Desalination and Water Treatment
www.deswater.com
doi: 10.5004/dwt.2022.28018

246 (2022) 315–324
January

Biosorbents based on residual biomass of *Lactobacillus* sp. bacteria consortium immobilized in sodium alginate for Orange 16 dye retention from aqueous solutions

Daniela Șuteu[a], Carmen Zaharia[b,*], Alexandra Cristina Blaga[a], Anisoara Catalina Peptu[c]

[a]*Department of Organic, Biochemical and Food Engineering, Faculty of Chemical Engineering and Environmental Protection, "Gheorghe Asachi" Technical University of Iasi-Romania, "Cristofor Simionescu" 73A Prof. Dr. Docent D. Mangeron Blvd., 700050, Iasi, Romania, emails: danasuteu67@yahoo.com (D. Șuteu), acblaga@tuiasi.ro (A.C. Blaga)*
[b]*Department of Environmental Engineering and Management, Faculty of Chemical Engineering and Environmental Protection, "Gheorghe Asachi" Technical University of Iasi-Romania, "Cristofor Simionescu" 73A Prof. Dr. Docent D. Mangeron Blvd., 700050, Iasi, Romania, email: czah@ch.tuiasi.ro (C. Zaharia)*
[c]*Department of Natural and Synthetic Polymers, Faculty of Chemical Engineering and Environmental Protection, "Gheorghe Asachi" Technical University of Iasi-Romania, "Cristofor Simionescu", 73A Prof. Dr. Docent D. Mangeron Blvd., 700050, Iasi, Romania, email: catipeptu@yahoo.co.uk (A.C. Peptu)*

Received 30 September 2021; Accepted 29 November 2021

ABSTRACT

Residual biomass from the lactic acid production (fermented dairy industry) represents a viable and important source of valuable compounds (nutrient, material) and energy which can be used as raw material for the production of a wide range of new added-value products and in certain valorisation processes and sanitation services. The aim of this work is to use the prepared biosorbent from the residual biomass (a *Lactobacillus plantarum* and *Lactobacillus casei* bacteria consortium, separated after the biosynthesis process by centrifugation and dried at 80°C) immobilized in sodium alginate (by cell inclusion technology) (in form of spherical beads with 0.5 and 1.5 mm in diameter) into a specific biosorption treatment step applied for certain aqueous solutions containing the reactive Orange 16 dye (28.96–231.68 mg/L). The biosorption potential of this imobilized residual bacteria consortium for the reactive Orange 16 dye retention from aqueous solutions was studied in a batch system. The preparation and characterization of the obtained biosorbent (SEM and FTIR spectra), the analysis of different equilibrium isotherm (Langmuir, Freundlich and Dubinin–Radushkevich – D–R) to estimate the quantitative characteristic biosorption parameters, thermal effect and possible action mechanism were investigated, and the modeling of experimental data was satisfactorily achieved. Langmuir was found the most suitable isotherm model (L II) and the basic mechanism is found to be of physical type, being spontaneous (probably exothermic) after the calculated values of the free biosorption energy (E = 13 kJ/mol, from D–R equation), free Gibbs energy ($\Delta G°$ = –1.845 – –1.768 kJ/mol) and biosorption enthalpy ($\Delta H°$ = –6.512 kJ/mol). Analysis of SEM images and FTIR spectra suggests that Orange 16 reactive dye is retained on biosorbent granules by a physical adsorption mechanism. The obtained results conclude that this residual bacterial biomass immobilized in sodium alginate can be a good biosorbent in static operating system for reactive organic dye-containing effluents.

Keywords: Aqueous medium; Biosorption; *Lactobacillus* sp. bacteria consortium imobilized; Reactive dye

* Corresponding author.

1. Introduction

Water remains today a major problem in the development of humanity and the ensurance of safe life quality. Water consumption has significantly increased since the last century, which has led to the depletion of natural water resources, the deterioration of fauna and certain aspects of the quality of life. Developing of advanced, environmentally friendly, low-cost and high efficient strategies for water treatment is one of today's leading global concerns [1]. Anthropogenic activity and industrialization have increased pressure on the environment by generating large amounts of hazardous effluents containing toxic metals, metalloids, radionuclides as well as various organic pollutants.

In order to eliminate a number of chemical pollutants (e.g., organic pollutants) from water, several physico-chemical and biological processes have been developed, such as adsorption, coagulation, flocculation, sedimentation, filtration (even biofiltration), membrane technologies and biological treatments [2]. Most of these processes involve the transfer of pollutants between the different phases, the use of additional chemicals, or require large amounts of energy during the process. Some of them also leads to the generation of waste and by-products that require treatment at later stages.

The release of organic dyes into the environment by certain industries such as the textile industry, the pulp and paper industry, the food industry, induces risks to human health and ecosystems [3–5].

Many of the used dyes are aromatic compounds that have a low biodegradability capacity and one of the effects is to reduce the photosynthesis activity of aquatic organisms by limiting the penetration of light when they are in water [4,6,7].

A new alternative to classical adsorption is represented by biosorption [8], which is an increasingly studied process based on the encouraging results obtained in the removal of persistent, inorganic and organic pollutants in small or medium concentrations in watery effluents, by using inactive and dead biomass, such as microorganisms, algae, agro-industrial waste [8–14]. The valorisation of different by-products and waste, as new product or raw material in different preparations of industrial products with added value, or in application of certain treatment systems for watery effluents (especially for pollutants removal), is an actual increasing demand, being stringently required in the action plan for 'circular' economy and 'waste hierarchy' principle implementation [15,16]. Therefore, building a sustainable vision on waste management requires taking into account the 'natural model', respectively the model that takes place in natural systems in the case of processing of natural residues/waste resulted from the activity of living organisms.

Simplely, in nature, the waste from a process is always a nutrient, a material or a source of energy for another process, and thus the solution for the environmental challenges regarding pollution but also for the social and economic challenges considering the shortcomings, can be found in the application of the models that we can observe in a natural ecosystem. Thus, residual biomass can be used as a source of nutrient, material (e.g., (bio)adsorptive material, among other), or bioenergy required in certain processes or treatments (e.g., loaded effluents with toxic/dangerous pollutants) in the natural environment (air, water, soil/subsoil).

The use of residual biomass as a biosorbent in treatment processes for retaining chemical species from aqueous environments is a variant of capitalization of this by-product in the food or biosynthesis industries. In order to facilitate the handling of this material and to make the experimental work much efficient, it was tried to bring it in a much more practical and easy to handle form. This was achieved by various immobilization techniques in natural polymeric matrices (alginate, gelatin, chitosan), the biomass being converted into spherical granules with a certain mechanical properties that make them suitable for work in static or dynamic system [17–21].

