Desalination and Water Treatment
www.deswater.com
doi: 10.5004/dwt.2023.29611

306 (2023) 39–50
September

Wickerhamomyces anomalus biofilm supported on wood husk and zeolite 13X for the treatment of chromium in aqueous solutions and a tannery effluent

Meryem Asri[a,b,*], Redouane Ouafi[c], Soumya Elabed[c], Wifak Bahafid[a], Saad Ibnsouda Koraichi[a], Teresa Tavares[d], Naïma El Ghachtouli[a]

[a]*Laboratory of Microbial Biotechnology and Bioactive Molecules (LM2BM), Faculty of Sciences and Techniques of Fez, Sidi Mohammed Ben Abdellah University, Morocco, Tel.: (+212) 669 111 680; email: meryem.asri@usmba.ac.ma (M. Asri) http://orcid.org/0000-0002-0886-9582, Tel.: (+212) 667 827 230; email: wifak.bahafid@gmail.com (W. Bahafid), Tel.: (+212) 664 772 800; email: saad.ibnsouda@usmba.ac.ma (S.I. Koraichi), Tel.: (+212) 655 559 261; email: naima.elghachtouli@usmba.ac.ma (N. El Ghachtouli)*
[b]*High Institute of Nursing and Technical Health (ISPITS), Ministry of Health, Fez, Morocco*
[c]*Laboratory of Electrochemistry Engineering, Modeling and Environment, Faculty of Science, Dhar El Mahraz, University Sidi Mohamed Ben Abdallah, Fez, Morocco, Tel.: (+212) 676 087 963; email: redouane.ouafi@usmba.ac.ma (R. Ouafi), Tel.: (+212) 663 450 648; email: soumya.elabed@usmba.ac.ma (S. Elabed)*
[d]*CEB-IBB – Centre of Biological Engineering, Institute for Biotechnology and Bioengineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal, Tel.: (+351) 963 001 961; email: ttavares@deb.uminho.pt (T. Tavares)*

Received 7 September 2022; Accepted 25 May 2023

ABSTRACT

Chromium is one of the most common environmental pollutants due to its numerous industrial applications. In this work, *Wickerhamomyces anomalus* biofilm attached to two different supports (wood husk and zeolite 13X) was tested to remove chromium from aqueous solutions and a tannery effluent. Fourier-transform infrared spectroscopy analysis allowed the identification of their chemical composition. The theoretical prediction of microbial adhesion showed a favorable attachment of *Wickerhamomyces anomalus* to wood husk and an unfavorable one to zeolite 13X. The predictions were confirmed by environmental scanning electron microscopy. The comparative study of chromium removal using both biofilms showed that the optimal contact time to reach maximal chromium removal was 36 h and 40 h for *W. anomalus* biofilm supported on wood husk and zeolite 13X, respectively. For both supports, the chromium removal efficiency decreased with the increase of the metal initial concentration. The lowest flow rate (5 mL·min^{-1}) and a support bed of 15 cm allowed the highest biosorption efficiency. The developed systems were therefore used for the treatment of a tannery effluent with an initial Cr(VI) and total Cr concentration of 7.23 and 42.56 mg·L^{-1}, respectively. The highest depollution performance was obtained by the consortium *W. anomalus* – *Bacillus* sp. attached to the wood husk. In a batch system, it allowed the removal of 97.52% of Cr(VI) and 98.12% of tot Cr initially contained in the tannery effluent after 48 h of treatment. In an open system, the removal of 90.34% of Cr(VI) and 95.33% of tot Cr was achieved after 50 h.

Keywords: Chromium; Biosorption; Biofilm; Consortium

* Corresponding author.

1. Introduction

Heavy metal pollution is one of the most significant global issues [1]. The disposal of these toxic compounds on water streams, as a consequence of industrial activities and technological development, is posing a serious threat to environment and human health [2]. Chromium (Cr) is one of the most toxic heavy metals of major concern. Hexavalent chromium (Cr(VI)) is known to have 100-fold more toxicity than its other forms [3]. It is introduced into the environment through tremendous industrial activities. The main sources of contamination are tanneries, electroplating and metal finishing industries [4]. Increasing awareness of chromium pollution and its long-term effects has encouraged intensive efforts for its remediation.

Conventional chromium treatment methods such as sludge separation, chemical precipitation, electrochemical process and membrane separation were often impractical and expensive. However, biosorption is considered a cost-effective and ecofriendly treatment that brings chromium within permissible limits before the effluent disposal [5,6].

Biosorption refers to several modes of non-active metal uptake by microbial biomass, mainly adsorption, ion exchange, complexation, and diffusion through cell walls and membranes. The mechanisms may differ depending on many parameters such as the microbial species and the solution chemistry [7,8]. Biosorption consists of the use of harmless and native microorganisms for the removal of hazardous compounds. Their Cr removal ability does not require the use of toxic chemical reagents or high energy consumption. This constitutes a major advantage in overcoming traditional processes for the treatment of Cr wastewater [9]. Microbial cells are characterized by their important contact interface and high surface area-to-volume ratio due to their small size, which offer a high capacity for metals sorption from the surrounding environment [10].

Biofilm can be defined as an assemblage of microbial cells attached to a surface and enclosed in a self-synthetized matrix of extracellular polymeric substances (EPS) [11]. Compared to planktonic cells, microorganisms existing in biofilm mode show greater tolerance to pollution and environmental stress. Varied harsh pollutants have been remediated using microbial consortia of biofilms including heavy metals, petroleum, explosives, and pesticides [2,12,13]. Biofilm mode offers microbes a great ability to degrade various pollutants via diverse catabolic pathways and a high level of protection from predation and stress due to the EPS matrix. Thus, the synthesis of EPS is of extreme importance in the development of biofilm in general, particularly in the biofilm that can be operated in bioremediation applications [8,14]. The adhesion to surfaces and the accumulation of elements from the environment are two key functions of EPS in biofilm-based bioremediation processes [15].

The aim of this work is the comparison of the biosorption properties of a *Wickerhamomyces anomalus* biofilm supported on wood husk or zeolite 13X for the removal of chromium from wastewater. The effect of the initial concentration of metal and the contact time between the yeast strain and the support were tested. The polysaccharides production of the *Wickerhamomyces anomalus* was quantified. Fourier-transform infrared spectroscopy (FTIR) characterization was carried out in order to confirm the presence of functional groups in the biomass for its role in biosorption process. The application of both systems to the treatment of real effluent provided by a tannery was studied.

2. Materials and methods

2.1. Materials

A chromium resistant and removing yeast *W. anomalus* was obtained from a previous isolation from wastewater samples, contaminated with chemical industrial wastes including those from tanning processing in Fez, Morocco, was used in this study. The pure culture was seeded on yeast medium agar (1% peptone, 1% yeast extract, 2% glucose, and 1.5% agar) plates and incubated for 48 h at the optimal temperature of 30°C. The yeast strain showed a great Cr(VI) removal percentage [16].

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 smashed, dried at 60°C, and sieved through a 1–5 mm size before further usage as biofilm support.

Zeolite 13X (13X MS Bead 5.0–8.0 mm) was obtained from Xiamen Zhongzhao Imp. & Exp. Co., Ltd., (China). It was dried at 200°C for 48 h under a dry air stream prior to use as biofilm support. The chemical composition of zeolite 13X is given in Table 1.

2.2. Contact angle measurements

Preparation of yeast strains suspension for cell surface contact angle measurements (CAM) was carried out following the protocol by the study of Mohd-Al-Faisal et al. [17] with slight modifications as described by the study of Asri et al. [18]. Microbial lawns suitable for CAM were prepared as described by the study of Elabed et al. [19] and realized in triplicate with separately cultured microbes.

2.2.1. Hydrophobicity

According to Vogler's approach [20], 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.

Table 1
Chemical composition of zeolite 13X [71]

Constituent	%
SiO_2	48.26
Al_2O_3	31.85
Fe_2O_3	3.2
TiO_2	0.08
CaO	0.38
Na_2O	15.7
K_2O	0.07

By contrast, van Oss [21] 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 (ΔGiwi). If the interaction between the two surfaces is stronger than the interaction between each surface with water (ΔGiwi < 0), the surface is considered hydrophobic and conversely (ΔGiwi > 0), the surface is considered hydrophilic.

