Research Paper

Experimental analysis and mathematical prediction of Cd(II) removal by biosorption using support vector machines and genetic algorithms

Raluca Maria Hlihor[1], Mariana Diaconu[1], Florin Leon[2], Silvia Curteanu[3], Teresa Tavares[4] and Maria Gavrilescu[1,5]

[1] "Gheorghe Asachi" Technical University of Iasi, Faculty of Chemical Engineering and Environmental Protection, Department of Environmental Engineering and Management, 73 Prof. Dr. Docent Dimitrie Mangeron Street, 700050 Iasi, Romania
[2] "Gheorghe Asachi" Technical University of Iasi, Faculty of Automatic Control and Computer Engineering, Department of Computer Engineering, 27 Prof. Dr. Docent Dimitrie Mangeron Street, 700050 Iasi, Romania
[3] "Gheorghe Asachi" Technical University of Iasi, Faculty of Chemical Engineering and Environmental Protection, Department of Chemical Engineering, 73 Prof. Dr. Docent Dimitrie Mangeron Street, 700050 Iasi, Romania
[4] University of Minho, Department of Biological Engineering, Campus de Gualtar, 4710-057 Braga, Portugal
[5] Academy of Romanian Scientists, 54 Splaiul Independentei, RO-050094 Bucharest, Romania

We investigated the bioremoval of Cd(II) in batch mode, using dead and living biomass of *Trichoderma viride*. Kinetic studies revealed three distinct stages of the biosorption process. The pseudo-second order model and the Langmuir model described well the kinetics and equilibrium of the biosorption process, with a determination coefficient, $R^2 > 0.99$. The value of the mean free energy of adsorption, E, is less than 16 kJ/mol at 25°C, suggesting that, at low temperature, the dominant process involved in Cd(II) biosorption by dead *T. viride* is the chemical ion-exchange. With the temperature increasing to 40–50°C, E values are above 16 kJ/mol, showing that the particle diffusion mechanism could play an important role in Cd(II) biosorption.

The studies on *T. viride* growth in Cd(II) solutions and its bioaccumulation performance showed that the living biomass was able to bioaccumulate 100% Cd(II) from a 50 mg/L solution at pH 6.0.

The influence of pH, biomass dosage, metal concentration, contact time and temperature on the bioremoval efficiency was evaluated to further assess the biosorption capability of the dead biosorbent. These complex influences were correlated by means of a modeling procedure consisting in data driven approach in which the principles of artificial intelligence were applied with the help of support vector machines (SVM), combined with genetic algorithms (GA). According to our data, the optimal working conditions for the removal of 98.91% Cd(II) by *T. viride* were found for an aqueous solution containing 26.11 mg/L Cd(II) as follows: pH 6.0, contact time of 3833 min, 8 g/L biosorbent, temperature 46.5°C. The complete characterization of bioremoval parameters indicates that *T. viride* is an excellent material to treat wastewater containing low concentrations of metal.

Introduction

Today, health and environmental impacts and risks continue to be generated by the discharge of heavy metals in the environment. They are released from various industrial, agricultural and domestic sources, and constitute a continuing challenge for policy and decision makers, as well as for society as a whole. Cadmium, lead, mercury, nickel, zinc, copper and chromium were of particular concern in the past decades in terms of environmental pollution

Corresponding author: Gavrilescu, M. (mgav@tuiasi.ro, mgav_ro@yahoo.com)

and public health because of their ability to migrate among environmental compartments, their accessibility for biological uptake and availability to accumulate in living tissue through the food chain [1–5]. United States Environmental Protection Agency (U.S. EPA) has classified cadmium as a probable human carcinogen, the chronic exposure to cadmium resulting in kidney dysfunction, while high levels of exposure could result in death [6]. Cadmium maximum intake by humans recommended by the World Health Organisation (WHO) is 0.4–0.5 mg week^{-1}, while the maximum admissible concentration in drinking water specified by the U.S. EPA is 0.005 mg/L [7].

