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# Chromium removal by newly developed microbial consortia supported on wood husk

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**ABSTRACT**

The resistance of microbial consortia to environmental challenges offers great stability to its members. In this work, biofilms of heavy-metal resistant microbial consortia supported on wood husk were studied for Cr(VI) removal. The theoretical biofilm adhesion of microorganisms on the support was studied using Extended Derjaguin–Landau–Verwey–Overbeek. The theoretical results were confirmed by scanning electron microscopy visualization. Batch experiments were conducted to investigate the biosorption of Cr(VI) from aqueous solutions onto microbial cells. The yeast–yeast consortia showed an antagonistic relationship reducing the chromium removal efficiency, while a synergetic action was noted between bacterial and yeast strains. Compared to the pure yeast culture, the microbial consortia demonstrated greater efficiency for chromium remediation. It allowed the reduction of the treatment time by 20%, signifying a complementary interaction among microbial strains. In the open system, the Cr(VI) elimination of the pure culture of *Wickerhamomyces anomalus* was compared to that obtained with *W. anomalus* – bacteria consortia. The best performance was obtained by *W. anomalus* – *Bacillus* sp. consortium. It was able to eliminate 97% of the Cr(VI) at an initial concentration of 50 $mg \cdot L^{-1}$. Our results demonstrate that the employment of microbial consortia is advantageous in the chromium removal from contaminated environments.

*Keywords:* Chromium; Biofilm; Consortium; Biosorption; Wood husk

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## 1. Introduction

Chromium contaminated water is still a growing concern constantly threatening the environment. This pollutant is mainly characterized by a marked persistence against chemical or biological transformation, with high environmental mobility and strong tendency for bioaccumulation in the food chain. It is hence posing an alarming threat to environment and public health [1]. Major sources of this metal are tanneries, electroplating and metal finishing industries [2].

Microbial biosorption is a fast growing field in metal remediation where microorganisms are involved in order to decontaminate and degrade one or various pollutants to nontoxic or less harmful compounds [3]. It provides many advantages over other commonly used technics for this purpose [4].

In contrast to pure culture, the use of mixed cultures for biosorption aims has several advantages [5]. In fact, microorganisms are working either individually or coordinately within microbial consortia, which are natural microbial associations including different species acting together in a complex system. Within a microbial consortium, the conduction of the cyclic growth and the nutrient flow is more efficient and effective [6]. Indeed, a microbial consortium has shown a better remediation performance of textile effluent decolorizing and heavy metals recovery compared to individual bacteria [7]. An earlier work has reported the potential use of an environmental bacterial consortium to remove many heavy metals including chromium from a synthetic wastewater effluent [5]. It was also reported that bacteria within mixed cultures used for Cr(VI) removal are presenting a higher survival and stability, especially in highly contaminated areas [8]. The use of bacterial consortia for heavy metals remediation is reported in many papers [9,10]. However, the works involving yeast-bacteria consortia are relatively rare. Nonetheless, yeast strains have shown a prominent efficiency in the bioremoval of various pollutants including heavy metals [11,12].

The pertinent use of microbial biofilms in heavy metals remediation has been intensively highlighted. It has been used efficiently for metal removal from industrial effluents [13,14]. It presents beyond any doubt many advantages because of greater tolerance to pollutants, environmental stress, and ability to degrade various pollutants via diverse catabolic pathways [15,16].

Different materials were involved in microbial cell attachment in this technology [17,18]. Among these materials, the wood husk provides a great ability to attach microbial cells and constitutes a source of cellulose for these [19]. Hence successfully employed for remediation application [17].

Among the biofilm formation steps, biofilm adhesion is certainly a crucial one [20]. During its functional lifetime, biofilm attachment plays an essential role in the long-term stability of the microbial system. It hence constitutes a key factor for its performance [21].

The aim of the present investigation was to evaluate the recovery of chromium ions from aqueous solutions of Cr(VI) by microbial consortia supported on the wood husk. Thus, microbial biofilm consortia constituted of bacteria and yeasts previously isolated from contaminated environments were developed.

Interestingly, the use of wood husk as biofilm support offers several advantages including cost effectiveness, high efficiency and minimization of chemical/biological sludge. Moreover, to our knowledge, the studies dealing with biofilm-based bioremediation overlook the crucial importance of microbial adhesion in system stability. Furthermore, despite their potential use in the treatment of wastewater, yeasts remain unsufficiently exploited. Keeping in view the above background, the aims of this study were to: (i) the theoretical prediction of the adhesion ability of the microorganisms to wood husk surface, using the Extended Derjaguin–Landau–Verwey–Overbeek (XDLVO) predictive theory, (ii) the study of the contact time between wood husk and microbial cells, for its direct influence on the biofilm stability and (iii) the comparison, in batch experiments, of Cr(VI) removal by the different biofilm consortia with the single culture of the yeast.

## 2. Materials and methods

### 2.1. Microorganisms and culture conditions

Three chromium-resistant and removing yeast *Cyberlindnera fabianii*, *Wickerhamomyces anomalus* and *Candida tropicalis* and four bacteria *Bacillus* sp., *Bacillus cereus*, *Enterococcus faecium* and *Enterococcus mundtii* were studied in this work. These microorganisms were previously isolated from sediment and wastewater samples, contaminated with chromium resulting from chemical industrial including tanning processing in Fez, Morocco [22–24]. The used culture media were Luria broth (LB) medium agar plates (1% peptone, 1% NaCl, 0.5% yeast extract, and 1.5% agar) and the yeast medium agar plates (1% peptone, 1% yeast extract, 2% glucose and 1.5% agar) for bacterial and yeast strains, respectively. They were then incubated for 48 h at 30°C.

### 2.2. Development of microbial consortia

The mono-biofilm of *W. anomalus* has previously proved a remarkable Cr(VI) resistance and high Cr(VI) removal ability [25]. In this work, we investigate its chromium removing ability associated with bacterial and yeast strains within consortia biofilms.

Consortia were prepared according to Kurade et al. [26] by aseptically transferring 100 µL of *W. anomalus* culture grown at 30°C in 100 mL modified yeast medium (20% glucose, 1% peptone, 1% yeast extract) and a volume of 100 µL of the culture biomass of bacteria or yeast strains grown in Luria Broth (LB) medium for bacteria and yeast medium (YPG) for yeast strains, grown for 24 h at 30°C to the 250 mL Erlenmeyer flask containing the biofilm support (wood husk).

