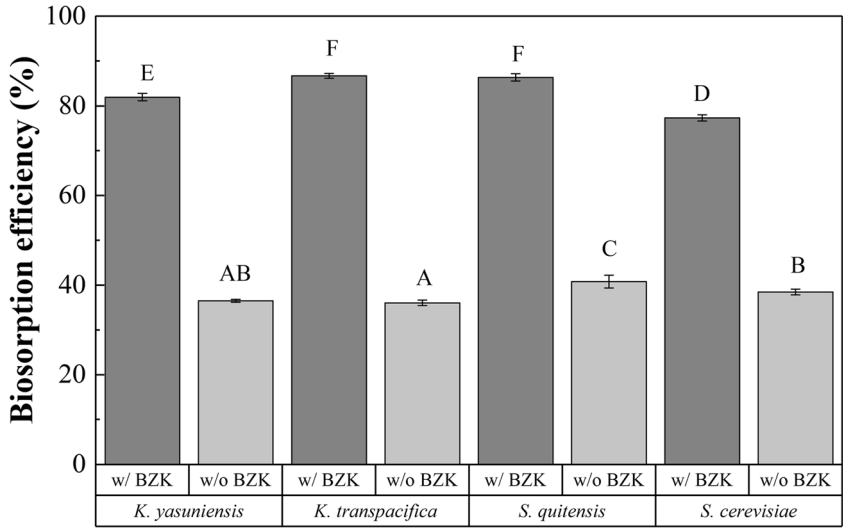

Fig. 1 Impact of cationic surfactant pretreatment on Cr(VI) biosorption efficiency. "w/" and "w/o" belzalkonium chloride (BZK) indicate pretreated and non-pretreated samples, respectively. Values correspond to the average of three repetitions. Means not sharing any letter are significantly different by Tukey's HSD test at the 5% level of significance (grouped according to isolate × surfactant interaction)