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

where 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.

2.2.2. 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 systems obtained from the application of the Young–Dupre equation to each probe liquid [21], by using three different liquids with known surface parameters values γ_L^{LW}, γ_L^{+}, and γ_L^{-}, 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.

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

where 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.

2.2.3. Calculation of free energy adhesion of the yeast strain to wood surface by Extended Derjaguin–Landau–Verwey–Overbeek theory

In the Extended Derjaguin–Landau–Verwey–Overbeek (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:

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

where *d* refers to the separation distance between a cell and a substratum:

$$\Delta G^{LW}=\left(\left(\gamma_M^{LW}\right)^{1/2}-\left(\gamma_s^{LW}\right)^{1/2}\right)^2-\left(\left(\gamma_M^{LW}\right)^{1/2}-\left(\gamma_L^{LW}\right)^{1/2}\right)^2-\left(\left(\gamma_s^{LW}\right)^{1/2}-\left(\gamma_L^{LW}\right)^{1/2}\right)^2 \quad (3a)$$

and

$$\Delta G^{AB}=2\left[\begin{array}{c}\left(\gamma_L^{+}\right)^{1/2}\left(\left(\gamma_M^{-}\right)^{1/2}+\left(\gamma_S^{-}\right)^{1/2}-\left(\gamma_L^{-}\right)^{1/2}\right)\\ +\left(\gamma_L^{-}\right)^{1/2}\left(\left(\left(\gamma_M^{+}\right)^{1/2}+\left(\gamma_S^{+}\right)^{1/2}-\left(\gamma_L^{+}\right)^{1/2}\right)-\left(\gamma_L^{+}\gamma_s^{-}\right)^{1/2}\right.\\ \left.-\left(\gamma_L^{+}\gamma_S^{-}\right)^{1/2}\right)\end{array}\right] \quad (3b)$$

The usage of a suspension liquid with high ionic strength (KNO_3 0.1 M) allows the negligence of electrostatic interaction free energy ΔG^{EL} as done before [22,23].

2.3. Biosorption experiments

The microbial growth was obtained at 30°C, using yeast extract, peptone, and glucose (YPG) medium at pH 3.72 [16], on a rotary shaker at 150 rpm. After 24 h of incubation, yeast cells were harvested by centrifugation at 7,000 g for 10 min, at 4°C. The cell concentration was adjusted to an optical density of 0.450 at 550 nm (A_0) (approximately 10^7 CFU·mL^{-1} cell density).

In order to study the chromium biosorption potential of microbial strains, the residual Cr(VI) concentration was determined using diphenylcarbazide method [24]. Chromium solution was prepared in sterile distilled water, containing an initial concentration of metal ions of 25, 50 and 100 mg·L^{-1} of Cr(VI) as $K_2Cr_2O_7$. Real effluent was obtained from a traditional tannery in Fez, Morocco.

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

2.4. Open system assays

The open-system assays were performed in Plexiglas columns (height: 33 cm, internal ø 3.5 cm) (Fig. 1) filled with 96 cm^3 of wood husk or zeolite 13X supporting a *W. anomalus* biofilm, while two control assay columns were filled only with wood husk or zeolite 13X. The biofilm attachment on the wood husk or the zeolite was carried out following the protocol by the study of Quintelas et al. [25]. The wood husk and zeolite 13X (15 g) were placed in 250 mL Erlenmeyer flasks with 150 mL of distilled water and sterilized at 120°C for 20 min to release the air inside the pores of the support. Then, these materials were placed in columns

of Plexiglas for open system studies. The culture of microorganisms and the culture medium were pumped (up-flow) at a rate of 25 mL·min^{-1}. Then, the modified YPG medium was used for the development of the microorganism on the support for 3 d, in order to optimize the microbial adhesion. The high flow rate allows the formation of a compact biofilm and therefore resistant to erosion stresses resulting from hydrodynamic forces. Afterwards, the metal aqueous solutions with initial concentrations of 10, 25, 50 and 100 mg·L^{-1} were pumped through the columns with a flow rate of 5 mL·min^{-1} and a pH value of 3.72 [16]. The total chromium and Cr(VI) concentrations in the outflow were periodically evaluated.

2.5. Quantification of polysaccharides

In order to evaluate the effect of the support on EPS production by yeast biofilm supported on wood husk or zeolite 13X. The quantification of polysaccharides was realized following the protocol by the study of Azeredo and Oliveira [26]. The assay of exopolysaccharides and total polymers takes place in three steps: (i) solubilization of the polysaccharide and the polymer network using glutardialdehyde for 2 d at a low speed of rotation, (ii) dialysis of the obtained solution and (iii) precipitation of dialysis. This precipitation step is carried out with phenol and sulfuric acid for the quantification of polysaccharides carried out by spectrometry at 440 nm. The quantification of the total polymers is obtained by precipitation of the dialyzed nitron solution, followed by centrifugation and drying. The residual material is finally weighed.

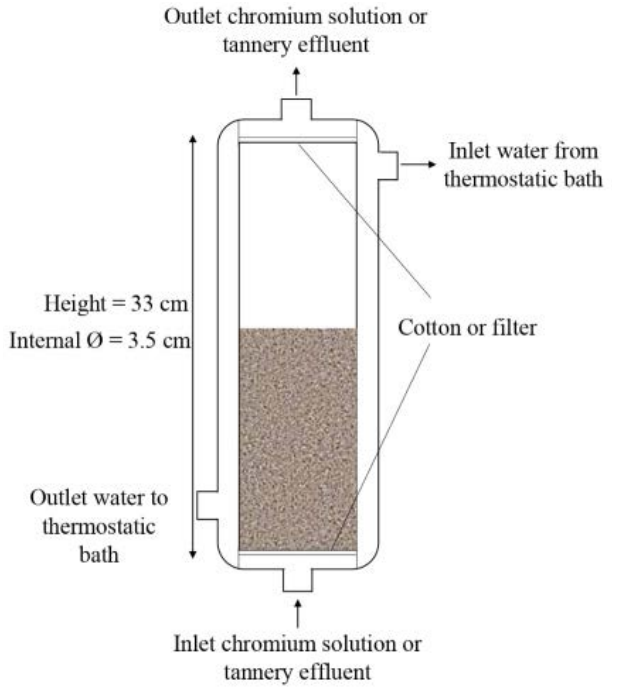

Fig. 1. Schematic illustration of the Plexiglas columns for chromium treatment open system. The biofilm is first formed on the support (wood husk/zeolite 13X) and the Cr solution is pumped with different concentration at flow rate of 5 mL·min^{-1}.

2.6. Fourier transform infrared spectrometer characterization and environmental scanning electron microscopy observation

Samples of the formed biofilms on both zeolite and wood husk were analyzed with environmental scanning electron microscopy and Fourier-transform infrared spectrometer (FTIR), after dehydration using gradual concentration of ethanol. Samples were then gold coated before scanning electron microscopy (SEM) observation using (Leica Cambridge S360, USA).

For the FTIR analysis, the support-biofilm was finely grounded. Then, 10 mg of powder of the smashed biofilm was encapsulated in 100 mg of KBr forming transparent pellets and infrared spectrum of the unloaded and Cr-loaded wood husk and zeolite were obtained using a Fourier-transform infrared spectrometer (FTIR BOMEM MB 104) in the 4,000–500 cm^{-1} range by averaging 20 scans at a maximum resolution of 10 cm^{-1}.

2.7. Statistical analysis

All the experimental work was performed in triplicate. The results were subjected to statistical calculations for means comparison using XLSTAT software [27].

