Wickerhamomyces anomalus biofilm supported on wood husk for chromium wastewater treatment

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ABSTRACT

A *Wickerhamomyces anomalus* biofilm supported on wood husk was used to remediate water bodies contaminated with chromium (Cr), in batch and open systems. The favorable adhesion ability of the chromium-resistant yeast strain on the wood husk was predicted by XDLVO theory and confirmed by environmental scanning electronic microscopy. The chromium decontamination was then optimized in a batch mode using a central composite design (CCD). Analysis of variance (ANOVA) showed a high coefficient of determination (R^2) value of 0.93–0.91 for Cr(VI) and total Cr removal, respectively, ensuring a satisfactory fitting of the second-order regression model to the experimental data. In batch system, the concentration of biomass exhibited the minimal effect on the process. An acidic pH of 3.72 and 5.48, an initial chromium concentration of 10 and 16.91 mg/L and a support dose of 6.95 and 8.20 g/L were optimal for Cr(VI) and total Cr removal, respectively. The breakthrough curves were determined in open system for different initial chromium concentrations. The study of glucose concentration effect on the yeast extracellular polymeric substances (EPS) production showed that a medium exempt of glucose allowed maximal EPS production and minimal chromium removal efficiency, while 20 g/L glucose concentration of presented the optimal condition for chromium removal.

1. Introduction

Toxic heavy metals are the most harmful pollutants increasingly produced by natural and anthropogenic means [1]. Chromium is one of the main pollutants discharged in the environment due to its extensive use in various industries [2,3]. It occurs in different degrees of toxicity depending on its oxidation state. Cr (III) and hexavalent Cr (VI) forms are of significant concern in environmental issues [4].

Hexavalent chromium Cr(VI) is a highly toxic water pollutant. It can originate from different anthropogenic activities such as leather tanning, pigment synthesis, electroplating and metal finishing. Chromium-contaminated wastewaters directly discharged into the environment cause serious threat to human beings and present harmful ecological effects [5].

The United States Environmental Protection Agency determined the value of 0.1 mg/L as a maximum contaminant level for total chromium in the environment. Hence, chromium removal is gaining importance in the environmental research community [6].

In order to alleviate water stress, there is a pressing need to seek suitable technological systems for effective metal recovery before the discharge of contaminated wastewater. Owing to its numerous advantages, biosorption has aroused a great interest. It has been defined as the ability of biomaterials to selectively bind ions or molecules from aqueous solutions [7]. It is considered as a cost-effective and efficient method for chromium removal from aqueous environment [8–10].

Recently, biofilm-based biosorption is considered as an attractive process that has been used effectively for metals removal from industrial effluents [11–13]. This technology involved different materials as a support for microbial cells attachment [14,15]. Among these materials, wood husk was successfully reported for remediation application [14]. This material is known to have a great ability to attach microbial cells, providing also a source of cellulose for these ones [16]. Biofilm adhesion is a crucial step for biofilm formation [17]. It was reported that the biofilm attachment during its functional life time is a key factor for its performance, since it assures the long-term stability of the system [18].

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Among theoretical models of adhesion prediction, XDLVO theory remains a reliable tool for the evaluation of the biofilm initial attachment capacity, taking into account Van der Waals and acid-basic interactions. Numerous reports have proved its ability to describe microbial adhesion to different solid surfaces [17,19].

In the biofilm growth mode, microbial cells are immobilized in a heterogeneous layer of extracellular polymeric substances (EPS). It refers to polysaccharides, proteins, nucleic acids and other biopolymers located outside the cell [20]. The natural ability of cells to produce these substances is a major element in biofilm formation [21].

Some works have reported the major role of EPS in the sorption of inorganic substances. In fact, the application of the microbial EPS in heavy metals biosorption and immobilization was extensively reported [21–23]. The production of these biopolymers is mainly influenced by the substrate type, nutrient content and external conditions [23].

Experimental design is a useful tool allowing the optimization of a process conditions while studying the possible interactions between numerous influencing variables at limited number of experimental trials [24]. It is a methodological strategy, widely performed in experiments influenced by several variables where it is necessary to evaluate the combined effect of the factors on a response [25,26]. The response surface methodology (RSM) is routinely used for the efficiency optimization of several industrial processes in order to study the combined influence of independent variables [4].