Lactic acid bacteria are industrially important microorganisms used in the production of fermented vegetable products, wine and dairy industry, comprising spherical (cocci) or rod-shaped (bacilli), gram-positive bacteria, with the following characteristics: catalase-negative, immobile, nonsporulating and anaerobic [22]. Lactic acid (2-hydroxypropanoic acid) is a colourless, organic liquid, with a variety of applications in the food (accepted by FDA as Generally Recognized as Safe – GRAS), chemical (used for the synthesis of polylactic acid – biodegradable materials as alternative for conventional plastic polymers), and pharmaceutical industries. Its increasing demand led to the development of improved fermentation process, from which the biomass results as a by-product and its use as biosorbent could increase the process durability [23–26].

The aim of this paper is to investigate the biosorptive properties of a new proposed residual biomass of *Lactobacillus* sp. bacteria consortium imobilized in sodium alginate. In this context, it was proposed an experimental study methodology that supposed the following key elements: (1) preparation and physical-chemical characterization of the prepared biosorbent based on residual biomass (*Lactobacillus* sp. bacteria consortium) immobilized in sodium alginate; (2) the analysis of the experimental biosorption data using different (bio)adsorption equilibrium isotherms in order to estimate the quantitative characteristic parameters, thermal effect and possible action mechanism in this biosorptive treatment of dye-containing watery effluents.

2. Materials and methods

2.1. Materials

2.1.1. Adsorbate

A reactive dye, such as Orange 16 (O16 – C.I. 18097, from Sigma-Aldrich) (MW = 617.54 g/mol, λ_{max} = 495 nm) with chemical structure showed in Fig. 1a, was selected as chemical pollutant of aqueous system for this study (reference model of reactive anionic dye). It was prepared a stock solution (with concentration of 724 mg dye/L) using a commercial salty form of the dye with analytical reagent purity grade, and distilled water. For working, solutions were prepared starting from the stock solution by appropriate dilution with distilled water.

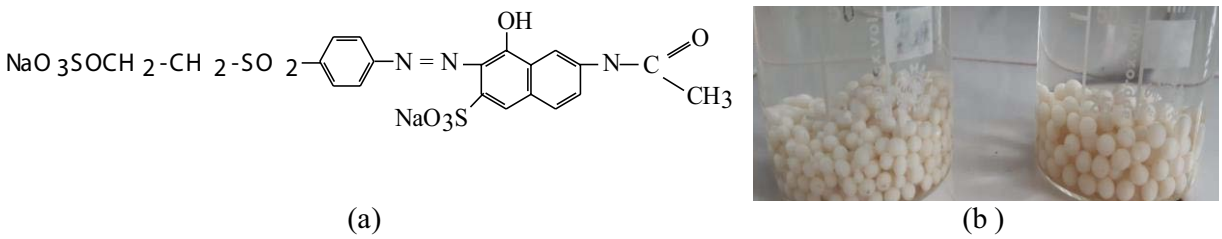

Fig. 1. (a) Reactive dye Orange 16 (C.I. 18097) and (b) granules of *Lactobacillus* sp. bacteria consortium immobilized in sodium alginate.

2.1.2. Biosorbent

The biomass used to obtain the biosorbent is a mixture of two strains: *Lactobacillus plantarum* and *Lactobacillus casei* obtained in an anaerobic process from a 1L fermentor, with the purpose of lactic acid production. After the biosyntesis process, the biomass is separated by centrifugation and the fermentation broth is processed for the lactic acid separation. The cetrifuged biomass (at 8,000 rpm) is used for the biosorbent production. The inactive powder obtained does not require any protection, or special conditions in handling. The powder of residual biomass was dried at 80°C (to increase its adsorptive properties) and immobilized by cell inclusion into sodium alginate. For immobilization, 1% sodium alginate solution prepared in distilled water at 70°C was mixed with 5% residual biomass and dripped after complete homogenisation into 1% calcium chloride (prepared in distilled water at 5°C) through two different capillaries, thus obtaining spherical beads with Φ1 = 0.5 mm and Φ2 = 1.5 mm in diameter (Fig. 1b). The obtained immobilized biosorbent contained 5% dry weight (dw) of bacterial cells.

All the other chemicals required in our experimental part were of analytical purity, used without further purification and purchased from Chemical Company SA (Iasi, Romania).

2.2. Batch biosorption equilibrium studies

The batch biosorption studies were performed using 100 mL-Erlenmeyer flasks in which were introduced 0.2 g of immobilized biomass (8 g/L with 4.7277% dry matter (dw)) and 25 mL of dye solution with different initial dye concentrations (in range of 28.96–231.68 mg of dye/L) at three pH values (adjusted with 1 N HCl solution), and a certain selected temperature (5°C, 20°C, 45°C in a thermostatic bath) with a corresponding contact time of solid-aqueous phases of about 24 h. After reaching equilibrium, the dye content in the supernatant was spectrophotometrically determined using the samples taken from the tested solution (directly, or after phase separation by filtration) and reading the absorbance at the maximum dye wavelength of 495 nm, with a JK-VS-721N VIS spectrophotometer.

The biosorption capacity of the prepared biosorbent was appreciated using the amount of biosorbed dye (q, mg of dye/g of biosorbent) calculated as follows:

$$q = \frac{C_0 - C}{G} \times V \tag{1}$$

where C_0 and C are the initial and equilibrium (residual) concentration of dye in solution (mg/L), G is the amount of biosorbent (dry matter (dw) from alginate granule) (g) and V is the volume of solution (L).

For the processing and analysis of the experimental data were applied three biosorption equilibrium models: Freundlich (F), Langmuir (L) and Dubinin–Radushkevich (D–R), shortly synthesized in Table 1 [19,23–26].

2.3. Advanced analysis methods

2.3.1. Scanning electron microscopy (SEM)

The surface morphology of the biosorbent based on *Lactobacillus* sp. bacteria consortium immobilized in alginate was examined before and after the biosorption process by using the scanning electron microscopy (SEM) technique. Prior to the SEM investigation, the biosorbent granules were lyophilized using the Labconco lyophilizer. SEM images were recorded with a HITACHI SU 1510 (Hitachi SU-1510, Hitachi Company, Japan) Scanning Electron Microscope, MNPs were fixed on aluminum stub and coated with a 7 nm thick gold layer using a Cressington 108 device before observation.

2.3.2. Fourier transform infrared (FT-IR)

It was used to identify the functional groups existing in the biosorbent material in the two states of the process: before and after biosorption. FTIR spectra were registered using a Bruker Vertex 70 FT-IT spectrophotometer in total attenuated reflectance mode in the wavenumber range of 4,000–400 cm^{-1} with a resolution of 2 cm^{-1} and 32 acquisitions, at room temperature.

2.4. Thermodynamic analysis of the biosorption

Some characteristic thermodynamic parameters were determined using the well-known equations as [10,11,20,21]:

$$\Delta G = -RT \ln K_L \tag{2}$$

$$\ln K_L = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} \tag{3}$$

where ΔG is the free energy (kJ/mol), ΔH is the enthalpy (kJ/mol) and ΔS is the biosorption entropy changes (kJ/mol K), R is the universal gas constant (8.314 J/mol K), T is the absolute temperature of solution (K) and K_L is the value of Langmuir constant (L/mol).