3. Results and discussion

3.1. FTIR analysis of wood husk and zeolite 13X

3.1.1. Wood husk

The FTIR spectrum of the wood husk is illustrated in Fig. 2. The peak 3,334 cm^{-1} corresponds to the elongation vibration of the O–H bond of the aromatic and aliphatic structures of phenol, lignin group and cellulose [28]. The band between 2,975 and 2,800 with a peak at 2,921 cm^{-1} corresponds to the asymmetric elongation vibration of the cellulose C–H bond [29]. The peak 1,728 cm^{-1} is attributed to the C=O valence vibration of carboxylic acids and/or xylan esters present in lignin and hemicellulose [30]. The peak at 1,506 cm^{-1} corresponds to the C=C deformation of aromatic rings of lignin. The peak 1,324 cm^{-1} corresponds to

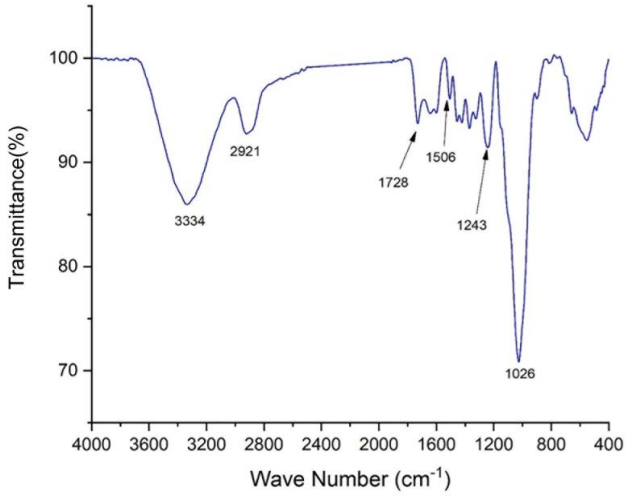

Fig. 2. Fourier-transform infrared spectrum of wood husk.

the valence vibration (C–H) of the cellulose and hemicellulose. Peak 1,243 could be attributed to the elongation vibration of the C–O bonds of lignin and xylan [31]. The peak 1,026 cm^{-1} is assigned to C–O stretching in cellulose, hemicellulose and lignin [31]. Peak 894 and the band between 7,200 and 400 cm^{-1} are assigned to C–H group elongations of cellulose [32].

3.1.2. Zeolite 13X

The obtained FTIR spectrum of the used zeolite 13X represented in Fig. 3 is consent with the previously reported values [33]. The peak 561 cm^{-1} is attributed to double six-member rings (D6R), symmetric stretch (668 cm^{-1}) and asymmetric stretch (978 cm^{-1}) are identified (where T = Si or Al). The band at 1,640 cm^{-1} is attributed to H_2O deformation mode due to incomplete dehydration of the samples. The observed strong band at 3,526 cm^{-1} corresponded to OH-stretching of water molecules present in the zeolite channel [34].

3.2. Physico-chemical surface characteristics of wood husk, zeolite 13X and W. anomalus cells

The physicochemical surface characteristics of the used yeast strain and supports were obtained using CAM method. The characterization of *W. anomalus* and wood husk was reported in our previous work [16]. In this work, we mainly focus on zeolite 13X characterization.

Zeolite are extensively used in the bio-depollution of many pollutants. They are used as adsorbents or as support of microbial biofilm aimed for wastewater treatment [2]. The catalytic and adsorption properties of zeolite are mainly due to their acidity, molecular sieving ability and large contact areas [35]. The result of physicochemical characterization of the used supports show that zeolite 13X presents an hydrophilic character (Table 2).

Our results are in agreement with previous work showing that zeolite 13X is among the hydrophilic types of zeolite [36]. It is well known that the silica content is the main parameter determining the hydrophobic properties of zeolite. Thus, zeolite with high-silica present hydrophobic surfaces properties, while low-silica zeolite such as 13X and NaY preferentially adsorb polar molecules and hence exhibit hydrophilic character [37]. Zeolite 13X are naturally exhibiting an hydrophilic nature, as the substitution of Si for Al in the zeolite structure creates a negative charge which is neutralized by cations. Thus, it is inducing a strong electrostatic field on the zeolite surface. Thus, depending on the Si/Al ratio, water molecules (or any polar molecules) are adsorbed on zeolite 13X surface. This adsorption occurs through dipole-field interaction as well as hydrogen bonds with residual hydroxyl groups. With the increase of the Si/Al ratio, the amount of required cations to neutralize the charge decreases and hence the hydrophobicity increases [35].

The zeolite 13X show a dominant donor electron character (43.24 ± 2.91 $mJ{\cdot}m^{-2}$) and a weak acceptor electron character (2.31 ± 0.83 $mJ{\cdot}m^{-2}$). Similar results were reported by the study of Costanzo et al. [38]. In fact, it was reported that clay minerals typically have weak values of acceptor electron character γ^+ and a donor electron character ranging from 3 to 60 $mJ{\cdot}m^{-2}$ [39]. The Lewis base component of the surface tension is mainly due to the lone electron pairs of the oxygen atoms present in the zeolite extra frameworks [40].

Table 2 shows also the interaction free energies between *W. anomalus* and the wood husk or zeolite 13X. In other words, ΔG^{XDLVO} show the ability of the attachment of the

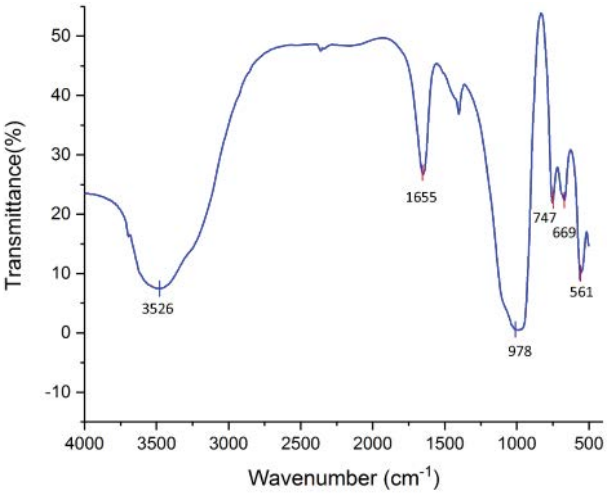

Fig. 3. Fourier-transform infrared spectrum of zeolite 13X.

Table 2
Contact angle values using water (θ_W), formamide (θ_F) and diiodomethane (θ_D), Lifshitz–van der Waals (γ^{LW}), electron–donor (γ^-) and electron–acceptor (γ^+) parameters, surface energies (ΔGiwi) of wood husk, zeolite 13X and *Wickerhamomyces anomalus* and the total energy of adhesion between yeast cells and the support (ΔG^{XDLVO})

Material	Contact angles (°)			Surface tension: components and parameters ($mJ{\cdot}m^{-2}$)			Surface energies	Interaction free energy ΔG^{XDLVO} ($mJ{\cdot}m^{-2}$)	
	θ_W	θ_F	θ_D	γ^{LW}	γ^+	γ^-	ΔGiwi ($mJ{\cdot}m^{-2}$)		
Wickerhamomyces anomalus	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		[16]
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 ± 3.86	–7.13	[16]
Zeolite 13X	30.8 ± 1.05	23.86 ± 4.48	50.83 ± 1.62	33.73 ± 0.91	2.31 ± 0.83	43.24 ± 2.91	19.11 ± 4.30	23.99	

studied strain to the support. The obtained results show that ΔG^{XDLVO} presents a negative value of –7.13 mJ·m^{-2}. According to the XDLVO predictive tool, the attachment between *W. anomalus* and wood husk is favored. While the adhesion ability between the yeast strain and the zeolite is predicted as unfavored with a positive value of ΔG^{XDLVO} 23.99 mJ·m^{-2}.

The comparison of the theoretical formation of yeast biofilm supported on wood husk with that of biofilm fixed to zeolite demonstrated that the yeast strain showed a better affinity to wood husk. The SEM observations were then performed in order to visualize the microbial adhesion. Fig. 4A shows the octahedral crystalline shape of 13X zeolite with a very weak adhesion of microbial cells, while Fig. 4B shows an abundant adhesion on the rough surface of wood husk.