Thus, this study aims: (i) the evaluation of the theoretical adhesion ability of the *Wickerhamomyces anomalus* yeast strain to wood husk surface, using XDLVO predictive theory; (ii) the optimization of chromium removal by the biofilm in batch system using central composite experimental design; (iii) the determination of breakthrough curves in open system under different initial chromium concentrations and (iv) the evaluation of extracellular polymeric substances production under different glucose concentrations.

2. Materials and methods

2.1. Yeast strain and growing conditions

A Chromium resistant and removing yeast *Wickerhamomyces anomalus* previously isolated from wastewater samples, contaminated with chemical industrial wastes including those from tanning processing in Fez, Morocco, was used in this study. It was seeded on yeast medium agar (1% peptone, 1% yeast extract, 2% glucose and 1.5% agar) plates and incubated for 48 h at optimal temperature of 30 °C. The yeast strain showed a Cr(VI) adsorption capacity of 28.14 mg. Cr(VI) g⁻¹ and a removal percentage of 100% at the optimal pH value of 4 [7].

2.2. Preparation of the support

Wood husk was obtained from a local wood industry. It was extensively washed under tap water in order to remove any particulate and sprayed with distilled water. This material was crushed, dried at 60 °C and sieved through a 1–5 mm size before further usage.

2.3. Contact angle measurements CAM

Preparation of yeast strains suspension for cell surface CAM was carried out following the protocol of [27] with slight modifications as described by [28]. Microbial lawns suitable for CAM were prepared as described by [16] and realized in triplicate with separately cultured microbes.

2.3.1. Hydrophobicity

According to Vogler's approach [29], the value of water contact angle θ_w permits to evaluate the hydrophobicity of a surface qualitatively. A θ_w value higher than 65° indicates a hydrophobic surface, conversely a θ_w value lower than 65° allows classifying a surface as

being hydrophilic.

By contrast, Van Oss approach seems to be more precise. It permits the determination of the absolute degree of hydrophobicity of a surface which is calculated using Eq. (1).

In this approach, the degree of hydrophobicity of a given material (i) is expressed as the free energy of interaction between two identical surfaces immersed in water (ΔG_{iwi}). If the interaction between the two surfaces is stronger than the interaction between each surface with water ($\Delta G_{iwi} < 0$), the surface is considered hydrophobic and conversely ($\Delta G_{iwi} > 0$), the surface is considered hydrophilic.

$$\Delta G_{iwi} = -2\gamma_{iw} = -2[(\gamma_i^{LW})^{1/2} - (\gamma_w^{LW})^{1/2})^2 + 2((\gamma_i^+ \gamma_i^-)^{1/2} + (\gamma_w^+ \gamma_w^-)^{1/2} - (\gamma_i^+ \gamma_w^+)^{1/2} - (\gamma_w^+ \gamma_i^-)^{1/2})] \quad (1)$$

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

2.3.2. Surface tension components

Once the contact angles were measured, the Lifshitz-van der Waals (γ^{LW}) and acid-base (γ^{AB}) surface tension components were obtained using the three equation system obtained from the application of the Young-Dupré equation to each probe liquid [30], by using three different liquids with known surface parameters values γ_1^{LW} , γ_1^+ , and γ_1^- , for example water, formamide and diiodomethane (Table 1). 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.

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

In this equation, θ is the measured contact angle and the subscripts (S) and (L) report to solid 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.

2.3.3. Calculation of free energy adhesion of the yeast strain to wood surface by XDLVO theory

In the XDLVO approach the total interaction energy between microbial cells (m) and substratum (s) through water (w) is described as a balance between attractive Lifshitz-van der Waals forces, repulsive or attractive electrostatic forces and acid-base interaction forces, being written as:

$$\Delta G^{XDLVO}(d) = \Delta G^{LW}(d) + \Delta G^{EL}(d) + \Delta G^{AB}(d) \quad (3)$$

In this equation:

d: the separation distance between a cell and a substratum

$$\Delta G^{LW} = ((\gamma_M^{LW})^{1/2} - (\gamma_S^{LW})^{1/2})^2 - ((\gamma_M^{LW})^{1/2} - (\gamma_L^{LW})^{1/2})^2 - ((\gamma_S^{LW})^{1/2} - (\gamma_L^{LW})^{1/2})^2 \quad (4a)$$

and

Table 1

Independent variables and levels considered for the removal of Cr (VI) and tot Cr using central composite design (CCD).

