A Wicherhamomyces 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 efficiency, while 20 g/L emptied of glucose allowed maximal EPS production and minimal chromium removal efficiency. 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 extent 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.

**Keywords:** Chromium, Biofilm, Biosorption, Yeast, Wood husk
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-base 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 condition 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 industrial conditions 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 θw permits to evaluate the hydrophobicity of a surface qualitatively. A θw value higher than 65° indicates a hydrophobic surface, conversely a θw 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).

\[
\Delta G_{\text{W}} = -2\gamma_w = -2\left[(\gamma_{\text{LW}}^{\text{L}})^{1/2} - (\gamma_{\text{w}}^{\text{L}})^{1/2}\right] + 2\left[(\gamma_{\text{L}}^{\text{L}})^{1/2} + (\gamma_{\text{w}}^{\text{L}})^{1/2} - (\gamma_{\text{L}}^{\text{L}})^{1/2} - (\gamma_{\text{w}}^{\text{L}})^{1/2}\right] (1)
\]

\(\gamma_{\text{w}}^{\text{L}}\) is the Lifshitz-van der Waals component, \(\gamma_{\text{L}}^{\text{L}}\) is the Lifshitz-van der Waals component of water, \(\gamma_w^+\) is the electron acceptor of a given material (i), \(\gamma_w^-\) is the electron donor of a given material (i), \(\gamma_w^{-}\) is the electron acceptor of water and \(\gamma_w^{+}\) is the electron donor of water.

2.3.2. Surface tension components

Once the contact angles were measured, the Lifshitz-van der Waals (\(\gamma_{\text{L}}^{\text{L}}\)) and acid-base (\(\gamma_{\text{AB}}^{\text{L}}\)) 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 \(\gamma_{\text{L}}^{\text{L}}\), \(\gamma_w^+\), and \(\gamma_w^-\), for example water, formamide and diiodomethane (Table 1). The unknown surface tension components of a solid surface \(\gamma_{\text{s}}^{\text{AB}}\), \(\gamma_{\text{s}}^+\), and \(\gamma_{\text{s}}^-\) or microbial surface \(\gamma_{\text{m}}^{\text{AB}}\), \(\gamma_{\text{m}}^+\), and \(\gamma_{\text{m}}^-\) can be estimated.

\[
\chi (\cos \theta + 1) = 2\left[(\gamma_{\text{L}}^{\text{L}})^{1/2} + (\gamma_{\text{w}}^{\text{L}})^{1/2} + (\gamma_{\text{m}}^{AB})^{1/2}\right] (2)
\]

In this equation, \(\theta\) is the measured contact angle and the subscripts (S) and (L) report to solid and liquid phases, respectively. \(\gamma_{\text{L}}^{\text{L}}\) is the Lifshitz-van der Waals component of the surface free energy, \(\gamma_w^+\) and \(\gamma_w^-\) are the electron acceptor and electron donor parameters, respectively, of the Lewis acid-base component (\(\gamma_{\text{AB}}^{\text{L}}\)). The surface free energy is expressed as: \(\gamma_{\text{s}} = \gamma_{\text{s}}^{\text{AB}} + \gamma_{\text{s}}^+\) and \(\gamma_{\text{m}} = 2(\gamma_{\text{m}}^{\text{AB}})^{1/2}\) 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:

\[
\Delta G_{\text{XDLVO}} (d) = \Delta G_{\text{LW}} (d) + \Delta G_{\text{EL}} (d) + \Delta G_{\text{AB}} (d) (3)
\]

In this equation:

\(d\) the separation distance between a cell and a substratum

\[
\Delta G_{\text{LW}} = \left((\gamma_{\text{L}}^{\text{L}})^{1/2} - (\gamma_{\text{w}}^{\text{L}})^{1/2}\right)^2 - \left((\gamma_{\text{m}}^{\text{AB}})^{1/2} - (\gamma_{\text{w}}^{\text{L}})^{1/2}\right)^2 (4a)
\]