3.3. Effect of time contact between yeast strain and support on chromium removal

It is well known that the microbial adhesion is a complicated phenomenon influenced by various factors [41]. Among these latter, the contact time between the support and the microbial cells is a prominent parameter affecting considerably the biofilm attachment and stability [42]. It is strongly required for the irreversible adhesion of microbial cells to the substratum [43]. However, works evaluating the effect of this parameter are aiming mainly to eliminate or prevent the adhesion of undesirable biofilms in food industries for example [44,45]. Thus, in this study, we evaluate its effect on *W. anomalus* adhesion on both supports, in order to optimize the biofilm stability that would lead to a better depollution efficiency. The experimental assays evaluating the effect of the contact time on *W. anomalus* Cr removal were conducted at the optimal pH of the strain and an initial Cr(VI) concentration of 50 mg·L^{-1} as represented in Fig. 5. The obtained results showed a contact time of 36 h was required for *W. anomalus* supported on wood husk to reach a Cr(VI) removal percentage of 98.02% ± 2.36% (Fig. 5A). For the *W. anomalus* biofilm supported on zeolite 13X, the needed time was 40 h to reach 66.25% ± 3.12% of Cr(VI) removal (Fig. 5B). Our results are in agreement with previous works proving that depending on the support, the required time for the attachment of the microbial cells may widely vary. For instance, Beresford et al. [46] showed that the required time to *Listeria monocytogenes* attachment on polychlorure de vinyle was higher than on stainless steel.

Fig. 5 shows that the removal of chromium by *W. anomalus* biofilms increased significantly with the increase of contact time from 0 to 48 h until reaching a maximum value which remains contact or slightly decreases over time. Our results are in agreement with works reporting that the increase of the contact time is accompanied by the increase of microbial adhesion and the metal uptake efficiency [42,47].

3.4. Effect of initial metal concentration on yeast chromium removal efficiency

The obtained results of the effect of metal initial concentration on chromium removal efficiency are represented in Fig. 6. At a chromium concentration of 10 mg·L^{-1}, a total

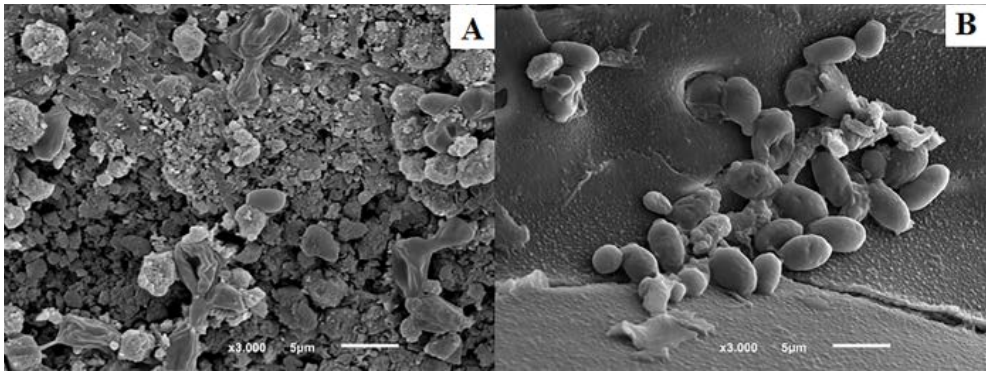

Fig. 4. Scanning electron microscopy of *Wickerhamomyces anomalus* biofilm (3,000×) (A) zeolite 13X and (B) wood husk.

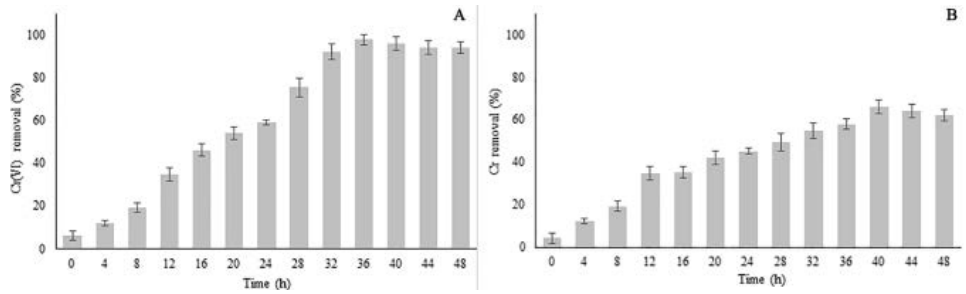

Fig. 5. Effect of time contact between *Wickerhamomyces anomalus* and supports on chromium removal (A) wood husk and (B) zeolite 13X.

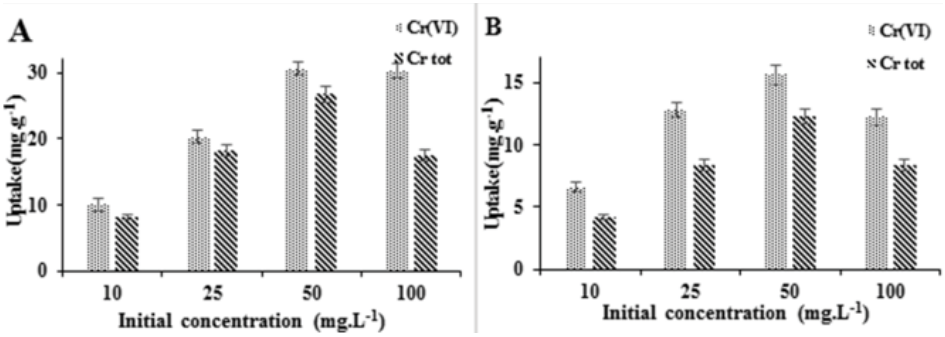

Fig. 6. Uptake values (mg/$g_{biosorbent}$) obtained for batch experiments by *Wickerhamomyces anomalus* biofilm (A) supported on wood husk and (B) supported on zeolite 13X.

removal was obtained for *W. anomalus* biofilm supported on wood husk. While an adsorption quantity of 6.62 mg·g^{-1} was removed by cells attached to the zeolite 13X at the same metal initial concentration.

At an initial concentration of 25 mg·L^{-1}, *W. anomalus* biofilm attached to wood husk and zeolite 13X was able to remove 20.30 and 12.81 mg·g^{-1} of Cr(VI), respectively.

At an initial concentration of 50 mg·L^{-1}, the percent Cr(VI) removal achieved by cells attached to wood husk and zeolite 13X was 30.56 and 15.65 mg·g^{-1}, respectively.

The lowest removal efficiency (30.25 and 12.24 mg·g^{-1} for *W. anomalus* biofilm supported on wood husk and zeolite 13X, respectively) was obtained with the highest concentration of 100 mg·L^{-1}. These results are in agreement with previous works [48], where the removal efficiency in batch experiments decreased with the increase of the metal initial concentrations. This can be ascribed to the increase of the number of ions competing for the available binding sites on the biomass and to the lack of binding sites at higher concentration levels [49].

3.5. Quantification of polysaccharides and total polymers

The attachment of microbial cells to a solid surface is a prominent stage in the biofilm formation [50]. Besides the implication of physico-chemical properties of both cells and the surface [51], a major player in this stage is the natural ability of microbial cells to produce EPS [15]. In microbial initial adhesion, the EPS function as cementation agents, aiding the firm adhesion of the microorganisms to each other and to the support [52].

The quantification polysaccharides revealed a value of 8.37 mg/$g_{biosorbent}$ for the wood husk supported biofilm and 1.59 mg/$g_{biosorbent}$ for the zeolite supported biofilm. For the total polymers, the quantification revealed a value of 61.2 mg/$g_{biosorbent}$ for the wood husk supported biofilm and 11.8 mg/$g_{biosorbent}$ for the zeolite supported biofilm.

The production of polysaccharide and total polymers has been reported to give important information about the biofilm formation capacity of the microorganism [53]. In this case, the important production of EPS by wood husk supported biofilm was correlated to its good adhesion to this substratum. Inversely, a low production of EPS by the zeolite 13X supported biofilm was accompanied by a weak adhesion to its surface. This may explain a good part of the difference of the chromium removal efficiency between the two systems. Indeed, besides assuring a good attachment and nutrients sequestration, the binding sites in EPS enables the biofilm to remove toxic metals in biological wastewater treatment [54].

3.6. Effect of flow rate

The effect of flow rate on the Cr removal capacity of *W. anomalus* biofilm was investigated. The initial metal concentration (50 mg·L^{-1}) and the bed height (15 cm) were kept constant and the flow rate was varied from 5 to 20 mL·min^{-1}. For both supports, the lowest flow rate (5 mL·min^{-1}) allowed the highest biosorption efficiency, while the highest flow rate (20 mL·min^{-1}) resulted in lowest removal efficiency (Fig. 7). Similar results were previously reported by the study of Ko et al. [55]. This may be ascribed to the insufficient time for the solute inside the column and the diffusion limitations into the pores of the biofilm system at high flow rates [56].