Table 1
Characteristics of the three selected (bio)adsorption isotherm models

Biosorption isotherm model	Nonlinear/Linear form of equation and characteristic parameters
Freundlich (F) – is based on the surface heterogeneity and exponential distribution of active sites of the adsorbent.	$q = K_F \cdot C^{1/n} / \log q = \log K_F + \frac{1}{n}\log C$ where K_F and $1/n$ – are constants associated to the biosorption capacity and intensity (efficiency), respectively; a favorable biosorption corresponds to a value of $1 < n < 10$.
Langmuir (L) – considers that the maximum biosorption corresponds to a monolayer of solute molecules on the biosorbent surface, containing a finite number of energetically equivalent sites.	$q = \frac{K_L \cdot C \cdot q_0}{1 + K_L \cdot C}$ The two models being differentiated by the way of presenting the linearized equation from the nonlinear equation, modalities that can offer a better modeling of the experimental data: L1: $\frac{1}{q} = \frac{1}{q_0} + \frac{1}{K_L \cdot q_0} \cdot \frac{1}{C}$ L2: $\frac{C}{q} = \frac{1}{q_0 \cdot K_L} + \frac{C}{q_0}$ where q_0 is the maximum amount of sorbed solute (mg/g) and K_L is the constant related to the binding energy of solute (L/mg).
Dubinin–Radushkevich (D–R) – allows the characterization of the biosorption process nature (physical or chemical) based on the free energy value: $E < 8$ kJ/mol characterizes a physical biosorption mechanism and values between 8 and 16 kJ/mol indicates an ion exchange mechanism	$q = q_0 \exp\left(-B \cdot \varepsilon^2\right) / \ln q = \ln q_0 - B \cdot \varepsilon^2$ $\varepsilon = RT \ln\left(1 + \frac{1}{C}\right) / E = \frac{1}{\sqrt{2B}}$ where q_0 is the maximum biosorption capacity (mg/g); B is the activity coefficient related to mean biosorption energy; ε is the Polanyi potential and E is the mean free energy of biosorption (kJ/mol).

3. Results and discussion

3.1. Biosorbent analysis

The prepared residual lactic acid bacteria-based biomass granules (a *Lactobacillus plantarum* and *Lactobacillus casei* sp. bacteria consortium) used in a biosorption step of an aqueous dye-containing solution treatment is shown in Fig. 1b. All biomass granules of residual inactive bacteria consortium have uniform size and are stable with no coalescence, or adherence episode manifested between them in the aqueous solution at normal laboratory room temperature.

3.1.1. SEM analysis of residual biomass-based biosorbent

Scanning electron microscopy (SEM) was used to study the surface morphology of the granules obtained by immobilization of the *Lactobacillus* sp. bacteria consortium in sodium alginate (Fig. 2). The SEM images show the existence of an internal mesoporous structure and the fact that the biomass is distributed in a relative homogenized form in the polymer mass, immobilization being successfully processed.

3.1.2. FT-IR spectra analysis of residual biomass-based biosorbent

The study of the FTIR spectra from Fig. 3 highlights certain aspects mentioned below.

For the FTIR spectra of dried residual biomass-based biosorbent (Fig. 3), before and after the dye biosorption, a series of characteristic absorption peaks remarks as

- for the stretching (extinction) vibrations: broad peaks at frequency of ~3,285.59 cm^{-1} (before) and 3,335.74 cm^{-1} after the dye biosorption onto biosorbent surface are specific for the O–H alcoholic, or phenolic groups, the peak displacement is due to the adsorbed dye amount which has a similar characteristic vibration usually recorded at 3,437.14 cm^{-1}), or
- for stretching vibrations of CH bond associated to sp^3 C–H streching (methyl or ethyl groups): sharp IR peaks at ~2,927.81 cm^{-1} (before) and 2,928.74 cm^{-1} (after dye adsorption) found in the biosorbent matrix, related to that of the dye peak (2,931.79 cm^{-1}); the peak displacement and attenuation indicate that the reactive dye was adsorbed onto the biosorbent surface by possible C–H or hydrogen bondings,
- for stretching (deformation) vibrations of aromatic ring (C=C, C=N bonds): IR peak at 1,626.89 cm^{-1} only found on the biosorbent with adsorbed dye due to the adsorbed dye presence which has a quite similar characteristic peak at 1,627.92 cm^{-1}); it is indicated clearly the presence of dye onto the biosorbent surface;
- for stretching vibrations of C–C bonds, or CH_2 scissor: IR peaks at ~1,571.92 cm^{-1} (before) and 1,527.56 cm^{-1} (after dye adsorption) found in the biosorbent matrix, related to that of the registered dye peak (1,589.34 cm^{-1});

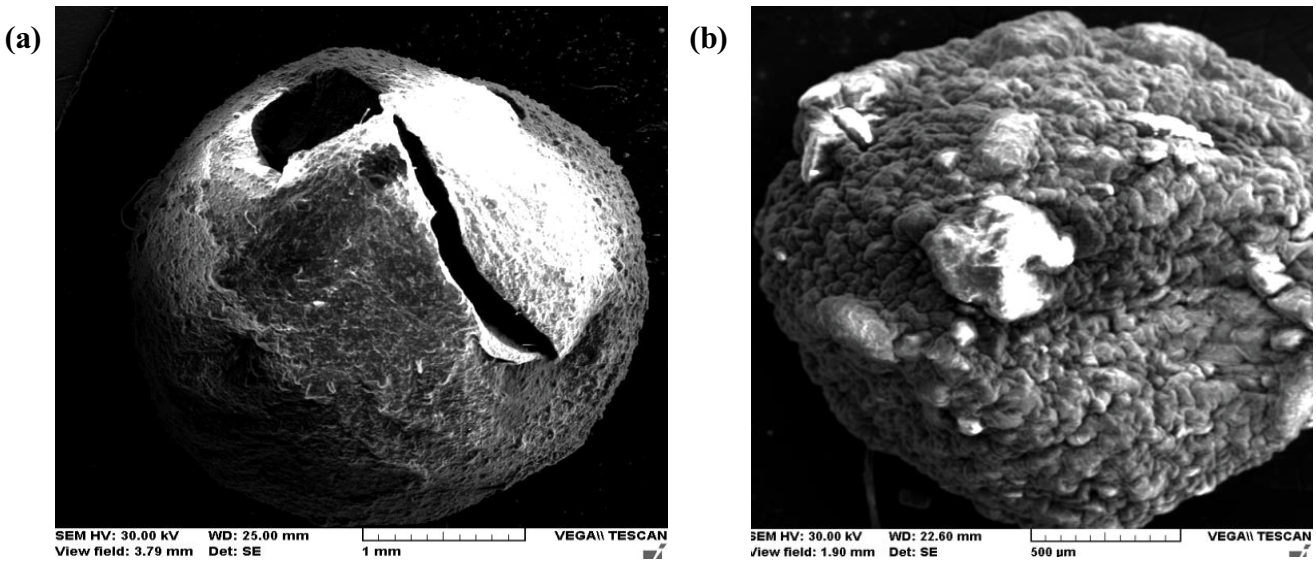

Fig. 2. SEM images of the biosorbent before (a) (1 mm) and after (b) Orange 16 dye biosorption (500 µm).