Variable	Symbol	Coded variable levels				
		-α	-1	0	1	+α
pH	x1	2	2	5	8	8
Initial Cr(VI) concentration (mg/L)	x2	10	10	55.5	100	100
Biomass concentration (g/L)	x3	1	1	3	5	5
Support dose (g/L)	x4	0.5	0.5	5.25	10	10

$$\Delta G^{AB} = 2[(\gamma_L^+)^{1/2}((\gamma_M^-)^{1/2} + (\gamma_S^-)^{1/2} - (\gamma_L^-)^{1/2}) + (\gamma_L^-)^{1/2}((\gamma_M^+)^{1/2} + (\gamma_S^+)^{1/2} - (\gamma_L^+)^{1/2}) - (\gamma_L^+\gamma_S^+)^{1/2} - (\gamma_L^+\gamma_S^-)^{1/2}] \quad (4b)$$

The usage of a suspension liquid with high ionic strength (KNO₃ 0.1 M) allows the negligence of electrostatic interaction free energy ΔG^{EL} as done before [31,32].

2.4. Batch system assays

2.4.1. Biosorption experiments

All laboratory glassware was washed twice with deionized water after being soaked in nitric acid (60%, v/v) bath overnight.

Yeast strain growth was obtained at 30 °C using YPG on a rotary shaker at 130 rpm. After 24 h of incubation, yeast cells were harvested by centrifugation at 7000g for 10 min.

Batch experiments were performed in modified YPG medium (0.2% peptone, 0.2% yeast extract, 2% glucose) prepared with sterile distilled water as proposed by [7]. The medium contained different initial concentrations (10, 55, 100 mg/L) of Cr(VI) as K₂Cr₂O₇ and final solution pH was adjusted to 2, 5 or 8 by adding either 0.1 M HCl or 0.1 M NaOH. An uninoculated medium prepared strictly using the same procedures served as a control to determine the extent of abiotic Cr(VI) reduction. Yeast biomass was supported on different wood husk amounts (0.5, 5.25, 10 g/L) and some other assays were also carried out with different concentrations of biomass (1, 3, 5 g/L). Experiments were conducted in 250 Erlenmeyer flasks with continuous stirring (130 rpm) in a shaker incubator at 30 °C [33]. Aliquots of 1 ml were taken periodically, centrifuged and residual Cr(VI) concentration was determined by colorimetry using the diphenylcarbazide method [34]. The biosorption potential percentage was calculated as:

$$\text{Biosorption potential\%} = \frac{(\text{Initial concentration of Cr} - \text{Observed concentration of Cr}) \times 100}{\text{Initial concentration of Cr}} \quad (5)$$

The total Cr in samples was quantified by inductive coupled plasma, ICP-OES (Optima 8000, Perkin-Elmer), at wavelength 283.5 nm using argon gas as the fuel.

2.4.2. Central composite design (CCD)

A 4 factor-5-level design, in which five coded levels were assigned to each factor (Table 1) was selected. The arrangement of central composite design is shown in Table 2 [26].

Statistical analysis and model fitting: The experimental data (Table 2) were subjected to a regression analysis to be fitted by a second-order polynomial model Eq. (5) describing the relationship between the studied factors and the responses.

$$Y = b_0' + \sum_{i=1}^n b_i X_i + \sum_{i=1}^n b_{ii} X_i^2 + \sum_{i=1}^n \sum_{j>1}^n b_{ij} X_i X_j \quad (6)$$

In here, Y is the predicted response, b_0' is the constant coefficient, b_i is the linear coefficient, b_{ij} is the interaction coefficient, b_{ii} coded values are the quadratic coefficient, and X are the process factors.

The analysis of variance (ANOVA) was also done to determine the significance of each factor in the fitting model and to determine the goodness of the fit. For this purpose, the software SAS.JMP v 8.0.1 (trial version) has been used. Graphical representation of the fitting polynomial has been given in the form of 3D plots.

2.5. Open system assays

The open-system assays were performed in plexiglas columns (height: 33 cm, internal ϕ 3.5 cm) filled with 96 cm³ of wood husk supporting a *W. anomalous* biofilm, while a control assay column was filled only with wood husk.

Table 2

Arrangement of the central composite design for independent variables (pH (x1), initial chromium concentration (x2), biomass concentration (x3), support dose (x4)).