3.7. Effect of bed height

In open system, the effect of bed height on the chromium removal by *W. anomalus* was reported to be a prominent parameter [56]. In the present work, support beds of 10 and 15 cm were constituted of wood husk or zeolite 13X in Plexiglas columns. The increase in bed height of both supports was accompanied with the increase of the chromium removal efficiency (Fig. 8). Indeed, the increase of depollution capacity may be attributed to the increase of the support amount and hence the increase in surface area and the adsorption sites available for the chromium uptake [47,57] have attributed this effect to the availability of more binding sites for metal adsorption.

3.8. Application of microbial biofilm on wood waste for the treatment of a tannery effluent

Several studies have focused on the application of microbial systems in the treatment of real effluents [58,59] and particularly those based on the use of biofilms [60]. The applicability of these processes has proven its efficiency for the depollution of various pollutants contained in industrial effluents [61–64]. In real effluents, the coexistence of

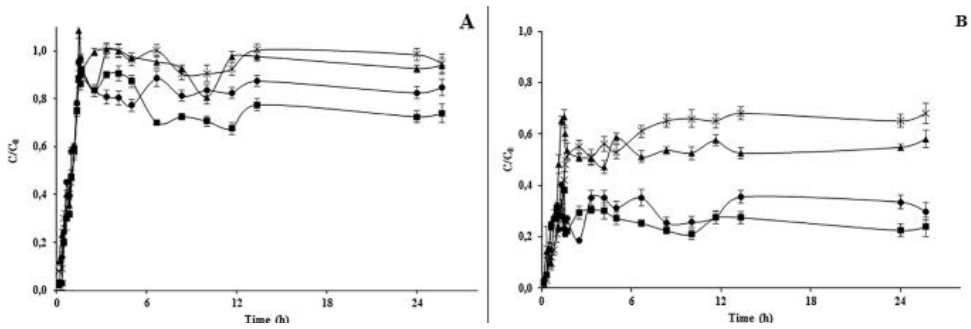

Fig. 7. Breakthrough curves for Cr(VI) onto *Wickerhamomyces anomalus* biofilm (A) supported on wood husk, (B) supported on zeolite 13X (bed height 15 cm, initial Cr concentration 50 mg·L^{-1}, pH = 3.72) at different flow rates (×) 5 mL·min^{-1}, (▲) 10 mL·min^{-1}, (●) 15 mL·min^{-1}, (■) 20 mL·min^{-1}.

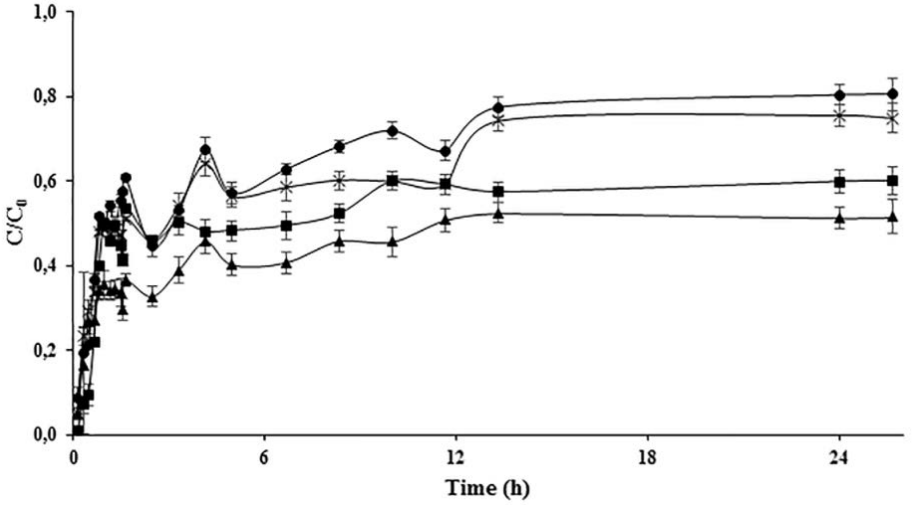

Fig. 8. Breakthrough curves for Cr(VI) onto *Wickerhamomyces anomalus* biofilm supported on wood husk or on zeolite 13X (flow rate 5 mL·min^{-1}, initial Cr concentration 50 mg·L^{-1}, pH = 3.72) at different bed heights (×) wood 10 cm, (▲) zeolite 13X 10 cm, (●) wood 15 cm, (■) zeolite 13X 15 cm.

ions and organic matter in aqueous solution makes catabolic conditions more difficult and complex [65]. These difficulties prompted researchers to consider the application of hybrid technologies, which involve the combination of various processes for treating actual effluents [65,66]. The use of microbial biofilms, in particular those involving consortia, has proven successful against various pollutants [67,68].

The efficiency of microbial biofilms for the treatment of a real tannery effluent has been studied. The depollution performance of the biofilm of *W. anomalus* attached to wood husk was compared on the one hand to the efficiency of cells attached to zeolite 13X and on the other hand to that of microbial consortia in both batch and open systems.

3.8.1. Batch system

In this study, the use of the *W. anomalus* strain in monobiofilm and within consortia attached to wood husk was studied for the treatment of a tannery effluent. The initial concentration of Cr(VI) and tot Cr in the effluent were 7.23 and 42.56 mg·L^{-1}, respectively.

Experiments realized with a real tannery effluent in batch system have shown a Cr(VI) elimination percentage values of 60.21% and 26.27% with the *W. anomalus* – wood husk and *W. anomalus* – zeolite 13X systems, respectively, after 48 h of treatment. For microbial consortia attached to wood husk, the obtained removal percentages of Cr(VI) were 81.17%, 83.13%, 90.56% and 97.52% with *W. anomalus* – *Enterococcus mundtii*, *W. anomalus* – *E. faecium*, *W. anomalus* – *Bacillus cereus* and *W. anomalus* – *Bacillus* sp., respectively (Fig. 9).

For tot Cr, the results showed removal percentages of 35.05%, 86.21%, 89.45%, 89.21%, 95, 52% and 98.12% by *W. anomalus* – zeolite 13X, *W. anomalus* – wood husk, *W. anomalus* – *E. mundtii*, *W. anomalus* – *Enterococcus faecium*, *W. anomalus* – *B. cereus* and *W. anomalus* – *Bacillus* sp., respectively (Fig. 9).

A previous study by the study of Sharma and Adholeya [69] reported the ability of the fungal strain *Paecilomyces lilacinus* to reduce 100% of Cr(VI) contained in the tannery effluent at an initial concentration of 1.24 mg·L^{-1} after 18 h of treatment. Similar results were obtained using *Bacillus*

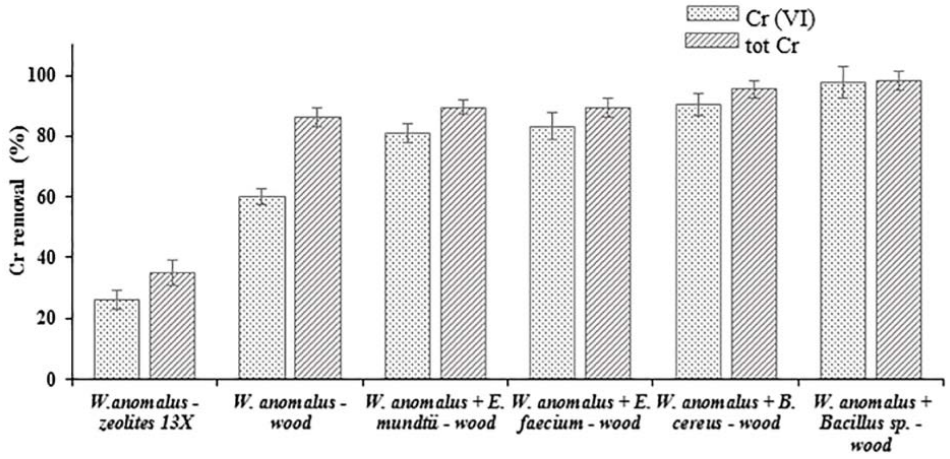

Fig. 9. Chromium removal percentages in batch system by the biofilm of *Wickerhamomyces anomalus* and the microbial consortia supported on wood husk and the biofilm of *Wickerhamomyces anomalus* on the zeolite 13X after 48 h of treatment.