### 2.3. Preparation of the support

Wood husk was obtained from a local wood industry Fez, Morocco. In order to remove any particulate, it was extensively washed and sprayed with distilled water. This material was crushed, dried at 60°C, and sieved through a 1–5 mm mesh sieve before its further usage as support for the microbial biofilm formation destined for Cr(VI) removal [25].

### 2.4. Contact angle measurements

The bacterial strains suspension for cell surface characterization using contact angle measurements (CAM) was prepared following the protocol of Mohd-Al-Faisal et al. [27] with slight modifications as described by the study of Asri et al. [28]. Microbial lawns suitable for CAM were prepared as described by the study of Soumya et al. [19] and carried out in triplicate with separated cultured microbes.

The physicochemical characterization of the studied yeast strains and the assessment of their adhesion to wood husk was carried out and reported in previous work [29].

#### 2.4.1. Hydrophobicity

Vogler's approach [30] expresses the qualitative hydrophobicity of a surface according to its water contact angle value ($\theta_W$). At a $\theta_W$ value higher than 65°, the surface is qualified as hydrophobic, while a $\theta_W$ value lower than 65° permits to classify the surface as being hydrophilic.

Eq. (1) of Van Oss' approach allows the evaluation of the absolute degree of hydrophobicity of a surface [31]. This parameter is expressed as the free energy of interaction between two identical surfaces in a humid environment (ΔGiwi). If ΔGiwi < 0, the surface is considered hydrophobic. If ΔGiwi > 0 the surface is considered hydrophilic.

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

where $\gamma_i^{\text{LW}}$ is the Lifshitz–van der Waals component, $\gamma_w^{\text{LW}}$ is the Lifshitz–van der Waals component of water, $\gamma_i^+$ is the electron acceptor of a given material (*i*), $\gamma_i^-$ 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.

#### 2.4.2. Surface tension components

The contact angle measurements allows also the determination of the Lifshitz–van der Waals ($\gamma^{\text{LW}}$) and acid–base ($\gamma^{\text{AB}}$) surface tension components [31]. By using three different liquids with known surface parameters values $\gamma_L^{\text{LW}}$, $\gamma_L^+$, and $\gamma_L^-$, the unknown surface tension components of a solid surface ($\gamma_s^{\text{LW}}$, $\gamma_s^+$, and $\gamma_s^-$) or microbial surface ($\gamma^{\text{LW}}$, $\gamma^+$, and $\gamma^-$) can be determined.

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

where θ is the measured contact angle; $\gamma^{\text{LW}}$ is the Lifshitz–van der Waals component of the surface free energy; $\gamma^+$ and $\gamma^-$ are the electron acceptor and electron donor parameters; $\gamma^{\text{AB}}$ is the Lewis acid–base component. The surface free energy is expressed as:

$$\gamma_S = \gamma_S^{\text{LW}} + \gamma_S^{\text{AB}} \quad (3)$$

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

#### 2.4.3. Prediction of the adhesion of the yeast strain to wood surface by XDLVO theory

XDLVO approach describes the total interaction energy between a microorganism (m) and substratum (s) through water (w) as follows:

$$\Delta G^{\text{XDLVO}}(d) = \Delta G^{\text{LW}}(d) + \Delta G^{\text{EL}}(d) + \Delta G^{\text{AB}}(d) \quad (4)$$

In this equation, *d* refers to the separation distance between a cell and a substratum.

$$\begin{aligned} \Delta G^{\text{LW}} = &\left(\left(\gamma_M^{\text{LW}}\right)^{1/2} - \left(\gamma_S^{\text{LW}}\right)^{1/2}\right)^2 \\ &-\left(\left(\gamma_M^{\text{LW}}\right)^{1/2} - \left(\gamma_L^{\text{LW}}\right)^{1/2}\right)^2 \\ &-\left(\left(\gamma_S^{\text{LW}}\right)^{1/2} - \left(\gamma_L^{\text{LW}}\right)^{1/2}\right)^2 \end{aligned} \quad (5)$$

and

$$\Delta G^{\text{AB}} = 2\begin{bmatrix} \left(\gamma_L^+\right)^{1/2}\left(\left(\gamma_M^-\right)^{1/2} + \left(\gamma_S^-\right)^{1/2} - \left(\gamma_L^-\right)^{1/2}\right) \\ +\left(\gamma_L^-\right)^{1/2}\left(\left(\left(\gamma_M^+\right)^{1/2} + \left(\gamma_S^+\right)^{1/2} - \left(\gamma_L^+\right)^{1/2}\right) - \left(\gamma_L^-\gamma_S^+\right)^{1/2}\right) \\ -\left(\gamma_L^+\gamma_S^-\right)^{1/2} \end{bmatrix} \quad (6)$$

The usage of a suspension liquid with high ionic strength ($KNO_3$ 0.1 M) was reported to allow the negligence of electrostatic interaction free energy $\Delta G^{\text{EL}}$ as done before by the studies of Elabed et al. [20], Gallardo-Moreno et al. [32], Rijnaarts et al. [33].

### 2.5. Biosorption assays

#### 2.5.1. Batch system

All laboratory glassware was washed twice with deionized water after being soaked in nitric acid (60%, v/v) bath overnight [25].

Yeast strain growth was obtained at 30°C, using YPG under continuous shaking at 130 rpm. After 24 h of incubation, the biomass was centrifuged at 7,000 g for 10 min [25].

Batch experiments were realized in a modified YPG medium (0.2% peptone, 0.2% yeast extract, 2% glucose) prepared with sterile distilled water as proposed by the study of Bahafid et al. [22]. The initial concentrations of Cr(VI) as $K_2Cr_2O_7$ contained in the medium were 25, 50, and 100 mg·L$^{-1}$ and the final pH of the solution was adjusted by adding either 0.1 M HCl or 0.1 M NaOH. An uninoculated sterilized medium prepared rigorously using the same protocol served to determine the amount of the abiotic Cr(VI) removal. Experiments were conducted in 250 Erlenmeyer flasks under an agitation system (130 rpm) in a shaker incubator at 30°C [23]. Samples of 1 mL were taken periodically, centrifuged and the residual Cr(VI) concentration was determined by

colorimetry using the 1,5-diphenylcarbazide method [34]. The removal percentage was calculated as:

$$\text{Biosorption potential \%} = \frac{\left(\begin{array}{c}\text{Initial concentration of Cr}\\ -\text{ Measured concentration of Cr}\end{array}\right)}{\text{Initial concentration of Cr}} \times 100 \quad (7)$$

In order to evaluate the effect of contact time between the wood husk and microbial cells on the adsorption of Cr(VI) by developed consortia, batch assays were conducted by varying this parameter from 0 to 60 h, at the initial Cr(VI) concentration of 50 mg·L$^{-1}$. Samples were taken periodically after 0.5 h intervals for chromium quantification.