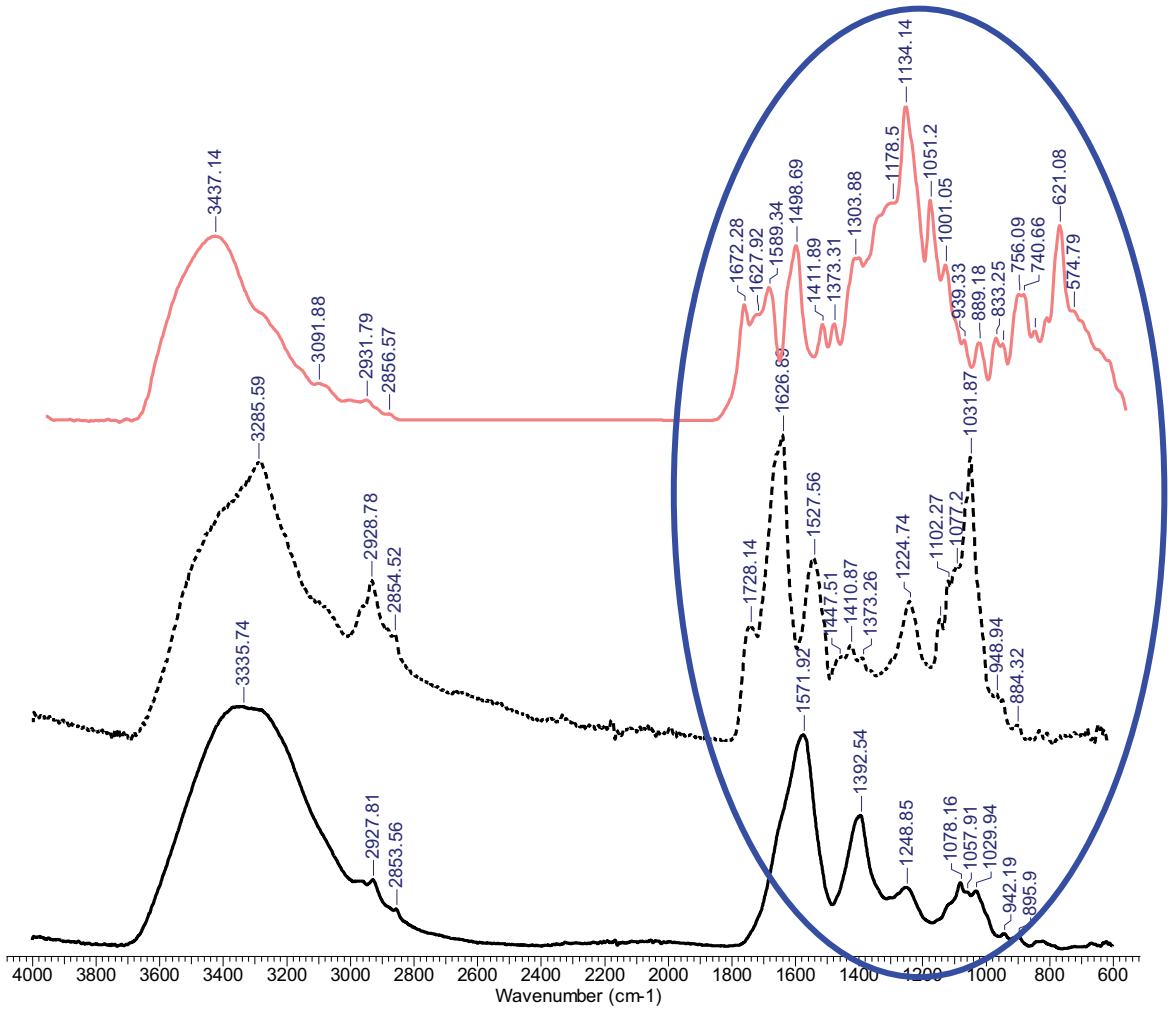

Fig. 3. FT-IR spectra of the reactive dye Orange 16 (red line) and of the biosorbent before (- the black line continues) and after (--- discontinuous black line) biosorption.

- for stretching (deformation) vibrations of OH groups, and also of nitro (N=O) groups: IR peak at ~1,447.51 cm^{-1} only found on the biosorbent with adsorbed dye due to the adsorbed dye presence which has a quit similar characteristic peak at 1,498.98 cm^{-1}); it is indicated clearly the presence of dye onto the biosorbent surface;
- for stretching vibrations of sulphonile, chloride, or sulphate groups: IR peak of biosorbent at ~1,392.54 cm^{-1} (before) and ~1,373.26 cm^{-1} (after) dye adsorption; attenuation of IR frequency peak is due to dye adsorption on biosorbent (attenuation of IR dye peak frequency recorded at ~1,373.31 cm^{-1}); thus it is indicated the presence of dye onto the biosorbent surface;
- for blending (extinction) vibrations of C–O bonds from phenolic and alcoholic compounds, or N–O bonds: IR peak of biosorbent at ~1,224.74 cm^{-1} (before) and ~1,228.85 cm^{-1} (after) dye adsorption; attenuation of IR peak is due to dye adsorption on biosorbent (attenuation of IR dye peak recorded at ~1,303.88 cm^{-1}).

In the characteristic absorption region of 880–1,180 cm^{-1} are numerous attenuations of peaks' frequency for the biosorbent after dye adsorption due to blending/extinction vibrations of C–C, C–O, C–N bonds (i.e., 1,102.27; 1,031.87; 948.94; 884.32 cm^{-1}) related to that of the biosorbent before adsorption (i.e., 1,078.16; 1,029.94; 942.19; 895.90 cm^{-1}) which indicates the dye adsorption onto biosorbent, the dye peaks being recorded at around 1,178.50; 1,051.20; 939.22; 889.18 cm^{-1}. In the absorption region of 600–800 cm^{-1} the peaks can be attributed to the groups of esters, S–OR or P–OR.

A series of new peaks appears as a result of the biosorption process (e.g., 1,626.89 and 1,447.51 cm^{-1}), and some frequency peaks in the biosorbent structure undergo a series of attenuations that revealed that the Orange 16 reactive dye was retained on the prepared biosorbent based on residual bacteria consortium biomass. The complexity of IR spectra in the 1,450 to 600 cm^{-1} region makes it difficult to assign all the adsorption bands, this region being named commonly the 'fingerprint' region.