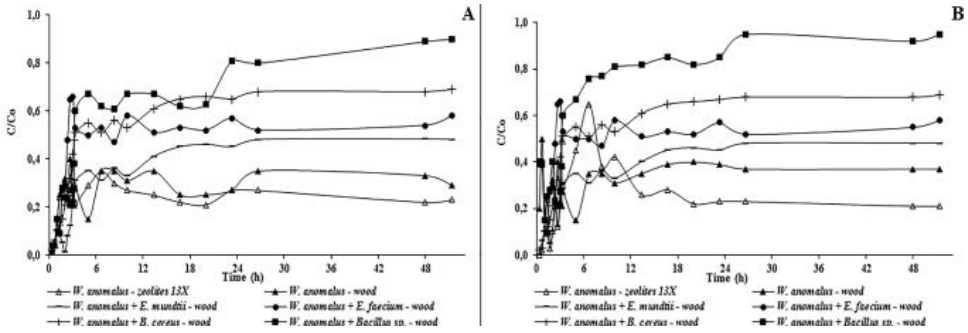

Fig. 10. Breakthrough curves of the removal of (A) Cr(VI) and (B) Cr tot, contained in the tannery effluent by the biofilm of *Wickerhamomyces anomalus* and the microbial consortia supported on wood husk and the biofilm of *Wickerhamomyces anomalus* on the zeolite 13X, in an open system.

coagulans biofilm attached to granulated activated carbon [8]. In fact, the obtained results with aqueous solutions of Cr(VI) are often more promising than those obtained with real effluents. This can be attributed to the inhibition of the metal removal process in the presence of other ions and compounds in the tannery effluent. Their presence leads to an interactive competitive effect for the same active sites [70].

The remarkable performance of the microbial consortia in terms of depollution toward Cr(VI) and Cr tot contained in the real effluent (especially that of 98.12% obtained by the consortium *W. anomalus* – *Bacillus* sp. attached to wood husk) would be due to the synergistic action between the two microbial species. This would be the result of the resistance conferred by the biofilm mode and the robustness and catabolic diversity within microbial consortia [12,67].

3.8.2. Open system

Studies carried out on the tannery effluent have shown Cr(VI) reduction values after 50 h, of 23.55%, 29.13%, 49.01%, 58.23%, 66.07% and 90.34% by *W. anomalus* – zeolite 13X, *W. anomalus* – wood waste, *W. anomalus* – *E. mundtii*, *W. anomalus* – *E. faecium*, *W. anomalus* – *B. cereus* and *W. anomalus* – *Bacillus* sp., respectively (Fig. 10A).

The removal of tot Cr by *W. anomalus* – zeolite 13X, *W. anomalus* – wood waste, *W. anomalus* – *E. mundtii*, *W. anomalus* – *E. faecium*, *W. anomalus* – *B. cereus* and *W. anomalus* – *Bacillus* sp. allowed percentages of 21.18%, 37.09%, 48.12%, 57.27%, 69.12% and 95.33%, respectively (Fig. 10B).

Compared to the depollution of aqueous chromium solutions, the metal removal process would have been inhibited by the presence of other compounds contained in the used effluent. The presence of a multiplicity of metals in the tanning effluent would lead to an interactive effect. It has been argued that these effects can be extremely complex and that three types of response can be expected: (1) the effects of the mixture are greater than those of the individual effects of the ions in the mixture (synergy); (2) the effects of the mixture are less than those of the individual effects of the ions in the mixture (antagonism); (3) no effects of the mixture (no interaction) are observed by the study of Salehizadeh and Shojaosadati [70].

4. Conclusion

W. anomalus biofilm on two different supports (wood husk and zeolite 13X) was tested to remove chromium from aqueous solutions and a real tannery effluent. The FTIR analysis of zeolite 13X and wood husk used as supports

for yeast strain was performed to identify their chemical composition. The theoretical prediction of microbial adhesion onto the support using XDLVO model showed a favorable attachment of *W. anomalus* to wood husk and an unfavorable one to zeolite 13X. The theoretical predictions were confirmed by the SEM visualization of experimental adhesion.

The comparative study of chromium removal using both biofilms showed that a contact time of 36 h was required for *W. anomalus* supported on wood husk to reach a Cr(VI) removal percentage of 98.02%. For the *W. anomalus* biofilm supported on zeolite 13X, the needed time was 40 h to reach 66.25% of Cr(VI) removal. The comparative study of the effect of initial concentration of chromium removal showed that the highest removal percentage (100% and 66.23% for *W. anomalus* biofilm supported on wood husk and zeolite 13X, respectively) was attained with the lowest initial concentration 10 $mg{\cdot}L^{-1}$. For both supports, the lowest flow rate (5 $mL{\cdot}min^{-1}$) allowed the highest biosorption efficiency, while highest flow rate (20 $mL{\cdot}min^{-1}$) resulted in lowest removal efficiency. Support beds of 15 cm allowed a better performance of chromium removal for both *W. anomalus* biofilms.

The systems developed were therefore used for the treatment of a tannery effluent with an initial Cr(VI) and total Cr concentration of 7.23 and 42.56 $mg{\cdot}L^{-1}$, respectively. The highest depollution performance was obtained by the consortium *W. anomalus* – *Bacillus* sp. attached to wood husk.

In batch system, this consortium allowed the removal of 97.52% of Cr(VI) and 98.12% of tot Cr tot initially contained in the tannery effluent, after 48 h of treatment. In an open system, the removal 90.34% of Cr(VI) and 95.33% of tot Cr was achieved after 50 h of treatment.

Taken together these results indicate that the biosorption using *W. anomalus* biofilm in pure culture or within consortia supported on wood husk is a promising treatment for chromium rich effluents.

Funding

This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UID/BIO/04469/2013 unit and COMPETE 2020 (POCI-01-0145-FEDER-006684) and BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by the European Regional Development Fund under the scope of Norte 2020 – Programa Operacional Regional do Norte.

Statements and declarations

The authors have no relevant financial or non-financial interests to disclose.

The authors have no competing interests to declare that are relevant to the content of this article.

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

The authors have no financial or proprietary interests in any material discussed in this article.

Authors' contributions Conceptualization: Meryem Asri; methodology: Meryem Asri, Redouane Ouafi, Wifak Bahafid; formal analysis and investigation: Meryem Asri, Redouane Ouafi, Saad Ibnsouda Koraichi; writing—original draft preparation: Meryem Asri; writing—review and editing: Naïma El Ghachtouli; funding acquisition: Teresa Tavares; resources: Meryem Asri; supervision: Naïma El Ghachtouli, Soumya Elabed, Teresa Tavares.

References

[1] M. Batool, Waqas-ud-Din Khan, Y. Hamid, M.A. Farooq, M.A. Naeem, F. Nadeem, Interaction of pristine and mineral engineered biochar with microbial community in attenuating the heavy metals toxicity: a review, Appl. Soil Ecol., 175 (2022) 104444, doi: 10.1016/j.apsoil.2022.104444.

[2] B. Silva, H. Figueiredo, C. Quintelas, I.C. Neves, T. Tavares, Zeolites as supports for the biorecovery of hexavalent and trivalent chromium, Microporous Mesoporous Mater., 116 (2008) 555–560.

[3] S. Chaturvedi, A. Khare, S.M.P. Khurana, Toxicity of Hexavalent Chromium and Its Microbial Detoxification Through Bioremediation, M.P. Shah, Ed., Removal of Emerging Contaminants Through Microbial Processes, Springer, Singapore, 2021, pp. 513–542.

[4] N. Tahri Joutey, W. Bahafid, H. Sayel, S. Ananou, N. El Ghachtouli, Hexavalent chromium removal by a novel *Serratia proteamaculans* isolated from the bank of Sebou River (Morocco), Environ. Sci. Pollut. Res., 21 (2013) 3060–3072.

[5] R. Jobby, P. Jha, A.K. Yadav, N. Desai, Biosorption and biotransformation of hexavalent chromium [Cr(VI)]: a comprehensive review, Chemosphere, 207 (2018) 255–266.