At optimal contact time, the biosorption capacity of the performant *W. anomalus* – bacteria consortia was evaluated at an initial Cr(VI) concentration of 100 mg·L$^{-1}$ in comparison to the pure culture of *W. anomalus*.

After a contact period of 48 h between wood and microbial strains, samples of the biofilm were taken and imaged by using environmental scanning electron microscopy (ESEM Quanta 200) after dehydration with different concentrations of ethanol [35].

#### 2.5.2. Open system

The open-system assays were executed in Plexiglas columns (height: 33 cm, internal ø 3.5 cm). They were filled with 96 cm$^3$ *W. anomalus* and the consortia biofilms attached to the wood husk, while a control assay column was filled with sterilized wood husk [25]. The biofilm formation on the substratum was carried out following the protocol of Quintelas et al. [35]. Afterwards, Cr(VI) aqueous solution at an initial concentration of 50 mg·L$^{-1}$ was pumped through the columns with a flow rate of 5 mL min$^{-1}$. The total chromium and Cr(VI) concentrations in the outflow were periodically evaluated [25].

## 3. Results and discussion

### 3.1. Wood surface characteristics

The characterization of the wood surface has been the subject of many works [19,36,37]. Its surface free energy presents a wide range of variations in the literature due to the chemical heterogeneity and the biodiversity of wood species. It contains mainly (37%–51%) cellulose, (20%–30%) hemicellulose, (20%–30%) lignin and (1%–5.5%) extracts [36].

In this work, the used wood exhibits a free surface energy value of –59.75 ± 1.04 mJ·m$^{-2}$, an electron donor character of 4.45 ± 0.43 mJ·m$^{-2}$ and an acceptor character of electrons with a value of 0.69 ± 0.10 mJ·m$^{-2}$ (Table 1). These results corroborate those found in previous work [19]. It has been reported that the wood surface exhibits an hydrophobic character, a dominant electron donor character and a low acceptor character of electrons. According to the literature, the electron acceptor character ($\gamma^+$) of wood is due to the presence of acidic hydrogen atoms on its macromolecules, principally phenolic functions of hemicellulose and lignin and carboxylic acids, while its electron donor character ($\gamma^-$) is in correlation with the oxygen/carbon ratio of lignin and polysaccharides present in the wood [37].

### 3.2. Microbial cell surface characteristics

For the characterization of microbial cell surface properties, several techniques such as contact angle measurements by the sessile drop can be used [38]. In this work, it served for the physicochemical characterization of the surfaces of four bacterial strains using the sessile drop of test liquids, which were diiodomethane ($\theta_D$), formamide ($\theta_F$), and water ($\theta_W$). This technique allows obtaining valuable information on cell surface hydrophobicity and electron donor/acceptor character.

The results of hydrophobicity expressed qualitatively ($\theta_W$) and quantitatively (in terms of ΔGiwi) are compiled in Table 2. It shows that the strain *C. fabianii* exhibits an hydrophobic character with a negative value of ΔGiwi –2.33 ± 1.06 mJ·m$^{-2}$.

On the other hand, *E. faecium*, *B. cereus*, *E. mundtii*, *Bacillus* sp., *C. tropicalis* and *W. anomalus* have an hydrophilic character with qualitative hydrophobicity values ranging from 12.87° ± 0.61° to 48.40° ± 0.74° and positive values of the free energy of interaction ΔGiwi ranging from 17.25 ± 4.05 to 38.79 ± 3.44 mJ·m$^{-2}$.

Numerous studies aimed to comprehend microbial cell surface properties. A strong correlation between bacterial surface hydrophobicity and the chemical composition of cell surface has been previously suggested. In fact, low protein/carbohydrate ratios were characteristic of the most hydrophilic microbial surfaces [39].

According to Mozes et al. [40], the hydrophobicity of bacteria is directly related to the concentration of carbon in the hydrocarbon form. This character is inversely related to oxygen concentration or to the N/P ratio, while for yeasts; hydrophobicity is directly related to the N/P ratio [39,41].

As for yeast strains, their surface hydrophobicity was strongly correlated to the surface protein concentration [41–43], while no relation was reported with hydrocarbon compounds or polysaccharide concentrations [44].

Results concerning electron donor/acceptor character are also listed in Table 1. All isolated strains are mostly

Table 1
Contact angle values using water $\theta_W$, formamide $\theta_F$, and diiodomethane $\theta_D$, Lifshitz–van der Waals $\gamma^{LW}$, electron donor $\gamma^-$ and electron acceptor $\gamma^+$ parameters and surface energies (ΔGiwi) of wood

| Material | Contact angles (°) | | | Surface tension: components and parameters (mJ·m$^{-2}$) | | | Surface energy |
|---|---|---|---|---|---|---|---|
| | $\theta_W$ | $\theta_F$ | $\theta_D$ | $\gamma^{LW}$ | $\gamma^+$ | $\gamma^-$ | ΔGiwi (mJ·m$^{-2}$) |
| Wood | 73.33 ± 0.35 | 39.57 ± 0.71 | 19.93 ± 0.49 | 47.70 ± 0.15 | 0.69 ± 0.10 | 4.45 ± 0.43 | –59.75 ± 1.04 |