3.2. Analysis of the biosorption isotherms

Our previous study have concluded that the biosorption process of Orange 16 dye onto residual biomass of *Lactobacillus* sp. bacteria consortium immobilized in sodium alginate proceeds with adequate results under the following conditions: pH = 2, contact time of 24 h, temperature of 20°C–25°C and the concentration of biosorbent in range of 0.0852–0.164 g/L depending on the diameter of the residual bacteria consortium biomass-based granules [13].

In this context, this study focuses on the analysis of the biosorption balance of the Orange 16 reactive dye on residual biomass-based biosorbent prepared by immobilization of a *Lactobacillus* sp. bacteria consortium (residual inactive bacterial biomass) in sodium alginate based on (i) some quantitative characteristic parameters that describe the process and allow the determination of some thermodynamic parameters and (ii) the assessment of biosorption mechanism as well as its association with the preliminary process optimization for highest dye retention performance.

The proposed equilibrium isotherms, presented in Fig. 4, were processed based on the isotherm models known in the scientific literature (Table 1) in order to determine the characteristic quantitative parameters, values that will provide a series of preliminary information about the mechanism involved in the studied process of biosorption.

As shown in Fig. 4a and b, the reactive dye retention on the granules of smaller size (Φ1) (Fig. 4a) is better than that on granules of (Φ2) size considering the biosorption capacity of biomass-based biosorbent for the reactive dye at the three tested temperatures. However, the allure of the curves indicates different types of isotherms, according to Gills' classifications [27]: the isotherms for the small diameter (Φ1) are of "S" type, subgroup 2 – suggesting a (bio) adsorption on the surface of the vertically oriented molecules, achieved by relative weak bonds, and those for larger diameter (Φ2) are of "L" type, subgroup 2 – which is the classic Langmuir type isotherm based on the surface (bio) adsorption of vertically oriented molecules but through particularly strong intermolecular bonds.

The experimental data were modeled by applying the linearized forms of each proposed (bio)sorption model (Freundlich, Langmuir I and II, Dubinin–Radushkevich) (Table 1). The obtained graphs are presented in Fig. 5 and the results based on the intercepts and slopes of the corresponding linear plots performed with residual biomass-based biosorbent of (Φ2) size are listed in Table 2.

Assessment of the best isotherm model that describes the biosorption process of the reactive Orange 16 dye on the immobilized bacteria consortium-based biosorbent (residual biomass of *Lactobacillus* sp. bacteria consortium

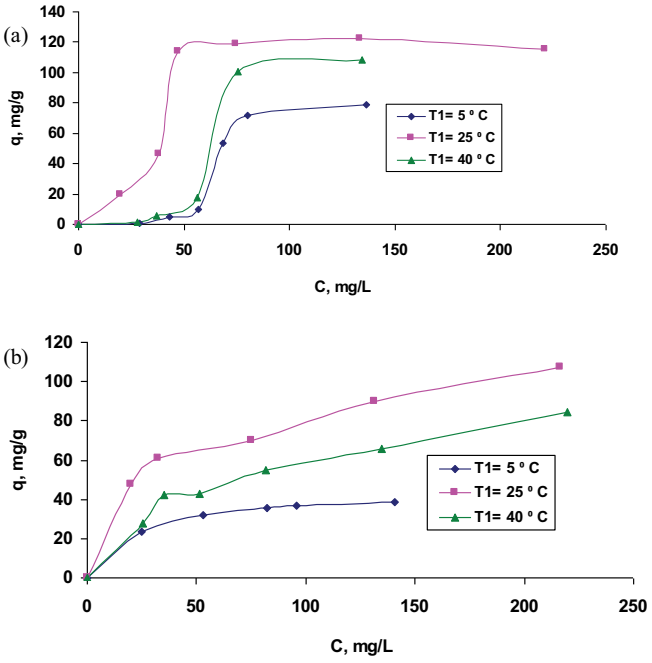

Fig. 4. Biosorption isotherms of reactive Orange 16 dye on residual *Lactobacillus* sp. bacteria biomass immobilized in sodium alginate. Conditions: pH = 2, contact time = 24 h, biosorbent content = 2 and 4 g/L (with 4.7277% dry substance); diameter of granules: Φ1 = 0.5 mm (a) and Φ2 = 1.5 mm (b).

in sodium alginate) was mainly based on the values of the correlation coefficients, R^2.

The analysis of the allure of the straight lines presented in Fig. 5 shows different behaviors of the biosorption systems depending on the sizes of the biosorbent granules. Although it leads to obtaining somewhat lower biosorption capacities, biosorbents with granules of larger size Φ2 provide the system with Langmuir behavior which allows the calculation of the characteristic parameters (Table 2).

A series of valuable information about the studied biosorption process of reactive Orange 16 dye onto residual biomass of *Lactobacillus* sp. immobilized in sodium alginate were obtained by analyzing the data presented in Table 2:

- All studied parameters were influenced by the temperature, but it is observed that the optimum temperature is 25°C. Temperatures higher than 40°C led to values of calculated characteristic adsorption parameters below those obtained at 25°C, the explanation can be given by the fact that, at temperatures higher than 25°C, the biomass denaturation occurs and thus decreases the biosorption capacity and other characteristic parameters. For the subsequent calculations, it was taken into account the system containing the biosorbent with larger granules (Φ2 = 1.5 mm).
- From Table 2, it is observed that the values of correlation coefficient higher than 0.970 indicate that the Langmuir model was more suitable to describe this biosorption process. Of the two forms of the Langmuir model, model II is best suited (to achieve a much better correlation degree of data, R^2) than model I.

The free adsorption energy (E), calculated by D–R equation, offers some preliminary information about the nature of the biosorption process (physical or chemical). The obtained values are around 13 kJ/mol, values characteristic for a physisorption process of the reactive dye onto the immobilized bacteria consortium-based biosorbent. The used biomass is inactive before preparation of the biosorbent, the biosorption being achieved by different physical interactions (more physical than chemical) between the dye and the functional groups from the cell wall and the plasma membrane of the cells.

The value obtained for the biosorption capacity, according to the Langmuir II model is comparable with other (bio) adsorption capacities reported in the literature for different types of bacterial biomass in free or immobilized state on various supports for removal of dyes from aqueous medium (Table 3).

3.3. Analysis of the proposed thermodynamic parameters

To evaluate the influence of temperature on the biosorption of reactive Orange 16 dye onto residual *Lactobacillus* sp. bacteria consortium-based biomass immobilized in sodium alginate and to establish the nature of the biosorption process, thermodynamic parameters were determined (Table 4) from the values of Langmuir constant, K_L (L/mol), and Eqs. (2) and (3) [33,34].

The processed data from Table 4 clearly indicate the negative values of Gibbs free energy, $\Delta G°$, which suggests that the biosorption of reactive Orange 16 dye onto the studied immobilized residual bacterial biomass-based biosorbent (immobilized *Lactobacillus* sp. bacteria consortium granules) is a spontaneous process. Moreover, considering the scientific literature information (i.e., $\Delta G°$ value between –20 and 0 kJ/mol indicates a physical mechanism of adsorption), the calculated $\Delta G°$ values suggest a possible physical mechanism for this studied biosorption process. These findings are in accordance with our preliminary information on the mean free adsorption energy (E), calculated by the D–R model equation.