[6] P. Sharma, A.K. Pandey, S.H. Kim, S.P. Singh, P. Chaturvedi, S. Varjani, Critical review on microbial community during *in-situ* bioremediation of heavy metals from industrial wastewater, Environ. Technol. Innovation, 24 (2021) 101826, doi: 10.1016/j.eti.2021.101826.

[7] G. Bayramoğlu, G. Çelik, E. Yalçın, M. Yılmaz, M.Y. Arıca, Modification of surface properties of *Lentinus sajor-caju* mycelia by physical and chemical methods: evaluation of their Cr^{6+} removal efficiencies from aqueous medium, J. Hazard. Mater., 119 (2005) 219–229.

[8] C. Quintelas, B. Fernandes, J. Castro, H. Figueiredo, T. Tavares, Biosorption of Cr(VI) by a *Bacillus coagulans* biofilm supported on granular activated carbon (GAC), Chem. Eng. J., 136 (2008) 195–203.

[9] C. Cervantes, J. Campos-García, S. Devars, F. Gutiérrez-Corona, H. Loza-Tavera, J.C. Torres-Guzmán, R. Moreno-Sánchez, Interactions of chromium with microorganisms and plants, FEMS Microbiol. Rev., 25 (2001) 335–347.

[10] M.D. Mullen, D.C. Wolf, F.G. Ferris, T.J. Beveridge, C.A. Flemming, G.W. Bailey, Bacterial sorption of heavy-metals, Appl. Environ. Microbiol., 55 (1989) 3143–3149.

[11] M.C. Sportelli, C. Kranz, B. Mizaikoff, N. Cioffi, Recent advances on the spectroscopic characterization of microbial biofilms: a critical review, Anal. Chim. Acta, 1195 (2022) 339433, doi: 10.1016/j.aca.2022.339433.

[12] A. Mitra, S. Mukhopadhyay, Biofilm mediated decontamination of pollutants from the environment, AIMS Bioeng., 3 (2016) 44–59.

[13] J.M. Sonawane, A.K. Rai, M. Sharma, M. Tripathi, R. Prasad, Microbial biofilms: recent advances and progress in environmental bioremediation, Sci. Total Environ., 824 (2022) 153843, doi: 10.1016/j.scitotenv.2022.153843.

[14] T. Siddharth, P. Sridhar, V. Vinila, R.D. Tyagi, Environmental applications of microbial extracellular polymeric substance (EPS): a review, J. Environ. Manage., 287 (2021) 112307, doi: 10.1016/j.jenvman.2021.112307.

[15] C. Quintelas, V.B. da Silva, B. Silva, H. Figueiredo, T. Tavares, Optimization of production of extracellular polymeric substances by *Arthrobacter viscosus* and their interaction with

a 13X zeolite for the biosorption of Cr(VI), Environ. Technol., 32 (2011) 1541–1549.
[16] M. Asri, N. El Ghachtouli, S. Elabed, S. Ibnsouda Koraichi, A. Elabed, B. Silva, T. Tavares, *Wicherhamomyces anomalus* biofilm supported on wood husk for chromium wastewater treatment, J. Hazard. Mater., 359 (2018) 554–562.
[17] N. Mohd-Al-Faisal, W.H.A. Wan Harun, F. Abdul Razak, An in vitro study on the anti-adherence effect of *Brucea javanica* and *Piper betle* extracts towards oral Candida, Arch. Oral Biol., 58 (2013) 1335–1342.
[18] M. Asri, A. Elabed, N. El Ghachtouli, S. Ibnsouda Koraichi, W. Bahafid, S. Elabed, Theoretical and experimental adhesion of yeast strains with high chromium removal potential, Environ. Eng. Sci., 34 (2017) 693–702.
[19] S. Elabed, M. Mostakim, F. Berguadi, H. Latrache, A. Houari, F. Hamadi, S. Ibnsouda koraichi, Study of microbial adhesion on some wood species: theoretical prediction, Microbiology, 80 (2011) 43–49.
[20] E.A. Vogler, Structure and reactivity of water at biomaterial surfaces, Adv. Colloid Interface Sci., 74 (1998) 69–117.
[21] C.J. van Oss, Interfacial Forces in Aqueous Media, Dekker, New York, 1996.
[22] A.M. Gallardo-Moreno, M.L. González-Martín, C. Pérez-Giraldo, J.M. Bruque, A.C. Gómez-García, The measurement temperature: an important factor relating physicochemical and adhesive properties of yeast cells to biomaterials, J. Colloid Interface Sci., 271 (2004) 351–358.
[23] H.H.M. Rijnaarts, W. Norde, J. Lyklema, A.J.B. Zehnder, DLVO and steric contributions to bacterial deposition in media of different ionic strengths, Colloids Surf., B, 14 (1999) 179–195.
[24] P. Pattanapipitpaisal, N.L. Brown, L. Macaskie, Chromate reduction and 16S rRNA identification of bacteria isolated from a Cr(VI)-contaminated site, Appl. Microbiol. Biotechnol., 57 (2001) 257–261.
[25] C. Quintelas, Z. Rocha, B. Silva, B. Fonseca, H. Figueiredo, and T. Tavares, Removal of Cd(II), Cr(VI), Fe(III) and Ni(II) from aqueous solutions by an *E. coli* biofilm supported on kaolin, Chem. Eng. J., 149 (2009) 319–324.
[26] J. Azeredo, R. Oliveira, A new method for precipitating bacterial exopolysaccharides, Biotechnol. Technol., 10 (1996) 341–344.
[27] A. Avellan, C. Levard, J. Rose, M. Auffan, M. Bertrand, L. Olivi, C. Santaella, W. Achouak, A. Masion, Influence of structural defects of Ge-imogolite nanotubes on their toxicity towards *Pseudomonas brassicacearum*, Environ. Sci. Nano, 3 (2016) 839–846.
[28] Y. Guo, R.M. Bustin, FTIR spectroscopy and reflectance of modern charcoals and fungal decayed woods: implications for studies of inertinite in coals, Int. J. Coal Geol., 37 (1998) 29–53.
[29] S. Benyoucef, D. Harrache, Microstructure characterization of scots pine "*Pinus sylvestris*" sawdust, J. Mater. Environ. Sci., 6 (2015) 765–772.
[30] M. Sain, S. Panthapulakkal, Bioprocess preparation of wheat straw fibers and their characterization, Ind. Crops Prod., 23 (2006) 1–8.
[31] A.K. Rana, R.K. Basak, B.C. Mitra, M. Lawther, A.N. Banerjee, Studies of acetylation of jute using simplified procedure and its characterization, J. Appl. Polym. Sci., 64 (1996) 1517–1523.
[32] K.K. Pandey, A.J. Pitman, FTIR studies of the changes in wood chemistry following decay by brown-rot and white-rot fungi, Int. Biodeterior. Biodegrad., 52 (2003) 151–160.
[33] D. Chen, X. Hu, L. Shi, Q. Cui, H. Wang, H. Yao, Synthesis and characterization of zeolite X from lithium slag, Appl. Clay Sci., 59–60 (2012) 148–151.
[34] Y. Ma, C. Yan, A. Alshameri, X. Qiu, C. Zhou, Synthesis and characterization of 13X zeolite from low-grade natural kaolin, Adv. Powder Technol., 25 (2014) 495–499.
[35] L. Liu, R. Singh, G. Li, G. Xiao, P.A. Webley, Y. Zhai, Synthesis of hydrophobic zeolite X@SiO_2 core-shell composites, Mater. Chem. Phys., 133 (2012) 1144–1151.
[36] B. Adnadjević, J. Jovanović, S. Gajinov, Effect of different physicochemical properties of hydrophobic zeolites on the pervaporation properties of PDMS-membranes, J. Membr. Sci., 136 (1997) 173–179.
[37] V. Ochoa-Herrera, R. Sierra-Alvarez, Removal of perfluorinated surfactants by sorption onto granular activated carbon, zeolite and sludge, Chemosphere, 72 (2008) 1588–1593.
[38] P.M. Costanzo, R.F. Giese, C.J. van Oss, The Determination of Surface Tension Parameters of Powders by Thin Layer Wicking, R.A. Williams, N.C. de Jaeger, Eds., Advances in Measurement and Control of Colloidal Processes, Butterworth-Heinemann, San Diego, USA, 1991, pp. 223–232.
[39] C.J. van Oss, R.F. Giese, The hydrophilicity and hydrophobicity of clay minerals, Clays Clay Miner., 43 (1995) 474–477.
[40] P. Dutournié, A. Said, T.J. Daou, J. Bikaï, L. Limousy, Hydraulic performance modifications of a zeolite membrane after an alkaline treatment: contribution of polar and apolar surface tension components, Adv. Mater. Sci. Eng., 2015 (2015) 524259, doi: 10.1155/2015/524259.
[41] R. Van Houdt, C.W. Michiels, Biofilm formation and the food industry, a focus on the bacterial outer surface, J. Appl. Crystallogr., 109 (2010) 1117–1131.
[42] J.M. Lundén, M.K. Miettinen, T.J. Autio, H.J. Korkeala, Persistent *Listeria monocytogenes* strains show enhanced adherence to food contact surface after short contact times, J. Food Prot., 63 (2000) 1204–1207.
[43] R.A.N. Chmielewski, J.F. Frank, Biofilm Formation and Control in Food Processing Facilities, Compr. Rev. Food Sci. Food Saf., 2 (2003) 22–32.
[44] H. Gibson, J.H. Taylor, K.E. Hall, J.T. Holah, Effectiveness of cleaning techniques used in the food industry in terms of the removal of bacterial biofilms, J. Appl. Microbiol., 1995 (1999) 41–48.
[45] B. Joseph, S.K. Otta, I. Karunasagar, I. Karunasagar, Biofilm formation by *Salmonella* spp. on food contact surfaces and their sensitivity to sanitizers, Int. J. Food Microbiol., 64 (2001) 367–372.
[46] M.R. Beresford, P.W. Andrew, G. Shama, *Listeria monocytogenes* adheres to many materials found in food-processing environments, J. Appl. Microbiol., 90 (2001) 1000–1005.
[47] A.K. Meena, K. Kadirvelu, G.K. Mishraa, C. Rajagopal, P.N. Nagar, Adsorption of Pb(II) and Cd(II) metal ions from aqueous solutions by mustard husk, J. Hazard. Mater., 150 (2008) 619–625.
[48] B. Silva, H. Figueiredo, C. Quintelas, I.C. Neves, T. Tavares, Improved biosorption for Cr(VI) reduction and removal by *Arthrobacter viscosus* using zeolite, Int. Biodeterior. Biodegrad., 74 (2012) 116–123.
[49] C. Quintelas, B. Fonseca, B. Silva, H. Figueiredo, T. Tavares, Treatment of chromium(VI) solutions in a pilot-scale bioreactor through a biofilm of *Arthrobacter viscosus* supported on GAC, Bioresour. Technol., 100 (2009) 220–226.
[50] Z. Lewandowski, J.P. Boltz, Biofilms in Water and Wastewater Treatment, P. Wilderer, Ed., Treatise on Water Science, Academic Press, Oxford, 2011, pp. 529–567.
[51] F. Costa, B. Silva, T. Tavares, 6 - Biofilm bioprocesses, C. Larroche, M.A. Sanromán, G. Du, A. Pandey, Eds., Current Developments in Biotechnology and Bioengineering: Bioprocesses, Bioreactors and Controls, Elsevier, Amsterdam, Netherlands, 2017, pp. 143–167.
[52] J.P. Bassin, M. Dezotti, Moving Bed Biofilm Reactor (MBBR), in: Advanced Biological Processes for Wastewater Treatment, Springer, Cham, 2018, pp. 37–74.
[53] C. Quintelas, B. Fernandes, J. Castro, H. Figueiredo, T. Tavares, Biosorption of Cr(VI) by a *Bacillus coagulans* biofilm supported on granular activated carbon (GAC), Chem. Eng. J., 136 (2008) 195–203.
[54] H. Liu, H.H.P. Fang, Characterization of electrostatic binding sites of extracellular polymers by linear programming analysis of titration data, Biotechnol. Bioeng., 80 (2002) 806–811.
[55] D.C.K. Ko, J.F. Porter, G. Mckay, Optimised correlations for the fixed-bed adsorption of metal ions on bone char, Chem. Eng. Sci., 55 (2000) 5819–5829.
[56] T.V.N. Padmesh, K. Vijayaraghavan, G. Sekaran, M. Velan, Biosorption of Acid Blue 15 using fresh water macroalga