Table 2
Contact angle values using water $\theta_W$, formamide $\theta_F$, and diiodométhane $\theta_D$, Lifshitz–van der Waals ($\gamma^{LW}$), electron donor ($\gamma^-$) and electron acceptor ($\gamma^+$) parameters and surface energies (ΔGiwi) of bacterial and yeast strains

| Bacterial strains | Contact angles (°) | | | Surface tension: components and parameters (mJ·m$^{-2}$) | | | Surface energy | References |
|---|---|---|---|---|---|---|---|---|
| | $\theta_W$ | $\theta_F$ | $\theta_D$ | $\gamma^{LW}$ | $\gamma^+$ | $\gamma^-$ | ΔGiwi (mJ·m$^{-2}$) | |
| *Enterococcus faecium* | 12.87 ± 0.61 | 13.13 ± 1.10 | 27.73 ± 0.21 | 45.04 ± 0.08 | 0.49 ± 0.03 | 54.53 ± 0.29 | 32.21 ± 0.02 | |
| *Bacillus cereus* | 18.93 ± 0.59 | 25.83 ± 2.56 | 30.77 ± 1.12 | 43.81 ± 0.47 | 0.18 ± 0.09 | 57.02 ± 1.83 | 38.79 ± 3.44 | [28] |
| *Enterococcus mundtii* | 17.20 ± 0.60 | 8.10 ± 1.22 | 27.47 ± 0.15 | 45.14 ± 0.06 | 0.69 ± 0.03 | 50.43 ± 0.49 | 26.13 ± 0.73 | |
| *Bacillus* sp. | 35.4 ± 0.325 | 44.33 ± 1.26 | 17.37 ± 1.64 | 48.41 ± 0.03 | 0.57 ± 0.11 | 55.59 ± 0.11 | 30.84 ± 1.36 | This work |
| *Wickerhamomyces anomalus* | 33.50 ± 0.84 | 49.30 ± 1.16 | 82.50 ± 1.03 | 16.18 ± 0.51 | 3.19 ± 0.34 | 60.14 ± 1.30 | 34.58 ± 2.24 | |
| *Cyberlindnera fabianii* | 51.00 ± 1.21 | 35.60 ± 1.90 | 81.10 ± 1.38 | 16.87 ± 0.69 | 9.85 ± 0.75 | 23.51 ± 1.30 | –2.33 ± 1.06 | [29] |
| *Candida tropicalis* | 48.40 ± 0.74 | 50.40 ± 2.14 | 74.20 ± 0.43 | 20.48 ± 0.24 | 2.49 ± 0.15 | 39.51 ± 2.85 | 17.25 ± 4.05 | |

electron donors with relatively high values of γ− ranging from 23.51 ± 1.30 to 60.14 ± 1.30 mJ·m$^{-2}$ and present a weak electron acceptor character with low values of $\gamma^+$ ranging from 0.18 ± 0.09 to 9.85 ± 0.75 mJ·m$^{-2}$.

In the same context, several studies have been devoted to the assessment of the chemical elements related to electron donor-acceptor characters. Several studies have investigated the correlation between these characters and the surface chemical composition using many techniques, notably X-ray spectrophotometry (XPS) [45,46]. The electron donor character has been correlated to the presence of basic groups on the microbial surface, such as carboxylate, hydroxyl, phosphate, amino and imidazole groups [47,48].

For yeast strains, the presence of phosphate groups in their cells wall offers a Lewis basic character to their surfaces [41,45].

### *3.3. Prediction of bacterial strains adhesion to a wood husk by XDLVO theory*

Wood is a material likely to be colonized by microbial biofilm causing much damage to its surfaces [49]. However, studies on microbial adhesion phenomena on wooden substratum are very restricted [19].

The determination of a stable state of the attached microbial biofilm remains a serious challenge for the scientific community. Although the mechanisms governing microbial adhesion are still poorly understood and have not been fully defined, it is recognized that the physicochemical properties of microbial membranes and supports are determinants of initial adhesion [50,51]. Thus, several theoretical models have been proposed to predict the phenomena of adhesion and stability of microbial biofilms. This prediction serves either for the prevention of harmful biofilm formation (for example, causing nosocomial infections in hospital areas) or for the enhancement of beneficial biofilms such as in bioremediation applications.

Theoretical physico-chemical predictive models have been proven useful in microbial attachment explication, taking into account the physicochemical properties of both microbial cells and support surfaces [49]. Nevertheless, very few works did employ the XDLVO theory approach to study the interactions between the support and microbial cells with the aim of developing a new bioprocess for wastewater treatment [29]. Indeed, among the most reviewed predictive approaches, XDLVO developed by van Oss has proved to predict and explain the results of experimental adhesion [20,49]. This theory has been established by the addition of AB interactions, comprising both the electron-donating and electron-accepting energies. Hence, it takes into account the three types of physicochemical interactions responsible for initial adhesion: Van der Waals, acid–base and electrostatic.

In this investigation, the theoretical bacterial adhesion ability on wood was studied using XDLVO approach. This would contribute to the evaluation of the compatibility microorganism-support aiming the long-term stability of the bioremediation system. According to XDLVO approach, the adhesion of a microorganism is energetically favored only when $\Delta G^{TOT}$ is negative. The obtained results show that the values of $\Delta G^{TOT}$ are –5.66, –5.35, –7.56 and –5.29 mJ·m$^{-2}$, respectively for bacterial strains *E. faecium*, *B. cereus*, *E. mundtii* and *Bacillus* sp. (Table 3). For yeast strains, the values of $\Delta G^{TOT}$ are –0.73, –10.42 and –9.34 mJ·m$^{-2}$ for *W. anomalus*, *C. fabianii* and *C. tropicalis*, respectively. These negative values show that the attachment of the tested bacterial strains to the used wood is favorable. This material might therefore be appropriate to be recycled in the wastewater treatment process as a support for microbial biofilm development.