The calculated negative value of the biosorption enthalpy ($\Delta H°$), considering the slope of the linear dependence of $\ln K_L$ vs. $1/T$, underlines the exothermic nature of reactive Orange 16 dye biosorption onto immobilized *Lactobacillus* sp. bacteria consortium granules but its value is corresponding to a physical biosorption and not proper for a chemical biosorption (commonly, the $\Delta H°$ value for a chemical biosorption must be in the range of –800 to –40 kJ/mol [32]). Information from the biosorption reports mentioned that both physical and chemical forces can be involved in the (bio)adsorption process of organic pollutant (reactive dye) onto the solid surface of biosorbent, but the required energy is different for each one (i.e., physical forces: 4–10 kJ/mol – for Van der Waals bonding, 5 kJ/mol – hydrophobic bonding, 2–29 kJ/mol – dipole bonding, 40 kJ/mol – coordination bonding, and for covalent chemical forces: >60 kJ/mol [35]). However, the value calculated in the case of the reactive Orange 16 dye biosorption onto residual *Lactobacillus* sp. bacteria consortium immobilized in sodium alginate as biosorbent suggests, in agreement with the biosorption energy at low coverage (deduced from D–R equation), that the process is more physical than chemical.

For a better understanding of the reactive dye adsorption onto residual biomass-based biosorbent, a few additional information must be mentioned. Thus, the cell wall of lactic acid bacteria (Gram-positive cells) contains: peptidoglycan, teichoic acids, polysaccharides, and proteins. In the peptidoglycan's structure the aminoacid sequence of the stem peptide is L-Ala-γ-D-Glu-X-D-Ala, while the third amino acid (X) is a di-aminoacid, that can react with the anionic dye. Also the side chains of many aminoacids (histidine, lysine and arginine) from the peripheric proteins can interact with the anionic dye. The interaction onto the residual immobilized lactic acid bacteria-based biosorbent with the reactive Orange 16 dye molecule is due to a complex combination of different interactions as (i) physical crosslinking in different manners such as electrostatic interaction (e.g., opposite charges of polycations from biosorbent aminoacids ($^-$OOC-R-NH_4^+) and anionic dye aminoacids ($^-_3$OS-Dye, -O-Dye), or small cations – biosorbent, or polymer-biosorbent by H-bond or hydrophobic interaction), (ii) anions exchanges, or ions exchange, (iii) coordination complex crosslinking (due to nitrogen from –N=N–, N–C bonds), (iv) covalent crosslinking among functional moieties of biosorbent chains and/or functional groups of reactive dye, or polymer (covalent bond between CH_3-CO-Dye and biosorbent groups) sometimes with the help of crosslinkers, among others.

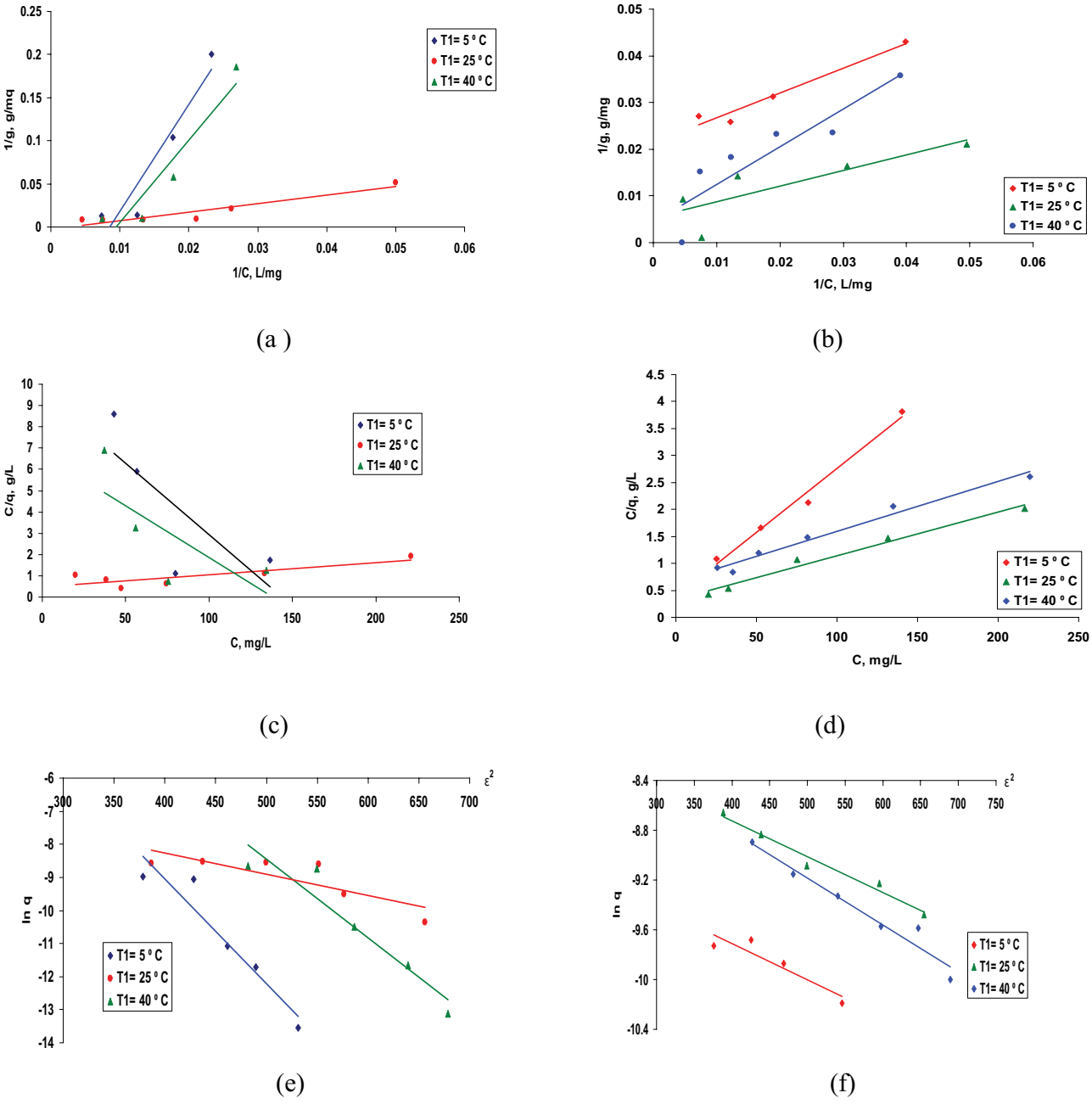

Fig. 5. Linearized form of Freundlich (a, b), Langmuir (c,d) and D–R (g, h) plots for the reactive Orange 16 dye biosorption onto immobilized residual biomass-based biosorbent (residual *Lactobacillus* sp. bacteria consortium biomass immobilized in alginate). Conditions: pH = 2, contact time = 24 h, biosorbent dose = 2 and 4 g/L (4.7277% dw); diameter of granules: Φ1 = 0.5 mm (a, c, e) and Φ2 = 1.5 mm (b, d, f) at three temperatures: 5°C, 25°C and 40°C.