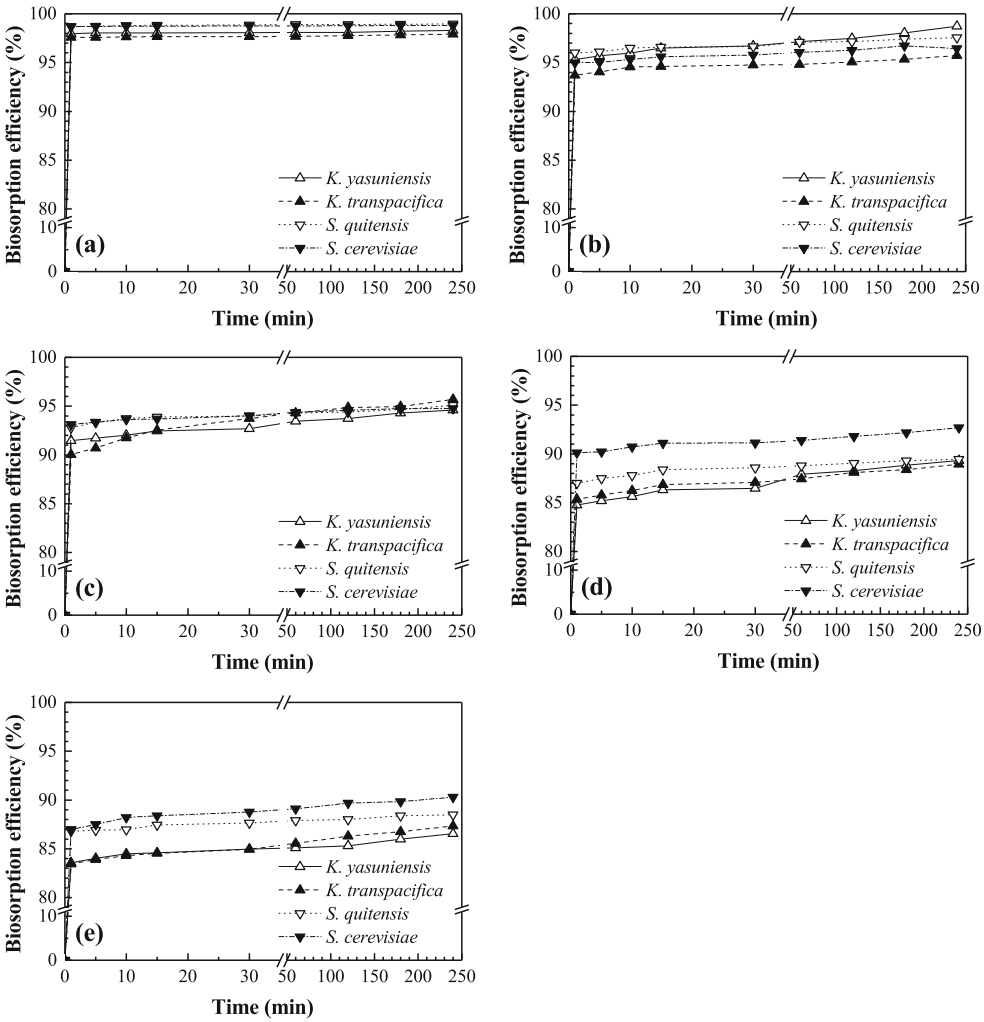

Fig. 2 Biosorption kinetics at different Cr(VI) concentrations for the four yeast isolates: **a** 10 mg/L; **b** 25 mg/L; **c** 50 mg/L; **d** 75 mg/L; and **e** 100 mg/L. Average values across three repetitions are shown, and standard deviations have been omitted for clarity purposes. Coefficients of variation (CV) generally remained below 5%, although CVs of up to 20% were obtained in some cases

Table 1 Pseudo-second order kinetic model parameters

Yeast species	C_o	Pseudo-second order parameters		
		q_e	k_2	R^2
K. yasuniensis	10	6.545	0.096	0.9999
	25	5.974	0.330	0.9993
	50	25.575	0.034	0.9999
	75	39.683	0.020	0.9999
	100	97.087	0.005	1
K. transpacifica	10	6.557	0.092	0.9999
	25	9.009	0.127	0.9997
	50	21.598	0.049	0.9999
	75	51.020	0.012	0.9999
	100	77.519	0.007	1
S. quitensis	10	17.637	0.014	1
	25	15.038	0.050	0.9999
	50	43.860	0.012	0.9999
	75	104.167	0.003	1
	100	200.000	0.001	1
S. cerevisiae	10	35.088	0.004	1
	25	15.576	0.044	0.9999
	50	67.568	0.005	1
	75	68.493	0.007	1
	100	125.000	0.003	1

q_e, biosorption capacity (mg metal/g biosorbent)

Biosorption kinetics

The biosorption efficiencies of the four yeast isolates at different times (1, 5, 10, 15, 30, 60, 120, 180, and 240 min) and at a concentration of 100 mg Cr(VI)/L are shown in Fig. 2. Whereas the coefficients of determination for the pseudo-first order model (not shown) were very low, high R^2 coefficient values were obtained for the pseudo-second order kinetic model for all isolates (Table 1), indicating that it would be the best fit for the Cr(VI) biosorption process. This would be in good agreement with other studies on the biosorption of Cr(VI) onto various biosorbents (Arica et al. 2005; Machado et al. 2010; Dileepa Chathuranga et al. 2013; Xu et al. 2016), and suggests that chemical adsorption would be the rate-limiting factor in the first stage of the process (passive biosorption) (May and Holan 1993; Zeraatkar et al. 2016).

The biosorption process occurred rapidly, with optimal contact times ranging from 10 to 30 min. The first minutes corresponded to the first stage of biosorption (passive), in which a high percentage of efficiency was reached (80 to 90% of the Cr(VI) removal), followed by a second stage (active), in which the sorption percentages increased by values ranging from 2 to 5% (Zeraatkar et al. 2016).

As reported by Ye et al. (2010); Yin et al. (2008a, b), the biosorption process of *S. cerevisiae* requires between 20 and 30 min of contact for passive sorption. For comparison purposes, for fungi (e.g., *Trichoderma* spp.), the optimal contact time has been reported to be 80 min (Shukla and Vankar 2014); and for algae (e.g., *Spirogyra* spp.), the required contact time would be 180 min (Gupta et al. 2001).