### *3.4. Biosorption studies using the developed consortia at different initial Cr(VI) concentration*

Earlier reports have shown the resistance of the bacteria and fungi grown in a consortium to high metal concentrations compared to those tolerated by pure cultures. They also proved a high metal accumulation ability of these mixed cultures [52,53]. However, there are no earlier reports on an effective yeast-bacterial consortium attached to wood

Table 3
Lifshitz–van der Waals ($\Delta G^{LW}$) (mJ·m$^{-2}$), acid–base ($\Delta G^{AB}$) (mJ·m$^{-2}$) and total free energy of adhesion between bacterial and yeast cells and the support ($\Delta G^{XDLVO}$)

| | Interaction free energy $\Delta G^{XDLVO}$ (mJ·m$^{-2}$) | | | | | |
|---|---|---|---|---|---|---|
| | $\gamma^{LW}$ | $\gamma^{+}$ | $\gamma^{-}$ | $\Delta G^{LW}$ | $\Delta G^{AB}$ | $\Delta G^{TOT}$ |
| Wood | 47.7 | 0.69 | 4.45 | | | |
| *Enterococcus faecium* | 45.04 | 0.49 | 54.53 | –4.59 | –1.07 | –5.66 |
| *Bacillus cereus* | 43.81 | 0.18 | 57.02 | –4.38 | –0.97 | –5.35 |
| *Enterococcus mundtii* | 45.14 | 0.69 | 50.43 | –4.61 | –2.96 | –7.56 |
| *Bacillus* sp. | 48.41 | 0.57 | 55.59 | –5.14 | –0.15 | –5.29 |
| *Wickerhamomyces anomalus* | 16.81 | 3.19 | 60.14 | 2.90 | –3.63 | –0.73 |
| *Cyberlindnera fabianii* | 16.87 | 9.85 | 23.51 | 2.52 | –12.94 | –10.42 |
| *Candida tropicalis* | 20.48 | 2.49 | 39.51 | 0.65 | –9.99 | –9.34 |

husk utilized for Cr(VI) removal. Hence, in this work we evaluated the applicability of *W. anomalus* consortia within biofilms fixed to wood husk surfaces, for the removal of chromium from aqueous solutions.

*W. anomalus* is the yeast strain that showed the best chromium removal efficiency among the tested yeasts and a favorable attachment to the wood husk. Hence, it was selected to be studied among consortia for Cr(VI) depollution. Indeed, the Cr(VI) removal capacity by *W. anomalus* consortia consisting of the yeast *W. anomalus* mixed with the bacterial strain *Bacillus* sp., *B. cereus*, *E. mundtii* or *E. faecium* or with the yeast strains *C. fabianii* or *C. tropicalis* has been investigated.

The removal kinetics of Cr(VI) by each consortium compared to the single yeast culture at different initial chromium concentrations are shown in Fig. 1.

At an initial chromium concentration of 25 mg·L$^{-1}$ (Fig. 1A), the consortia consisting of strains *W. anomalus* – *E. faecium*, *W. anomalus* – *E. mundtii* and *W. anomalus* – *B. cereus* showed higher performances than *W. anomalus* pure culture. These mixed cultures were able to remove the total amount of chromium in only 24 h. *W. anomalus* – *Bacillus* sp. and *W. anomalus* – *C. fabianii* consortia showed a slightly lower efficiency. The removal of the total amount of Cr(VI) by these consortia required 48 h of treatment, whereas the pure culture of *W. anomalus* could clean up the total amount of chromium after 96 h of incubation. The *W. anomalus* – *C. tropicalis* consortium was not able to achieve more than 95% of Cr(VI) removal after 144 h. The efficiency of this consortium was markedly lower than the single culture of *W. anomalus*.

At an initial chromium concentration of 50 mg·L$^{-1}$ (Fig. 1B), the *W. anomalus* – bacterial consortia showed a significantly higher Cr(VI) removal capacity compared to the single culture of the yeast strain, while the yeast–yeast consortia showed noticeably lower chromium removal efficiencies.

At an initial chromium concentration of 100 mg·L$^{-1}$ (Fig. 1C), the *W. anomalus* – *E. faecium*, *W. anomalus* – *Bacillus* sp. and *W. anomalus* – *B. cereus* consortia showed comparable Cr(VI) removal capabilities and were able to reach a 100% of Cr(VI) of removal in 72 h. These three consortia in addition to the *W. anomalus* – *E. mundtii* consortium showed slightly lower efficiency than the other yeast-bacteria consortia.

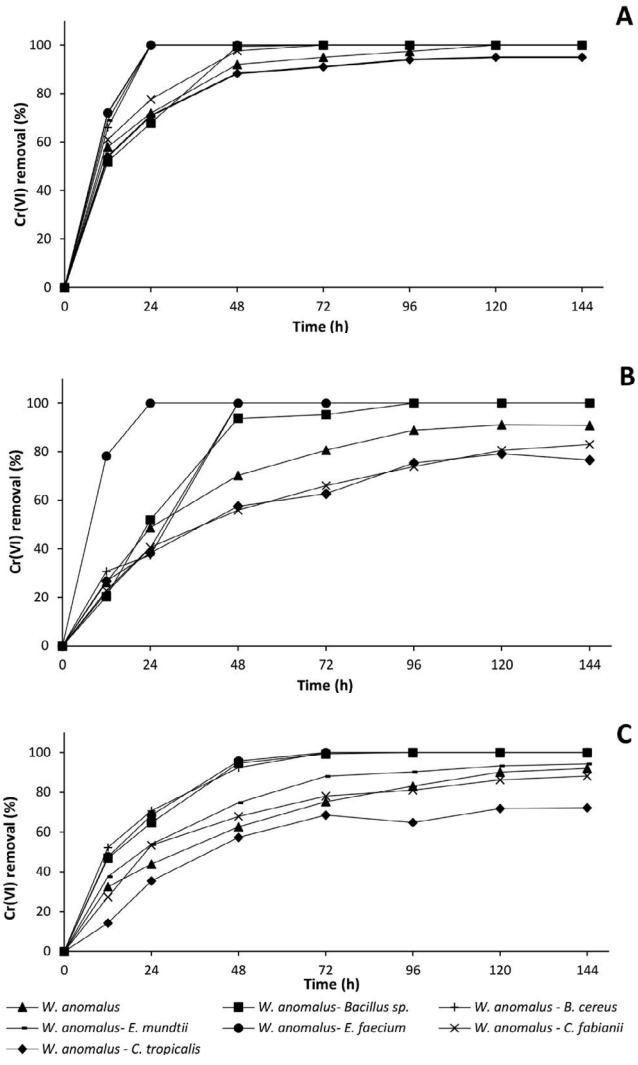


Fig. 1. Comparison of Cr(VI) removal efficiency between *Wickerhamomyces anomalus* single culture and consortia as a function of chromium initial concentration in batch system: (A) 25 mg·L$^{-1}$, (B) 50 mg·L$^{-1}$, and (C) 100 mg·L$^{-1}$.