Table 2
Characteristic parameters for the biosorption of reactive Orange 16 dye onto residual biomass of *Lactobacillus* sp. bacteria consortium biomass immobilized in sodium alginate

Isotherm	Φ2 = 1.5 mm		
	278 K	298 K	321 K
Freundlich			
K_F ((mg/g)(L/mg)$^{1/n}$)	9.7746	18.2684	7.1056
n	3.459	4.4248	2.1744
R^2	0.8355	0.9769	0.9485
Langmuir I ($1/q = f(1/C)$)			
q_0 (mg/g)	46.512	185.185	227.273
K_L (L/g)	0.0406	0.0161	0.00548
R^2	0.9561	0.6849	0.8163
Langmuir II ($C/q = f(C)$)			
q_0 (mg/g)	42.194	123.457	108.696
K_L (L/g)	0.0606	0.0245	0.01372
R^2	0.9851	0.980	0.9743
Dubinin–Radushkevich (D–R)			
q_0 (mg/g)	121.2	320.505	418.719
β (mol^2/KJ^2)	0.0029	0.0026	0.0036
E (kJ/mol)	13.13	13.867	11.471
R^2	0.8589	0.9737	0.9525

In addition, the recovery of the reactive dye from the 'spent' residual bacteria consortium biomass-based biosorbent is possible by treatment with low acid solution or diluted organic solvent followed by more repetitive washings with distilled water.

4. Conclusions

The experimental results underline that the studied residual bacteria consortium immobilized in alginate, can be considered an effective biosorbent in a static operating regime for reactive Orange 16 dye retention from aqueous solutions, especially when are present in the industrial dye-containing effluent treatment system. All working parameters in static regime at pH = 2 were varied in the range of 2–4 g/L for biosorbent concentration, 5°C–40°C for temperature, minimum 20 till 24 h for contact time of solid-water phases (certitude of equilibrium attainment in the aqueous system) and 28.96–231.68 mg/L for dye concentration. The best results were performed after 24 h of biosorption at pH = 2 and 25°C, and most suitable biosorption isotherm model is of Langmuir type, the Langmuir II model being better than the Langmuir I model. This biosorption process using the immobilized *Lactobacillus* sp. bacteria consortium applied for the reactive Orange 16 dye removal is of physical nature (physisorption), fact that is concluded after the calculated value of the free adsorption energy (E = 13 kJ/mol, from D–R model equation), the negative values of Gibbs free energy (ΔG° = –1.845 – –1.768 kJ/mol), the negative biosorption enthalpy (ΔH° = –6.512 kJ/mol), being considered as a spontaneous process, probably of exothermic origin. These preliminary experimental results organized in a static biosorption regime will be followed by a few optimization studies and also experimental biosorption testings in a dynamic working regime.

Table 4
Thermodynamic parameters of the biosorption of reactive Orange 16 dye onto residual *Lactobacillus* sp. bacteria consortium immobilized in sodium alginate

T (K)	K_L (L/g)	ΔG° (kJ/mol)	ΔH° (kJ/mol)	ΔS° (J/mol K)
278	0.04060	–1.845	–6.512	–4.004
293	0.01610	–1.805		
321	0.00548	–1.768		

Acknowledgement

This work was supported by a grant of the Ministry of Research, Innovation and Digitization, CNCS/CCCDI – UEFISCDI, project number 490PED/2020, within PNCDI III.

Table 3
Applications of various bacterial biomass-based biosorbents, in free or immobilized form, for different dyes removal

Biosorbent	Dye	Biosorption capacity, (mg/g)	Reference
Bacillus subtilis	Methylene blue	169.490 (298 K)	[28]
Cyanthillium cinereum (L) and *Paspalum maritimum*	Methylene blue	76.335 and 56.1798	[29]
Penicillium sp. immobilized in 2% sodium alginate	C.I. Reactive Red 2	120.480	[30]
Lentinus concinnus biomass immobilized to carboxymethyl cellulose (CMC), in the presence of $FeCl_3$	Disperse Red 60	92.600	[31]
Lentinus concinnus biomass immobilized in polyvinyl alcohol/polyethylene oxide hydrogels	Reactive Yellow 86	87.600	[32]
Bacillus subtilis sp. immobilized in sodium alginate	Brilliant Red HE-3B	588.235	[21]
Lactobacillus sp. immobilized in sodium alginate	Orange 16	123.459 (298 K)	This study

References

[1] I.O. Uribe, A. Mosquera-Corral, J.L. Rodicio, S. Esplugas, Advanced technologies for water treatment and reuse, AIChE J., 61 (2015) 3146–3158.

[2] G. Crini, E. Lichtfouse, Advantages and disadvantages of techniques used for wastewater treatment, Environ. Chem. Lett., 17 (2019) 145–155.

[3] I. Morosanu, C. Teodosiu, D. Fighir, C. Paduraru, Simultaneous biosorption of micropollutants from aqueous effluents by rapeseed waste, Process Saf. Environ. Prot., 132 (2019) 231–239.

[4] C. Zaharia, D. Suteu, Textile Organic Dyes – Characteristics, Polluting Effects, and Separation/Elimination Procedures From Industrial Effluents. A Critical Overview, T. Puzyn, A. Mostrag-Szlichtyng, Eds., Organic Pollutants – Ten Years After the Stockholm Convention – Environmental and Analytical Update, InTech Publisher, Rijeka, Croatia, 2012, pp. 55–86.

[5] C. Zaharia, D. Suteu, A. Muresan, Options and solutions for textile effluent decolourization using some specific physico-chemical treatment steps, Environ. Eng. Manage. J., 11 (2012) 493–509.

[6] G. Crini, Recent developments in polysaccharide-based materials used as adsorbents in wastewater treatment, Prog. Polym. Sci., 30 (2005) 8–70, doi: 10.1016/j.progpolymsci.2004.11.002.

[7] C. Zaharia, Decentralized wastewater treatment systems: efficiency and its estimated impact against onsite natural water pollution status. A Romanian case study, Process Saf. Environ. Prot., 108 (2017) 74–88.

[8] D. Suteu, C. Zaharia, A.C. Blaga, Biosorption – Current Bioprocess for Wastewater Treatment, Vol. I, "Al.I. Cuza" University Publishing House, Iasi, Romania, 2012, pp. 221–244. ISBN 978-973-703-797-8 General (ISBN 978-973-403-798-5 Vol. I)

[9] D. Suteu, A.C. Blaga, C. Zaharia, The Action of Microorganisms From Organic Pollutants in Water, Air, Soil, Vol. II, "Al.I. Cuza" University Publishing House, Iasi, Romania, 2013, pp. 259–274. ISBN 978-973-703-797-8 General (ISBN 978-973-703-939-2 Vol. II)

[10] L.I. Horciu, C. Zaharia, A.C. Blaga, L. Rusu, D. Suteu, Brilliant Red HE-3B dye biosorption by immobilized residual consortium *Bacillus* sp. biomass: fixed-bed column studies, Appl. Sci. (Switzerland), 11 (2021) 4498, doi: 10.3390/app11104498.