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 |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| 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 1 5 5      |
| Support dose (g/L)                   | x4              | 0.5 0.5 5.25 10 10 |
\[
\Delta G_{\text{BL}} = 2[(\gamma_1^T)^{1/2}(\gamma_{\text{bl}}^T)^{1/2} + (\gamma_2^T)^{1/2} - (\gamma_{\text{bl}}^T)^{1/2}]
+ (\gamma_1^T)^{1/2}((\gamma_2^T)^{1/2} + (\gamma_{\text{bl}}^T)^{1/2} - (\gamma_{\text{bl}}^T)^{1/2} - (\gamma_1^T)^{1/2})^{1/2} - (\gamma_1^T)^{1/2}
\] (4b)

The usage of a suspension liquid with high ionic strength (KNO₃ 0.1 M) allows the negligence of electrostatic interaction free energy \(\Delta G_{\text{BL}}\) 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 7000 g 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 procedure 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:

\[
\text{Biosorption potential\%} = \left( \frac{\text{Initial concentration of Cr - Observed concentration of Cr}}{\text{Initial concentration of Cr}} \right) \times 100
\] (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].

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
Experiment no. & Variable values & & & \\
\hline
\hline
x1 & x2 & x3 & x4 & \\
\hline
1 & 2 & 10 & 5 & 0.5 \\
2 & 2 & 10 & 1 & 10 \\
3 & 2 & 10 & 5 & 10 \\
4 & 2 & 100 & 5 & 10 \\
5 & 2 & 100 & 1 & 0.5 \\
6 & 2 & 100 & 5 & 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 & 10 & 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 & 1 & 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 \\
\hline
\end{tabular}
\caption{Arrangement of the central composite design for independent variables (pH (x1), initial chromium concentration (x2), biomass concentration (x3), support dose (x4)).}
\end{table}

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%) [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...
attachment support. Wood may be a good support for different biofilms especially for microbes able to breakdown wood macromolecules 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 adhesion 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 $-49.27 \pm 38.6$ mJ/m$^2$, a $\gamma$ value of 6.28 $\pm$ 1.56 mJ/m$^2$ and a $\gamma^+$ value of 0.29 $\pm$ 0.18 mJ/m$^2$ (Table 3). These findings are in line with other data found in previous works [16], where the wood surface exhibited a hydrophilic 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 ($\gamma$) is related to the oxygen/carbon ratio of lignin and polysaccharides present in wood [39].

### 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, YPC-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:

$$Y_1 = 85.28 - 19.92X_1 - 12.16X_2 + 0.69X_1 + 7.80X_1 - 7.24X_1 - 0.18$$

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:

$$Y_1 = 85.28 - 19.92X_1 - 12.16X_2 + 0.69X_1 + 7.80X_1 - 7.24X_1 - 0.18$$

$$X_2X_1 - 0.006X_1X_2 + 0.68X_1 + 3.49X_1 + 1.91X_1X_4 - 14.85X_1^2 + 4.07X_1^2 + 7.48X_1^2 - 8.28X_1^2$$

(7)

$$Y_2 = 65.61 - 1.03X_1 - 2.13X_2 + 1.54X_1 + 11.13X_4 - 7.89X_1X_2 + 1.33$$

$$X_2X_1 - 0.43X_2X_4 + 7.28X_1X_4 + 1.06X_2X_4 + 1.26X_2X_4 - 38.79X_1^2 + 2.58X_1^2 + 5.88X_1^2 - 9.93X_1^2$$

(8)

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

### 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 Cr$_2$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 Cr$_2$O$_7^{2-}$ ions are in equilibrium. At pH lower than 2.0 Cr$_3$O$_10^{2-}$ and Cr$_4$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...
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 cellulosic composition, as a direct relation between glucose and chromium reduction was reported [38]:

\[
\text{C}_6\text{H}_5\text{O}_7\text{Cr}^{3+} + 8\text{HCO}_3^- + 34\text{H}^+ \rightarrow 8\text{Cr}^{3+} + 6\text{HCO}_3^- + 20\text{H}_2\text{O}
\]

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
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].

Among the studied parameters, the culture medium composition can 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. anomalus 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. anomalus 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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