However, it showed a better depollution capacity compared to the pure culture of *W. anomalus*. On the other hand, the Cr(VI) removal efficiency of the yeast–yeast consortia was slightly lower than for the single culture of *W. anomalus*. Indeed, *W. anomalus* – *C. fabianii* and *W. anomalus* – *C. tropicalis* reached 88.12% and 72.15% of Cr(VI) removal, respectively, after 144 h of incubation.

From the obtained results, it can be noticed that the addition of these bacterial strains has a positive effect on the Cr(VI) removal capacity of the yeast *W. anomalus*. Previous studies have shown the better depollution ability of microbial consortia compared to the action of individual microorganisms. In the work of Jadhav et al. [7], the use of bacterial consortia attained a significantly higher reduction in color, chemical oxygen demand (COD), biological oxygen demand (BOD) and metal ions in a much shorter time. Barsing et al. [54] reported that aromatic amine mineralization was favored with the use of a consortium, compared to pure strains. Further work reported a high performance in the mineralization of dyes, achieved by the consortium system within a short time, compared to individual cultures [26].

These works explain the beneficial use of microbial consortia in wastewater treatment applications by the concerted metabolic activity of the microorganisms. It suggests a complementarity in the functioning (synergy) of the two or more different microbial species.

The use of *W. anomalus* – yeast consortia presented a negative effect on the *W. anomalus* Cr(VI) performance. This can be explained by an antagonistic relationship between the used yeast strains. This antagonism affects the viability and/or the metabolic activity of the microorganisms resulting in a decrease in the efficiency of chromium removal.

### 3.5. Effect of contact time

In biofilm formation, the contact time between the support and the microbial cells is a parameter of extreme importance. However, studies on this parameter are principally investigated within the elimination or the prevention of biofilms [55,56]. Hence, in this study, we proposed to evaluate the effect of this parameter on the depollution capacity.

The experimental runs evaluating the effect of contact time on the batch Cr(VI) removal at the optimal pH value of 3,72 [25] and an initial Cr(VI) concentration of 50 mg·L$^{-1}$ is shown in Fig. 2. Evidently, the Cr(VI) removal efficiency increased with the increase of contact time from 0.5 to 48 h until it reached a maximum value which remains constant over time.

The used consortium and the available adsorption sites on its surface are the main parameters influencing the required time to reach the equilibrium. In fact, for *W. anomalus* – *B. cereus*, the required contact time was 40 h to reach a Cr(VI) removal percentage of 89.12% ± 0.35%. For the consortium constituted of *W. anomalus* – *Bacillus* sp., the needed time was 48 h to reach 88.93% ± 0.42% of Cr(VI) removal. In the case of *W. anomalus* – *E. mundtii*, a contact time of 40 h was needed for equilibrium to be established at 80.26% ± 0.16%. For *W. anomalus* – *E. faecium*, the required time was 44 h to achieve 81.16% ± 0.23%. According to these results, for the subsequent batch experiments of Cr(VI) removal by the mixed cultures of *W. anomalus*, 48 h contact between wood and microbial cells was assumed to be appropriate.

### 3.6. Cr(VI) removal by W. anomalus – bacterial consortia: batch system

A comparison between the Cr(VI) removal capabilities of the single culture of *W. anomalus* and the yeast-bacteria consortia has been performed. This comparison was carried out under optimal pH, support dose and contact time conditions for Cr(VI) removal by *W. anomalus*. The obtained results are illustrated in Fig. 3.

The consortia were able to remove Cr(VI) at significantly higher levels as compared to that achieved by individual strain. Moreover, the treatment rate was significantly faster than the individual strains. The best performance was obtained by the consortium *W. anomalus* – *Bacillus* sp. This consortium could achieve 100% of Cr(VI) removal at an initial concentration of 100 mg·L$^{-1}$ in 77 h. The time needed to achieve the same performance by the consortium *W. anomalus* – *B. cereus* was 90 h. The consortia *W. anomalus* – *E. mundtii* and *W. anomalus* – *E. faecium* attained a Cr(VI) removal of 100% after 96 h, while the required time to remove the total amount of Cr(VI) in the solution by the pure culture of *W. anomalus* was 100 h.

This could be the result of the better ability for stress-handling ability by the consortium owing to the variation in

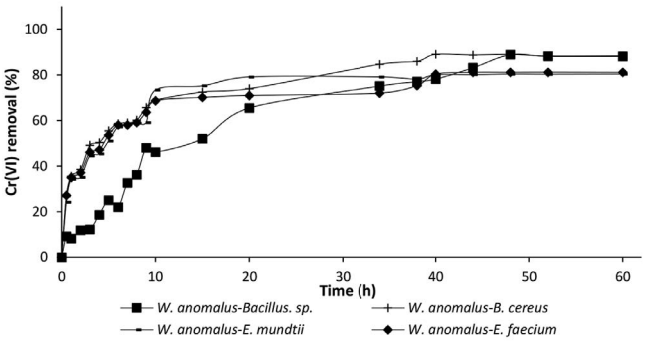


Fig. 2. Effect of contact time on the adsorption of Cr(VI) by the developed consortia: initial Cr(VI) concentration 50 mg·L$^{-1}$, pH 3,72.

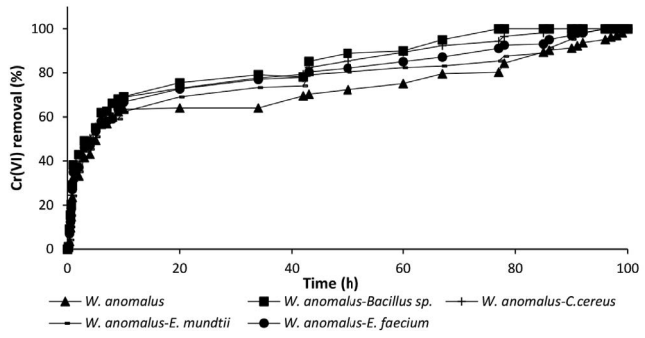


Fig. 3. Removal efficiency of Cr(VI), in batch system by *Wickerhamomyces anomalus* – bacterial consortia at optimal conditions compared to *Wickerhamomyces anomalus* single culture for an initial Cr(VI) concentration of 100 mg·L$^{-1}$.

toxicity response, chromium tolerance, growth inhibitions, and pollutant removal mechanism by different strains [57].