[11] I. Nica, C. Zaharia, D. Suteu, Hydrogel based on tricarboxi-cellulose and poly(vinyl alcohol) used as biosorbent for cobalt ions retention, Polymers, 13 (2021) 1444, doi: 10.3390/polym13091444.

[12] D. Suteu, L. Rusu, C. Zaharia, M. Badeanu, G.M. Daraban, Challenge of utilization vegetal extracts as natural plant protection products, Appl. Sci. (Switzerland), 10 (2020) 8913, doi: 10.3390/app10248913.

[13] I.L. Horciu, A.C. Blaga, C. Zaharia, S. Dascălu, D. Şuteu, Valorization of residual biomass as biosorbent: study of biosorption Brilliant Red dye from aqueous media, Bull. Inst. Polit. Iasi, Series: Chem. Chem. Eng., 65 (2019) 57–68.

[14] D. Suteu, A.C. Blaga, C. Zaharia, I.L.Horciu, Residual biomass of *Lactobacillus* sp. immobilized in alginate for Orange 16 dye retention from aqueous medium, Bull. Inst. Polit. Iasi, Series: Chem. Chem. Eng., 67 (2021) (in Press).

[15] C. Zaharia, Policies and Legislative Tools in the Waste Field (in Romanian: Politici si instrumente legislative in domeniul deseurilor), Performantica Ed., Iasi, 2018.

[16] J.C. Dernbach, J.A. Mintz, Environmental laws and sustainability: an introduction, Sustainability, 3 (2011) 531–540.

[17] D. Berillo, A. Al-Jwaid, J. Caplin, Polymeric materials used for immobilisation of bacteria for the bioremediation of contaminants in water, Polymers, 13 (2021) 1073–1079.

[18] A.C. Blaga, C. Zaharia, D. Suteu, Polysaccharides as support for microbial biomass-based adsorbents with applications in removal of heavy metals and dyes, Polymers, 13 (2021) 1440, doi: 10.3390/polym13172893.

[19] A. Adewuyi, Chemically modified biosorbents and their role in the removal of emerging pharmaceutical waste in the water system, Water, 12 (2020) 1551, doi: 10.3390/w12061551.

[20] I.L. Horciu, A.C. Blaga, L. Rusu, C. Zaharia, D. Suteu, Biosorption of reactive dyes from aqueous media using the *Bacillus* sp. residual biomass, Desal. Water Treat., 195 (2020) 353–360

[21] L.I. Horciu, C. Zaharia, A.C. Blaga, L. Rusu, D. Suteu, Brilliant Red HE-3B dye biosorption by immobilized residual consortium *Bacillus* sp. biomass: fixed–bed-column studies, Appl. Sci., 11 (2021) 4498, doi: 10.3390/app1110449.

[22] V. Capozzi, M. Tufariello, N. De Simone, M. Fragasso, F. Grieco, Biodiversity of oenological lactic acid bacteria: species- and strain-dependent plus/minus effects on wine quality and safety, Fermentation, 7 (2021) 24, doi: 10.3390/fermentation7010024.

[23] J.A. Mora-Villalobos, J. Montero-Zamora, N. Barboza, C. Rojas-Garbanzo, J. Usaga, M. Redondo-Solano, L. Schroedter, A. Olszewska-Widdrat, J.P. López-Gómez, Multi-product lactic acid bacteria fermentations: a review, Fermentation, 6 (2020) 23, doi: 10.3390/fermentation6010023.

[24] K. Wu, X. Pan, J. Zhang, X. Zhang, A. Salah Zene, Y. Tian, Biosorption of Congo red from aqueous solutions based on self-immobilized mycelial pellets: kinetics, isotherms, and thermodynamic studies, ACS Omega, 5 (2020) 24601–24612.

[25] M.N. Sahmoune, Evaluation of thermodynamic parameters for adsorption of heavy metals by green adsorbents, Environ. Chem. Lett., 17 (2019) 697–704.

[26] A.C. Blaga, C. Zaharia, D. Suteu, Polysaccharides as support for microbial biomass-based adsorbents with applications in effluent depollution, Polymers, 13 (2021) 1444, doi: 10.3390/polym13091444.

[27] C.H. Giles, T.H. MacEwan, S. Nakhwa, D.J. Smith, Studies in adsorption. Part XI. A system of classification of solution adsorption isotherms, and its use in diagnosis of adsorption mechanism and in measurement of specific surface areas of solids, J. Chem. Soc. London, 2 (1960) 3973–3993.

[28] A. Ayla, A. Çavuş, Y. Bulut, Z. Baysal, Ç. Aytekin, Removal of Methylene blue from aqueous solutions onto *Bacillus subtilis*: determination of kinetic and equilibrium parameters, Desal. Water Treat., 51 (2013) 7596–7603.

[29] F. Silva, L. Nascimento, M. Brito, K. da Silva, W. Paschoal Jr., R. Fujiyama, Biosorption of Methylene blue dye using natural biosorbents made from weeds, Materials (Basel), 12 (2019) 2486–2502.

[30] L.F. Zhang, M. Wang, Biosorption of C.I. Reactive Red 2 by immobilized fungal biomass, Adv. Mater. Res., 213 (2011) 432–436.

[31] G. Bayramoğlu, V. Cengiz Ozalp, M. Yakup Arıca, Removal of Disperse Red 60 dye from aqueous solution using free and composite fungal biomass of *Lentinus concinnus*, Water Sci. Technol., 72 (2017) 366–377.

[32] G. Bayramoglu, M. Yilmaz, Azo dye removal using free and immobilized fungal biomasses: isotherms, kinetics and thermodynamic studies, Fibers Polym., 19 (2018) 877–886.

[33] M. Ravi Kumar, E. Girma, Aklilu, Equilibrium and thermodynamic studies for dye removal using biosorption, Int. J. Sci. Res., 5 (2016) 905–908.

[34] V.V. Gedam, P. Raut, A. Chahande, P. Pathak, Kinetic, thermodynamics and equilibrium studies on the removal of Congo red dye using activated teak leaf powder, Appl. Water Sci., 9 (2019), doi: 10.1007/s13201-019-0933-9.

[35] A.O. Dada, F.A. Adekola, E.O. Odebunmi, F.E. Dada, O.M. Bello, B.A. Akinyemi, O.S. Bello, O.G. Umukoro, Sustainable and low-cost *Ocimum gratissimum* for biosorption of indigo carmine dye: kinetics, isotherm, and thermodynamic studies, Int. J. Phytorem., 22 (2020) 1524–1537.