Experiment no.	Variable values			
	x1	x2	x3	x4
1	2	10	5	0,5
2	2	10	1	10
3	2	10	5	10
4	2	100	5	10
5	2	10	1	0,5
6	2	100	1	0,5
7	2	55	3	5,25
8	2	100	1	10
9	2	100	5	0,5
10	8	10	5	0,5
11	8	100	1	0,5
12	8	10	5	10
13	8	10	1	0,5
14	8	55	3	5,25
15	8	100	5	10
16	8	100	1	10
17	8	100	5	0,5
18	8	10	1	10
19	5	55	3	10
20	5	55	3	0,5
21	5	55	5	5,25
22	5	100	3	5,25
23	5	10	3	5,25
24	5	55	1	5,25
25 (cp)	5	55	3	5,25
26 (cp)	5	55	3	5,25

The biofilm attachment on wood husk was carried out following the protocol of [12]. Afterwards, the metal aqueous solutions with initial concentrations of 10, 25, 50 and 100 mg/L were pumped through the columns with a flow rate of 5 mL/min and pH between 3.72 and 5.48 (optimum conditions according to experimental design). The total chromium and Cr(VI) concentrations in the outflow were periodically evaluated, as described above.

2.6. Quantification of polysaccharides

In order to evaluate the effect of glucose on extracellular polymeric substances production and Cr(VI) removal potential by yeast biofilm supported on wood husk, YPG-modified medium containing different glucose concentrations (0, 5, 10, 15, 20 g/L) were pumped during the open system assays. The quantification of polysaccharides was realized following the protocol of [34]. The Cr(VI) removal percentage was measured as previously described, at an initial chromium concentration of 25 mg/L.

3. Results and discussion

3.1. Physicochemical surface characteristics of wood husk and *W. anomalous* cells

3.1.1. Wood husk surface characteristics

Wood is a porous material, favorable for capillary penetration and microbial attachment. This material is characterized by a wide heterogeneity of its surface. Its roughness makes the liquid spreading more pronounced parallel than perpendicular to the wood grains orientation [35]. Wood is generally composed of cellulose (37–51%), lignin (20–30%), hemicellulose (20–30%) and extractives (1–5.5%) [36]. The extractives are not evenly distributed within the cell walls. Although their amount is very low in comparison to the other components of wood, they significantly affect the wettability of the material [36,37].

Wood husk is an available material, chosen to be the yeast

Table 3

Contact angle values using water (θ_w), formamide (θ_F) and diiodomethane (θ_D), Lifshitz-vander Waals (γ^{LW}), electron-donor (γ^-) and electron-acceptor (γ^+) parameters, surface energies (ΔG_{iwi}) of wood husk and *W. anomalous* and the total energy of adhesion between yeast cells and the support (ΔG^{XDLVO}).

Material	Contact angles (°)			Surface tension: components and parameters (mJ/m ²)			Surface energies ΔG_{iwi} (mJ/m ²)	Interaction free energy ΔG^{XDLVO} (mJ/m ²)
	θ_w	θ_F	θ_D	γ^{LW}	γ^+	γ^-		
Wood husk	81.46 ± 0.32	61.73 ± 1.70	51.13 ± 1.00	33.57 ± 0.18	0.29 ± 0.18	6.28 ± 1.56	-49.27 ± 386	-7.13
<i>W. anomalous</i>	23.8 ± 2.72	28.6 ± 1.70	30.9 ± 0.32	43.74 ± 0.28	0.13 ± 0.08	54.58 ± 1.19	36.27 ± 2.48	

attachment support. Wood may be a good support for different biofilms especially for microbes able to breakdown wood macropolymers and to use lignin-related products as nutrients [18]. It is a porous material offering a high surface area for microbial attachment. Furthermore, wood husk contains a relevant amount of glucose within cellulose. Thus, it contains easily substituted hydroxyl groups providing a weakly basic and acidic ion exchange condition, favorable to microbial attachment and growth [14,38]. The small particle size of the used wood husk (1–5 mm) provides high specific surface area for microbial attachment.

Wood surface characterization was the topic of numerous research works [16,36,39]. Its surface free energy presents a wide range of variation in literature due to the chemical heterogeneity and biodiversity of wood species [36].