### 3.7. Cr(VI) removal by W. anomalus – bacterial consortia: open system

In an open system, the consortia were able to eliminate Cr(VI) at levels higher than those reached by the pure strain of *W. anomalus*. The best performance was obtained by the consortium *W. anomalus* – *Bacillus* sp. This consortium was able to eliminate 97% of the Cr(VI) at an initial concentration of 50 mg·L$^{-1}$ in 48 h. The *W. anomalus* – *B. cereus* consortium achieved a performance of 82%. The consortia *W. anomalus* – *E. mundtii* and *W. anomalus* – *E. faecium* achieved a Cr(VI) elimination of 70% and 65%, respectively. While the pure culture of *W. anomalus* was able to eliminate 59% of Cr(VI), after 48 h of treatment (Fig. 4).

Concerning the Cr(VI) curve shape, the uptake is relatively rapid during the first 6 h, at the beginning of the biosorption process. This period was followed by a slower decay during the following 3 h. From that point, the Cr(VI) biosorption declines at a much lower rate toward the total removal. Hence, similarly to many previous investigations, the major part of the adsorption of Cr(VI) by microbial biomass takes place within the first phase of the process [58].

The curve shape is similar to many previous results reporting the removal of Cr(VI) by biological materials. Indeed, the removal of this heavy metal was found to be dependent on the initial concentration. The adsorbed amount of chromium increases with the increase of initial concentration [59]. Further, the adsorption is rapid in the early stages; it attains then an asymptotic value for a larger adsorption period [59].

Four mechanisms were proposed for the biosorption of chromium: (a) anionic adsorption to cationic functional groups, (b) adsorption-coupled reduction, (c) anionic and cationic adsorption and (d) reduction and anionic adsorption [60]. In fact, metal binding mechanisms by individual microbial cells are known, however, metals sorption by intact cells and cellular products such as biofilms is still poorly understood. This is due to the multiplicity of mechanisms and interactions involved in these processes [61].

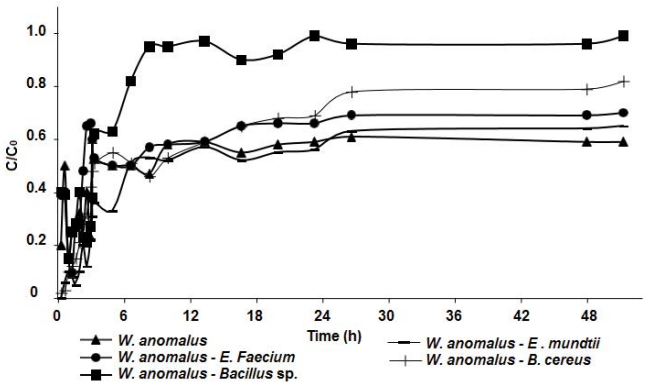


Fig. 4. Breakthrough curves for the elimination of Cr(VI) by consortia *Wickerhamomyces anomalus* – bacteria on wood husk after a contact time of 48 h, compared to the single culture of *Wickerhamomyces anomalus* (flow rate 5 mL·min$^{-1}$, initial Cr concentration 50 mg·L$^{-1}$).

From these experiments, we can conclude that the consortium system provides better results than the single culture. These findings corroborate previous works showing the advantage of the collaborative activity of microbial consortia offering different enzymatic machineries [26]. Indeed, the use of mixed cultures appears to be more feasible and reliable for wastewater treatment systems [62]. The biodiversity within microbial consortia can enhance environmental survival and multiply the catabolic pathways for the biodegradation of the pollutants [63,64]. The potential application of microbial consortia in the environment was previously reported. For instance, Yu et al. [65] have reported the potential use of bacterial consortia for the total degradation of polycyclic aromatic hydrocarbons contained in artificially contaminated sediments. Fungal consortia have also shown a good reduction in various pollution parameters including COD and heavy metals removal [66]. Moreover, the decolorization of textile industry effluents has been efficiently achieved using a bacterial-yeast consortium [26]. Yeast strains have been poorly studied in their use in heavy metals biosorption processes, specifically in Cr(VI) removal [67]. The applicability of yeast strains for environmental biotechnological strategies was mainly studied within single cultures while very few works evaluated the applicability of yeast strains within biofilms or consortia. However, they present numerous advantages such as being versatile microorganisms (they can develop in both aerobic and anaerobic environments) and having low nutritional supply requirements. Moreover, they are considered as safe microorganisms that can be used in both dead and alive states [68].

In many cases, the use of consortia appeared to be advantageous for bioremediation applications and more effective and reliable rather than single cultures. The planktonic mode of microbial growth in aqueous suspensions represents a unique possible survival strategy of microorganisms [69]. The alternative strategy which is immobilized consortia exhibits many advantages. In the work of Heinaru et al. [70], it was suggested that different metabolic pathways for aromatic compounds presented by dominating bacteria in mixed cultures optimized the response to contaminants. Hence, the use of microbial consortia is more effective due to the fact that the intermediates of a catabolic pathway of one strain may be further degraded by other strains [6,70].

Living in mixed cultures or consortia is not only assuring robustness to environmental fluctuations, but also promotes stability through time for the members of a community. Furthermore, consortia might be more resistant to invasion by other species [6]. Communities might also be more capable of resisting the invasion by other species rather than monocultures. They may be able to overcome nutrient limitations because of the diversity of metabolic pathways joined to the capacity to share metabolites inside the microbial community [71]. A three-member fungal consortium removed 100% of Cr(VI) and 81.60% Cu(II) from the metal mixture. This performance was higher than that accomplished individually by the different fungal strains. The complexity of metal-dye mixtures observed in the individual fungus run still stands for the mixed consortium, but the performance was unaltered [57]. Table 4. gives a comparison of the Cr(VI) removal efficiency using different adsorbents.