Azolla filiculoides: batch and column studies, Dyes Pigm., 71 (2006) 77–82.
[57] E. Malkoc, Y. Nuhoglu, Investigations of nickel(II) removal from aqueous solutions using tea factory waste, J. Hazard. Mater., 127 (2005) 120–128.
[58] M. Badia-Fabregat, D. Lucas, T. Tuomivirta, H. Fritze, T. Pennanen, S. Rodríguez-Mozaz, D. Barceló, G. Caminal, T. Vicent, Study of the effect of the bacterial and fungal communities present in real wastewater effluents on the performance of fungal treatments, Sci. Total Environ., 579 (2017) 366–377.
[59] R. De Philippis, G. Colica, E. Micheletti, Exopolysaccharide-producing cyanobacteria in heavy metal removal from water: molecular basis and practical applicability of the biosorption process, Appl. Microbiol. Biotechnol., 92 (2011) 697–708.
[60] E. Blanchet, E. Desmond, B. Erable, A. Bridier, T. Bouchez, A. Bergel, Comparison of synthetic medium and wastewater used as dilution medium to design scalable microbial anodes: application to food waste treatment, Bioresour. Technol., 185 (2015) 106–115.
[61] F. Bourgeois, F. Monette, D.G. Cyr, Operational modifications for the development of nitrifying bacteria in a large-scale biological aerated filter and its impact on wastewater treatment, Water Sci. Technol., 78 (2018) 1–11.
[62] M.E. Casas, K. Bester, Can those organic micro-pollutants that are recalcitrant in activated sludge treatment be removed from wastewater by biofilm reactors (slow sand filters)?, Sci. Total Environ., 506–507 (2015) 315–322.
[63] C. Quintelas, B. Fernandes, J. Castro, H. Figueiredo, T. Tavares, Biosorption of Cr(VI) by three different bacterial species supported on granular activated carbon – a comparative study, J. Hazard. Mater., 153 (2008) 799–809.
[64] K. Weinberger, Process and Facility for Treating Ammonium-Containing Wastewater, U.S. Patent No. 9,969,637, U.S. Patent and Trademark Office, Washington, D.C., 2018.
[65] J. Wang, C. Chen, Biosorption of heavy metals by *Saccharomyces cerevisiae*: a review, Biotechnol. Adv., 24 (2006) 427–451.
[66] P.R. Gogate, A.B. Pandit, A review of imperative technologies for wastewater treatment I: oxidation technologies at ambient conditions, Adv. Environ. Res., 8 (2004) 501–551.
[67] I.E. Mejias Carpio, G. Machado-Santelli, S. Kazumi Sakata, S.S. Ferreira Filho, D.F. Rodrigues, Copper removal using a heavy-metal resistant microbial consortium in a fixed-bed reactor, Water Res., 62 (2014) 156–166.
[68] R. Pan, L. Cao, R. Zhang, Combined effects of Cu, Cd, Pb, and Zn on the growth and uptake of consortium of Cu-resistant *Penicillium* sp. A1 and Cd-resistant *Fusarium* sp. A19, J. Hazard. Mater., 171 (2009) 761–766.
[69] S. Sharma, A. Adholeya, Detoxification and accumulation of chromium from tannery effluent and spent chrome effluent by *Paecilomyces lilacinus* fungi, Int. Biodeterior. Biodegrad., 65 (2011) 309–317.
[70] H. Salehizadeh, S.A. Shojaosadati, Removal of metal ions from aqueous solution by polysaccharide produced from *Bacillus firmus*, Water Res., 37 (2003) 4231–4235.
[71] B. Jha, D.N. Singh, Basics of Zeolites, In: Fly Ash Zeolites, Advanced Structured Materials, Vol. 78, Springer, Singapore, 2016, pp. 5–31.