The used wood husk exhibits a negative surface free energy value of -49.27 ± 386 mJ/m², a γ^- value of 6.28 ± 1.56 mJ/m² and γ^+ value of 0.29 ± 0.18 mJ/m² (Table 3). These findings are in line with other data found in previous works [16], where the wood surface exhibited an hydrophobic character, a strong electron-donor component and a weak electron-acceptor component. It was reported that the electron-acceptor component of wood results from the acidic hydrogen atoms on its macromolecules, mainly carboxylic acids and phenolic functions of hemicellulose and lignin, while its electron-donor component (γ^-) is related to the oxygen/carbon ratio of lignin and polysaccharides present in wood [39].

3.1.2. *W. anomalous* cells surface characteristics

W. anomalous cells exhibited hydrophilic character with a θ_w of $23.8^\circ \pm 2.72$ and a ΔG_{iwi} of 36.27 ± 2.48 mJ/m² (Table 3). These findings agree with previous results reporting the hydrophilic character of yeast cells [40,41]. Yeast surface hydrophobicity has been shown to be related to surface protein concentration [42–44].

W. anomalous cells appear to behave predominantly as electron donors/Lewis bases with high values of γ^- , 54.58 ± 1.19 mJ/m². A previous study showed that microbial cell surfaces are mainly electron donating, while electron-accepting cell surfaces are rarely found [45]. This character may be attributed to the presence of phosphate groups in their wall [44], as suggested before by [46]. Previous FTIR analyses of *W. anomalous* confirm the presence of phosphate groups on its cell surface [33].

3.2. Free energy adhesion of the yeast strains to wood species by XDLVO theory

The use of biofilm reactors for wastewater treatment is becoming a common application of environmental biotechnology. However, studies dealing with the stability of these systems as a function of the support-cells compatibility are still very few.

The attachment of microbial cells to a solid surface is the first and most important stage of the biofilm formation [47,48]. A good control of the interactions between microbial cells and their support within the biofilm system is of extreme importance to assure its long-term stability [18].

On this basis, the adhesion ability between the yeast strain and the support was first considered theoretically and experimentally evaluated. Many efforts were oriented to understand the biofilm formation on solid surfaces, either to prevent harmful biofilms formation or to control useful biofilms in biotechnological applications such as wastewater treatment.

Among theoretical models of adhesion prediction, XDLVO theory remains a reliable tool for the evaluation of the biofilm initial attachment, taking into account Van der Waals and acid-basic interactions. Numerous reports have proved its ability to describe microbial adhesion to different solid surfaces [17,19].

The XDLVO predictions of the free energy of adhesion between yeast strain and wood husk is presented in Table 3. According to this theory, positive values of the ΔG^{XDLVO} indicate unfavorable adhesion or repulsion. On the opposite, attraction between two interacting surfaces occurs when the total energy values ΔG^{XDLVO} are negative. In the present case, a negative total energy value ΔG^{XDLVO} of -7.13 mJ/m² was obtained, showing a favorable adhesion within the biofilm system. environmental scanning electronic microscopy images on Fig. 1, show the cellulose porous structure offering a high surface area for microbial adhesion and confirm the favorable attachment of yeast cells to wood husk.

3.3. Optimisation of chromium biosorption in batch system

The influence of four factors (medium pH, initial Cr concentration, biomass concentration and support dose) on measurable responses (Cr (VI) (y_1) and total Cr (y_2) removal percentage at equilibrium) was studied. For CDD assays, YPG-modified medium was used as previously suggested by [7]. Indeed, the use of a rich medium may cause an interference between the components which reduces the number of available sites for metals uptake.

The study intervals were set from the preliminary experiments. Table 2 shows the arrangement of central composite design experiments allowing the development of the appropriate empirical equations. Additional experiments were carried out at the centre point to estimate the overall error.

Using SAS JMP v 8.0.1 program, experimental data were fitted by a second-order model relating Cr(VI) removal percentage at equilibrium (y_1) and total Cr removal percentage at equilibrium (y_2) to the factors.