Biosorption isotherms

Three models of isotherms (viz., Henry's or lineal, Langmuir, and Freundlich isotherms) were tested to analyze the four yeast isolates sorption capabilities. Their respective equations and parameters are summarized in Table 2. Langmuir isotherm (Fig. 3) was found to be the best fit to the Cr(VI)

Table 2 Isotherm models parameters

Isotherm model	Yeast species	Parameters					
		K	q_{max}	b	K_f	n_F	R^2
Henry's	*K. yasuniensis*	1.424					0.7149
	K. transpacifica	1.758					0.9679
	S. quitensis	1.997					0.9723
	S. cerevisiae	1.911					0.9752
Langmuir	*K. yasuniensis*		114.94	0.029			0.9412
	K. transpacifica		476.19	0.005			0.9956
	S. quitensis		416.67	0.007			0.9968
	S. cerevisiae		120.48	0.033			0.9845
Freundlich	*K. yasuniensis*				5.156	1.328	0.8202
	K. transpacifica				2.853	1.130	0.9797
	S. quitensis				3.166	1.125	0.9829
	S. cerevisiae				4.534	1.312	0.9764

K (L solution/g biosorbent); q_{max} (mg Cr(VI)/g biosorbent); b (L solution/mg Cr(VI)); K_f (mg Cr(VI)·L solution/g biosorbent)

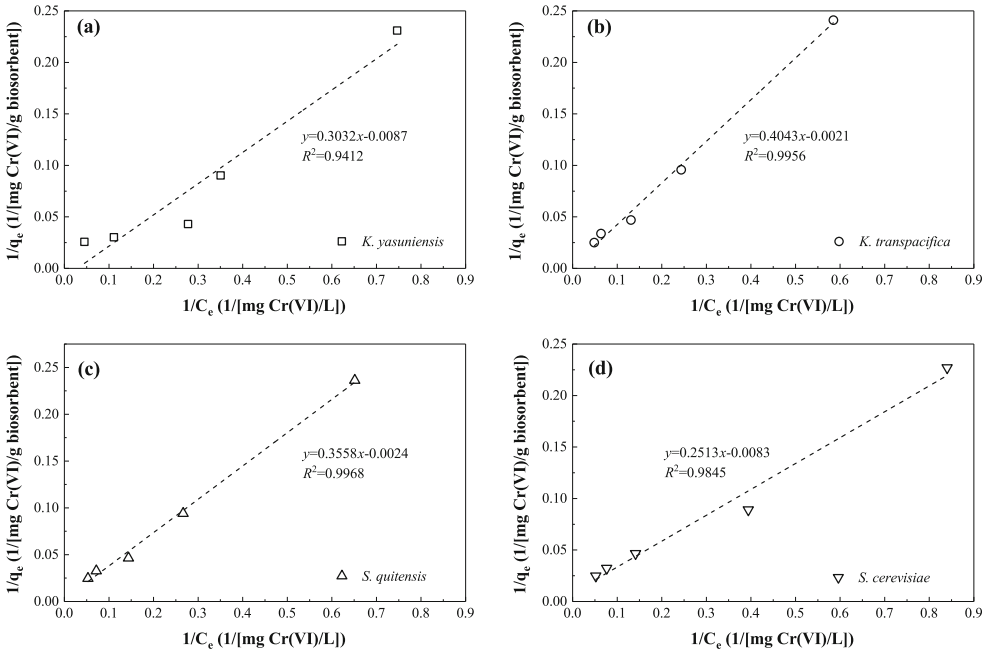

Fig. 3 Langmuir isotherm models for Cr(VI) biosorption: **a** *K. yasuniensis*; **b** *K. transpacifica*; **c** *S. quitensis*; **d** *S. cerevisiae*

biosorption activity for the four yeast isolates, on the basis of the R^2 coefficient values. These results are congruous with other studies in which yeasts were used as biosorbents (May and Holan 1993; Özer and Özer 2003; Bingol et al. 2004), although it should be clarified that those studies did not report cationic pretreatments for other yeasts apart from *S. cerevisiae*.