Table 4
Comparison of the Cr(VI) removal efficiency using different adsorbents

| Adsorbent | Metal initial concentration ($mg \cdot L^{-1}$) | Treatment efficiency | References |
|---|---|---|---|
| *Wickerhamomyces anomalus* | 50 | 59% | This work |
| *Wickerhamomyces anomalus* – *Bacillus* sp consortium | | 97% | |
| *Wickerhamomyces anomalus* – *Bacillus cereus* consortium | | 82% | |
| *Wickerhamomyces anomalus* – *Enterococcus mundtii* | | 70% | |
| *Wickerhamomyces anomalus* – *Enterococcus faecium* | | 65% | |
| *Sphaeroplea* sp. | 50 | 29.8 $mg \cdot g^{-1}$ | [72] |
| *Bacillus coagulans* | 100 | 5.34 $mg \cdot g^{-1}$ | [73] |
| *Arthrobacter viscosus* | 100 | 3 $mg \cdot g^{-1}$ | [2] |
| *Aspergillus niger* | 100 | 80% | [74] |
| *Brevibacillus laterosporus* | 100 | 92% | [75] |

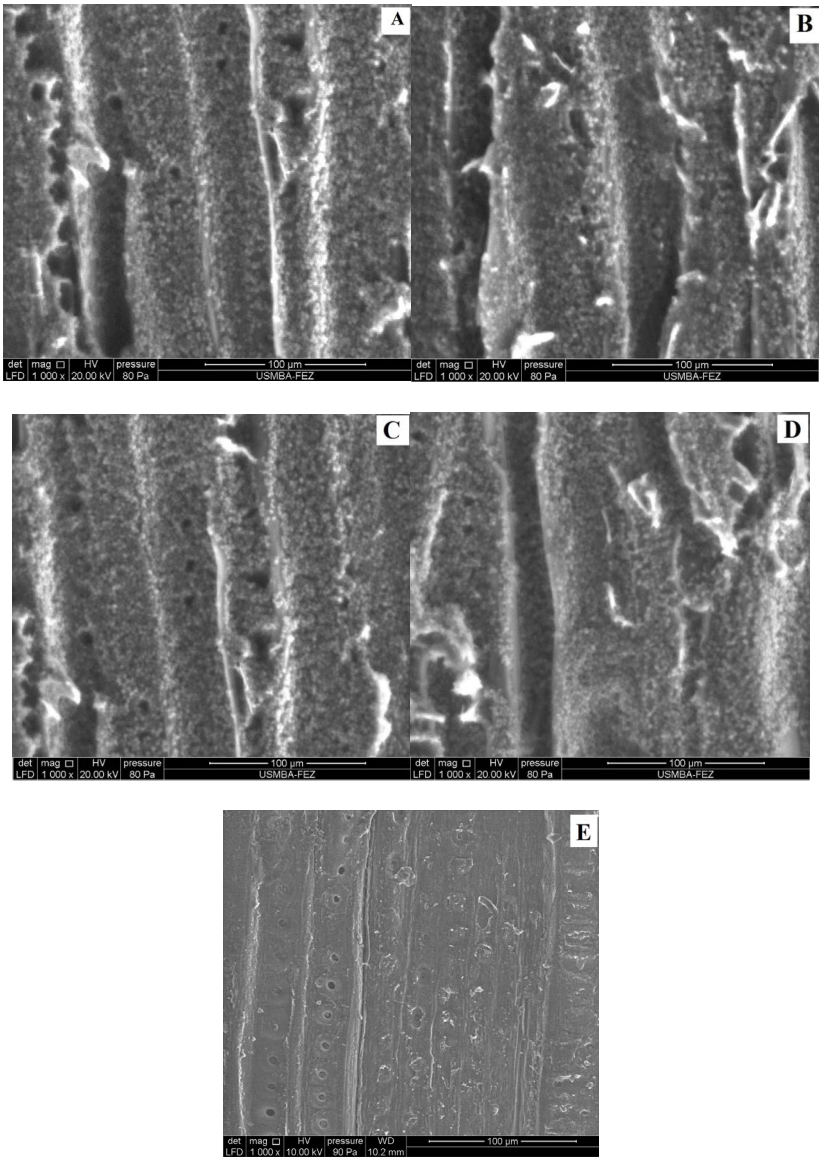


Fig. 5. Images of *Wickerhamomyces anomalus* consortia adhered onto wood husk, visualized by environmental scanning electron microscopy, after 48 h of contact period at 30°C. (A) *Wickerhamomyces anomalus* – *Bacillus* sp., (B) *Wickerhamomyces anomalus* – *Bacillus cereus*, (C) *Wickerhamomyces anomalus* – *Enterococcus mundtii*, (D) *Wickerhamomyces anomalus* – *Enterococcus faecium* and (E) control wood.

*3.8. Visualisation of the consortia adhesion on wood surface*

Scanning electron microscopy (SEM) was used to verify the consortia attachment after 48 h of contact between microbial cells and the wood husk surface. SEM images show the experimental adhesion behavior of the microbial cells on wood husk (Fig. 5) showing attractive interactions toward all tested bacterial strains according to the XDLVO predictions (Table 2). As can be noted from the SEM observations, the wood surface is characterized by the presence of depressions and grooves. This porous nature is conferring its microbial adhesion characteristic and a great number of binding sites for chromium ions fixation [17]. The obtained SEM images illustrate the immobilization of microbial consortia on the wood surface within a compact deposit where the cells were distributed relatively uniformly through the entire substratum surface (Fig. 5).

## 4. Conclusion

The microbial consortia of bacteria and yeasts isolated from heavy metal contaminated sites showed a higher uptake efficiency for Cr(VI) compared to the obtained results with a single culture. XDLVO as a performant predictive tool served to evaluate the thermodynamic compatibility between bacterial cells and wood husk and hence their biofilm formation ability. Batch sorption studies were conducted in order to study the relationship between *W. anomalus* and other yeasts or bacterial stains. Yeast strains showed an antagonistic relationship towards *W. anomalus*, while bacteria exhibited a synergetic one. The evaluation of the contact time showed that 48 h was the required time for the *W. anomalus* – bacterial consortia to attain the equilibrium. The maximum uptake capacity of 100% was obtained for *W. anomalus* – *Bacillus* sp. in 78 h of treatment. In the open system, the best performance was obtained by the *W. anomalus* – *Bacillus* sp. This consortium was able to eliminate 97% of the Cr(VI) at an initial concentration of 50 $mg{\cdot}L^{-1}$ in 48 h. The application of biofilm consortia allowed the reduction of the treatment time compared to the single culture of 20%. The obtained results show that the performant yeast-bacterial consortia designed herein could be employed for chromium removal from industrial effluents.

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## 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.

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## 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.

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