The models were described according to Eq. (6) as follows:

$$Y_1 = 85.28 - 19.92X_1 - 12.16X_2 + 0.69X_3 + 7.80X_4 - 7.24X_1X_2 - 0.18X_1X_3 - 0.006X_2X_3 + 0.68X_1X_4 + 3.49X_2X_4 + 1.91X_3X_4 - 14.85X_1^2 + 4.07X_2^2 + 7.48X_3^2 - 8.28X_4^2 \quad (7)$$

$$Y_2 = 65.61 - 1.03X_1 - 2.13X_2 + 1.54X_3 + 11.13X_4 - 7.89X_1X_2 + 1.33X_1X_3 - 0.43X_2X_3 + 7.28X_1X_4 + 1.06X_2X_4 + 1.26X_3X_4 - 38.79X_1^2 + 2.58X_2^2 + 5.88X_3^2 - 9.93X_4^2 \quad (8)$$

The results were then analyzed by ANOVA to assess the goodness of the fit. Statistical parameters obtained from the ANOVA for the reduced

Fig. 1. SEM of *W. anomalus* on wood husk (3000×). (a) Control yeast cells; (b) Metal loaded cells at initial concentration 25 mg/L.

models of chromium removal are given in Tables 4 and 5. Analysis of variance (ANOVA) showed a high coefficient of determination (R^2) value of 0.93 and 0.91 for Cr (VI) and total Cr respectively, ensuring a satisfactory fitting of the second-order regression model to the experimental data.

Table 4

Results of statistical central composite design analysis for Cr(VI) removal and tot Cr removal.

	y1		y2	
	Coefficientestimate	P-value	Coefficientestimate	P-value
Constant	85.278714	< 0.0001***	65.611857	< 0.0001***
x1	−19.91889	< 0.0001***	1.0288889	0.7067
x2	−12.15833	< 0.0001***	−2.132778	0.4403
x3	0.6922222	0.7706	1.5411111	0.5745
x4	7.8033333	0.0063**	11.126667	0.0015***
x1*x2	−7.240625	0.0133**	−7.894375	0.0175*
x1* x3	−0.183125	0.9419	1.328125	0.6475
x2* x3	−0.006875	0.9978	−0.430625	0.8816
x1* x4	0.679375	0.7872	7.284375	0.0257*
x2* x4	3.485625	0.1836	1.055625	0.7158
x3* x4	1.908125	0.4536	1.255625	0.6653
x1* x1	−14.84995	0.0341*	−38.79748	0.0002***
x2* x2	4.0750476	0.5205	−2.582476	0.7215
x3* x3	7.4800476	0.2486	5.8825238	0.4226
x4* x4	−8.279952	0.2046	−9.927476	0.1874

Level of statistical significance: *P < 0.05; **P < 0.01; ***P < 0.001.

Table 5

Analysis of variance for the reduced models of Cr(VI) and tot Cr removal.

Variable	% Cr (VI) removal	% tot Cr removal
R^2	0.925562	0.9137
R^2 adj	0.830824	0.8039
df	14	14
F_{exp}	9.7696	8.3245
Prob > F	0.0003***	0.0006***
Std. Dev.	9.825378	11.30101

* F_{exp} defined as the ratio of the mean square of the model to mean square of the error.

3.3.1. Effect of pH

Many works have confirmed that the biosorption was pH dependant [7,49]. In this study, acidic medium pH values were optimal for chromium removal by yeast biofilm. A pH of 3.72 was optimal for Cr(VI) removal and a pH of 5.48 was optimal for total Cr removal. It is known that in general low pH values enhance Cr(VI) biosorption from aqueous solutions [7,12]. Indeed, at high pH values the overall surface charge of the biomass becomes negative. This negative charge would generate repulsive forces towards chromate ions, appearing as anions (CrO_4^{2-} or $\text{Cr}_2\text{O}_7^{2-}$) in aqueous surrounding environment, while acidic solutions offer a positive overall charge to the microbial surface, promoting Cr (VI) anionic species binding to positively charged functional groups [50].

The study of interactions by the central composite design showed that the interactions between medium pH and Cr(VI) initial concentration displayed the most prominent effect on the responses. The response surface plots of the effect of this interaction on both responses are shown in the Fig. 2(a, b). The pH of the medium is absolutely a pertinent parameter in biosorption process, influencing the overall surface charge of the biomass by affecting the ionization states of functional groups on the biosorbent surface [51]. Moreover, pH was reported to strongly affect the chemistry of metallic ions in solution and their biosorption availability [52].

Depending on pH, chromium may exhibit different ionic species in aqueous solutions. Above pH 8.0, CrO_4^{2-} is the only chromium species present in solution. At pH range 2–6 HCrO_4^- and $\text{Cr}_2\text{O}_7^{2-}$ ions are in equilibrium. At pH lower than 2.0 $\text{Cr}_3\text{O}_{10}^{2-}$ and $\text{Cr}_4\text{O}_{13}^{2-}$ species are predominant [53].