The maximum biosorption capacity parameter (q_{max}) obtained from the Langmuir model for *S. cerevisiae* in this study was comparable to the one obtained by Bingol et al. (2004)

Table 3 Comparison of adsorption capacities and adsorption efficiencies of different yeast species used for Cr(VI) biosorption

Species	Biosorbent type	Adsorption capacity (mg/g)	Efficiency (%)	R^2	Reference
K. transpacifica	Cationic surfactant-modified living cells	476.19	85.80	0.99	This study
S. quitensis	Cationic surfactant-modified living cells	416.67	85.40	0.99	This study
K. yasuniensis	Cationic surfactant-modified living cells	114.94	80.70	0.94	This study
S. cerevisiae	Cationic surfactant-modified living cells	120.48	75.8	0.98	This study
S. cerevisiae	Lab-cultivated cells, then dried at 100 °C	32.6	–	0.98	(Özer and Özer 2003)
S. cerevisiae	Lab-cultivated, free cells	13.26	–	0.99	(Goyal et al. 2003)
S. cerevisiae	Cationic surfactant-modified living cells	94.34	99.5	0.98	(Bingol et al. 2004)
S. cerevisiae	Dead cells	–	44.2	–	(Park et al. 2005)
S. cerevisiae	Dried cells	6.607	–	–	(Nagendran 2008)
S. cerevisiae	Deactivated cells with ceramic membrane	–	99	–	(Vasanth et al. 2012)
S. cerevisiae	Immobilized cells in packed bed	1.7	60	–	(Ramírez Carmona et al. 2012)
S. cerevisiae	Gelatin impregnated with cells	500	–	0.99	(Mahmoud 2015)
S. cerevisiae	Immobilized cells in cellulose biopolymer	23.61	–	0.98	(Sathvika et al. 2015)
S. cerevisiae (*M1*)	Metallothionein recombinant cells	8.27	–	–	(Zhang 2017)
C. utilis (*M20*)	Living cells with medium	7.3	–	–	(Muter et al. 2001)
C. utilis (*CR-001*)	UV/HNO_2 mutant cells	–	98	–	(Yin et al. 2008a)
Candida sp. (*LMB2*)	Living cells with medium	–	100	–	(Juvera-Espinosa et al. 2006)
Y. lipolytica (*NCIM-3589*)	Centrifuged cells	109.75-	46.52	0.99	(Bankar et al. 2009)
Y. lipolytica (*NCIM-3590*)	Centrifuged cells	150.18	43.87	0.97	(Bankar et al. 2009)

Table 4 Effect of specific surface area on biosorption

Species	D (μm)	A_{sp} (m^2/L)	Efficiency (%)	q_{max} (mg Cr(VI)/g yeast)
K. yasuniensis	5.03	1192.67	80.70	114.94
K. transpacifica	4.04	1588.27	85.80	476.19
S. quitensis	4.07	1474.30	85.40	416.67
S. cerevisiae	8.29	731.49	75.8	120.48

(q_{max}: 94.34 mg/g), with a similar pretreatment; and q_{max} for *K. yasuniensis* was also in the same order of magnitude. Nonetheless, q_{max} values for the isolates of *K. transpacifica* and *S. quitensis* were almost four times higher. Moreover, they were higher than those reported in the literature for other yeasts (Table 3).

Apropos of *S. cerevisiae* biomass, in other studies it was subjected to a wide range of chemical and physical pretreatments (via the use of acids, methanol, ethanol, formaldehyde, cationic surfactants, free cells, dried cells, and protonated/unprotonated cells) for the sorption of a variety of heavy metals (including Cu(II), Ni(II), Cr(VI), Cd(II), and Pb(II)), and q_{max} reported values ranged from 11 to 270 mg metal/g yeast biomass (May and Holan 1993; Özer and Özer 2003; Bingol et al. 2004; Bankar et al. 2009; Mahmoud 2015). q_{max} values between 109 and 150 mg/g were reported for *Yarrowia lipolytica* (Bankar et al. 2009). Other biological materials, such biomass from vegetable residues (sawdust, maize, tamarind, walnut, banana, acorn, and others), have been reported to feature q_{max} values between 3 and 200 mg Cr(VI)/g biomass (Quiñones et al. 2014), also lower than those attained for *K. transpacifica* and *S. quitensis*.

Impact of specific surface area on biosorption

A comparison among the four yeast isolates in terms of cell diameter, specific surface area, efficiency, and maximum biosorption capacity is presented in Table 4. The efficiency of Cr(VI) sorption, as expected, was directly proportional to the specific surface area of the microbial particles ($y = 0.0122x + 66.696$; $R^2 = 0.9861$).