3.3.2. Effect of metal initial concentration

The removal of chromium experiments were performed with Cr(VI) concentrations ranging from 10 to 100 mg/L. Results showed that both

Fig. 2. Second-order response surface plot in the chromium removal (Cr(VI) (y1) and total Cr (y2)) by *W. anomalus* biofilm on wood husk. Dependence of the responses on: (a) the medium pH (x1), (b) initial chromium concentration (x2) and (c) support dose (x4).

responses were strongly affected by initial chromium concentration. Thus, complete chromium removal percentage was obtained with the concentration of 10 mg/L and the removal efficiency decreased with the increase of the initial chromium concentration. These observations are in line with previous results showing a better efficiency at low initial metal concentrations [54]. This may be explained by surface saturation that occurs with the increase of initial metal concentrations. At low

concentrations, available adsorption sites for chromium fixation are quickly occupied, while at higher concentrations, the slow metal ions diffusion into the biomass surface by intraparticle diffusion decreases chromium removal efficiency [55].

3.3.3. Effect of biomass concentration

Results of the experimental design showed that, out of the four tested factors, biomass concentration displayed the minimal effect on the process efficiency, at the opposite to planctonic form of the microbial cells assays where the biomass concentration affects strongly chromium removal efficiency [56]. Similar results were previously reported by [57] who stated that there is no evidence of correlation between wastewater treatment rate and dry biomass. In fact, biofilm systems are known to be complex and different parameters may influence their performance such as extracellular polymeric substances production, quorum sensing or metabolic pathways [58].

3.3.4. Effect of support dose

The support dose is one of the parameters that strongly affect the biosorption capacity within the biofilm system. The influence of the support dose on the biosorption capacity was studied for a concentration between 0.5 and 10 g/L (Fig. 2c). The increase in the chromium removal percentage with the increase in the support dosage may be due to the increase of microbial attachment available area. It may also be due to the increase of the amount of glucose in the medium contained in this substratum within its cellulolytic composition, as a direct relation between glucose and chromium reduction was reported [38]:

3.4. Open system assays

In open systems filled with *W. anomalus* biofilm-coated wood husk, the results showed Cr(VI) retention percentages of 7.5, 19.9, 28.2 and 57.82% and total Cr retention percentages of 7.1, 15.7, 19.43 and 40.02% for the initial concentrations of 10, 25, 50 and 100 mg/L, respectively (Fig. 3a). Fig. 3b illustrates the resulting breakthrough curves of Cr(VI) removal at different inlet concentrations. The concentration of chromium in the effluent during the first few minutes approaches zero, but starts to increase rapidly with the exposition of the biomass to metal ions, until an equilibrium between retained concentration of metal and the concentration in the effluent is established after 18 h. The retention capacity was strongly related to metal concentration, a higher percentage (57.82% and 40.03% for Cr(VI) and total Cr, respectively) of retained metal was observed with the highest chromium initial concentration (100 mg/L). This can be explained by the difference between the pollutant concentration in the solution and the pollutant concentration in the biosorbent, which is the main driving force conditioning the uptake process [59,60].

The shape of biosorption curves is due to the formation of a mass transfer zone in the column. The metal uptake process by microbial cells is known to occur in two distinct phases, the first one being a passive transport period mainly related to the metal ions adsorption or ion exchange on microbial surfaces. This phase corresponds to a rapid increase in the uptake during the first hours of cell-metal contact, followed by an active uptake which is a slower process due to the metabolism of living cells [50]. When chromium solution contacts the fresh layer of the biomass, metal ions are sorbed onto the biomass fresh layer until the sorbed amount is in equilibrium with the influent concentration. At this moment, the biomass is loaded to its full capacity and that portion of the biomass becomes exhausted. Sorption is occurring above this line progressing in the direction of the flow and metal ions in the solution are actively transferred onto the biomass. The mass transfer zone will move down through the column until it reaches the effluent port and then the heavy metal concentration in the effluent starts to

Fig. 3. (a) Breakthrough curves of Cr(VI) biosorption by *W. anomalous* biofilm supported on wood husk at different initial concentrations. (b) Uptake percentages obtained for column studies.

increase. In this process, the metallic solutions pass through the bed of active biomass, which would act as a series of batch contactors and consequently, the biomass would be loaded up to its maximum capacity [61].