The noticeable differences in the specific surface area among the three Ecuadorian isolates under study and the control (*S. cerevisiae*) can be readily ascribed to differences in particle size, as shown in the micrographs (Fig. 4): the diameter of the yeast cells of the control (8.29 μm) was approximately twice the diameter of the other isolates (4.04–5.03 μm).

Since, as noted above, biosorption capacity (q_e) not only depends on the surface charge of the cells but also on particle size/specific surface area, it is reasonable that *S. quitensis* and *K. transpacifica*—which had the highest specific surface areas—featured the highest biosorption capacities (416.67 and 476.19 mg Cr(VI)/g yeast, respectively), and the highest efficiencies (85.4 and 85.8%, respectively).

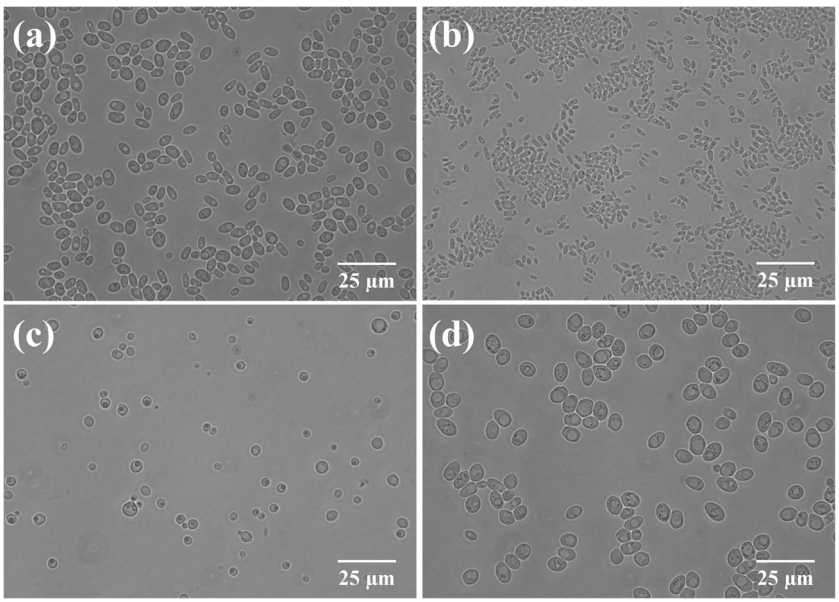

Fig. 4 Micrographs of **a** *K. yasuniensis*, **b** *K. transpacifica*, **c** *S. quitensis*, and **d** *S. cerevisiae*

However, the yeast's ability to adsorb Cr(VI) would not only depend on particle size/specific surface area, but on a range of other factors, including the architecture dynamics of the cell wall; the occurrence of functional groups on the yeast's surface (sulfates, phosphates, P-ligands, cysteine inserts, -S, and -N ligands); the secretion/excretion products passing through the cell wall; and physical-chemical phenomena that may influence the interaction of the yeasts and the metal ions in aqueous solution (Wang and Chen 2006). The lower q_{max} of *K. yasuniensis* as compared to the other two Ecuadorian isolates, in spite of its similar diameter and surface area of the isolate (5.03 μm and 1192.67 m^2/L, respectively), should be tentatively referred to one (or several) of aforementioned factors.

Conclusions

The use of a cationic surfactant as a yeast cell wall-conditioning strategy significantly enhanced the biosorption for all yeasts under study, almost doubling their Cr(VI) sorption efficiency. For the yeasts studied herein, the optimal contact time required to perform the Cr(VI) biosorption process ranged from 10 to 30 min, attaining efficiencies above 80% for Cr(VI) concentrations of up to 100 mg/L. While *Kazachstania yasuniensis* showed a biosorption capacity similar to that of *S. cerevisiae*, those of *Saturnispora quitensis* and *Kodamaea transpacifica* were almost four times higher (416.67 and 476.19 mg Cr(VI)/g yeast, respectively) due to their high specific surface areas (1474.30 and 1588.27 m^2/L, respectively), evidencing the impact of microbial particle size for the sorption of the metal ions. These two isolates may thus hold promise for the bioremediation of polluted bodies of water at a potentially low cost. Further research using real samples from chromium-polluted water bodies is underway.

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