The Cr(VI) may be reduced to the trivalent state by the biomass intervention following two different mechanisms. The first one considers that Cr(VI) is directly reduced by contact with electron-donor groups in microbial surface, particularly groups with lower reduction potential values than chromium (+1.3 V). The second mechanism considers the binding of chromium to the positively charged groups on the surface of biomass, where the adjacent electron-donor groups interfere to reduce Cr(VI) to Cr(III) which will be released in aqueous solutions due to electronic repulsion by the positive groups of the microbial surface. Cr(III) may also be complexed by microbial cells with adjacent Cr-binding groups [56].

3.5. Extracellular polymeric substances production

Extracellular polymeric substances (EPS) production is a common feature of many microbial cells. Research on microbial these substances has been mainly focused on bacterial species, whereas the EPS from yeast cells are especially more attractive due to their easier separation comparing to bacterial produced EPS [62].

Microbial EPS biosynthesis is likely stimulated in metallic solutions [63]. These saccharides offer a protective barrier against environmental stress conditions and hence increase the viability of microbial cells [64,65]. EPS matrix contains mainly sugars and mannuronic- and guluronic-acid complexes. It comprises also heteropolysaccharides,

Fig. 4. Effect of glucose concentration on chromium removal and extracellular polymeric substances production by *W. anomalous* biofilm on wood husk.

proteins and nucleic acids [63,66]. The presence of carboxyl, hydroxyl and phosphoryl functional groups is responsible for the negative charge of EPS. These groups are known to be involved in the metal uptake process by coordination, allowing the stability of ion polymer complexes [67].

EPS production has been the topic of numerous research works. Among the studied parameters, the culture medium composition can dramatically influence their production [68], especially glucose concentration in the medium proved to be of great impact [21].

Results in Fig. 4 show that the glucose-poor medium allowed a maximum EPS production (826 mg/g) but the lowest chromium removal (20.64%). The chromium uptake increased with the increase of glucose concentration in the medium. A maximum chromium removal was obtained in the glucose rich medium (20 g/L) with an uptake value of 64.8%. Inversely, the EPS production decreased with the increase of the glucose concentration in the medium.

Our results show that rich medium was favorable for microbial growth and chromium removal but unfavored EPS production. Obtained data also show that the maximum chromium uptake was attained with the minimum EPS production. Hence, EPS production seems to have no effect on chromium uptake. In fact [69], showed previously that EPS production has no significant effect on chromium retention efficiency, as no relevant difference on the concentration of main binding sites such as carboxyl and phosphate was noted. [63] Reported also that the EPS layer presented a negative effect on metal uptake. Indeed, copper adsorption was higher by rinsed bacteria comparing to those with intact EPS. The negative effect of EPS layer on metal uptake may be due to aggregation effect that EPS induce within microbial cells, reducing the surface interactive area. In addition, the release of large quantities of dissolved organic carbon from the EPS layer may cause a metal complexation [69].

4. Conclusion

The biofilm of *W. anomalous* supported on wood husk demonstrated its ability to remove chromium from dilute solutions and its potential applicability in wastewater remediation. The biofilm formation ability was first predicted by XDLVO theory. The negative value of the free energy of adhesion is indicative of a good attachment of the yeast strain onto the wood surface. The theoretical prediction was confirmed by visualization of the experimental adhesion using environmental electronic microscopy.

A biosystem based of *W. anomalous* biofilm supported on wood husk, in batch system showed a great chromium removal potential. A scale-up into an open system was then evaluated and optimized. Both systems

have proved the efficiency of the treatment and its biotechnological applicability for the remediation of chromium in aqueous solutions.

According to central composite design, the optimal conditions of Cr (VI) biosorption are 10 mg/L of Cr(VI) initial metal concentration, pH 3.72, 5 g/L of biomass and 6.95 g/L of support. On the other hand, optimal conditions for total Cr removal are 16.91 mg/L of Cr(VI) initial metal concentration, pH 5.48, 5 g/L of biomass and 8.20 g/L of support. The quantification of extracellular polymeric substances production related to the glucose concentration in the medium, showed that a glucose exempt medium allowed the maximal extracellular polymeric substances production but led to the minimal chromium removal efficiency.

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