All already established conventional methods for heavy metals removal have several disadvantages such as high consumption of reagents and energy, low selectivity, high operational costs and generation of secondary pollutants [8–11]. Biosorption can represent a sustainable alternative for metal removal and/or recovery, based on metal-sequestering properties of dead or living biomass [12–14]. Although, there are numerous studies and research carried out at laboratory and bench scales for biosorption of heavy metals, their behavior in contact with various biosorbents and the mechanism by which microorganisms take up metals is still relatively unclear. In this context it was considered opportune to fill some gaps in the knowledge by analyzing the features and advantages of *biosorption and bioaccumulation* of Cd(II) by *Trichoderma viride* and provide an *opportunity* for alternative perspectives.

Despite the well-known potential of fungi to remove heavy metal cations from aqueous solutions, only scarce information is available for the cadmium biosorption and bioaccumulation by *T. viride*, a mold of the family *Hypocreaceae*, order *Hypocreales* [15–19]. The mold could be considered in the category of fungal biocontrol agents (BCAs). These antagonistic fungi are the most common due to their multiple BCA characteristics, namely, antagonist and plant-growth simulation as it was found by Al-Taweil et al. [20], which have studied the optimization of *T. viride* cultivation for mass-scale production for industrial biotechnological uses on large scale.

Taking into consideration the aforementioned context, the objectives of our experimental program were: (i) to evaluate and discuss the effects of various factors (pH, biosorbent dosage, initial metal concentration, contact time, temperature) on Cd(II) removal efficiency and uptake capacity of dead an living *T. viride* in batch operating mode; (ii) to investigate the mechanism that governs Cd(II) removal by dead and living *T. viride* through equilibrium and kinetic evaluations combined with FTIR analysis; (iii) to develop a general and efficient methodology based on support vector machines (SVM) and evolutionary algorithms, particularly genetic algorithm (GA), for modeling and optimization of the removal process.

The simulation of Cd(II) removal process by biosorption can offer the advantage of accurate prediction or optimal working conditions. The importance of SVM combined with GA methodology can also be related to the lack of a phenomenological model capable to work into an optimal control procedure.

Support vector machine (SVM) is a statistical learning theory based machine learning method. SVM method has gained recognition due to its features and promising generalization performance, such as: (i) the ability to model non-linear relationships, (ii) the dimensionality of the input space does not affect the

generalization; (iii) the regression function is related to a quadratic programming problem whose solution is global and in general unique [21]. Open literature presents different combinations between SVM and GA with applications in different domains. For example, genetic algorithms can use a SVM classifier to evaluate the fitness functions of gene subset or for SVM parameter optimization [21–23].

In the present paper, SVM method is applied for modeling the biosorption process efficiency as function of pH, biomass dosage, initial concentration of metal solution, contact time and temperature. The model was associated with a GA as solving instrument of the optimization procedure, consisting in determining the optimum working conditions which lead to a maximum efficiency. In addition, GA was implemented with adaptive parameters (adaptive genetic algorithm, AGA), that is, adaptive crossover and mutation rates, resulting in an improvement of its performance. Accurate results of the modeling and optimization proved the reliability and efficiency of the SVM–AGA procedure.

Materials and methods

Microorganism, culture and growth conditions

The microbial biomass of *T. viride* used for heavy metals biosorption and bioaccumulation experiments was isolated from a forest soil collected from 8 to 10 cm deep, in Iasi city, Romania. The soil sample was diluted with sterile water and placed onto a medium containing 30 g/L glucose, 5 g/L yeast extract, 3 g/L NaNO₃, 1 g/L MgSO₄ 7H₂O, 0.5 g/L KCl and 20 g/L agar. Petri plates were incubated at $28 \pm 2°C$ for 168 hours. On the basis of color, diameter of the mycelia and microscopic observation of formation of spores the strain was identified as *T. viride* [24]. Subculturing of the stock culture from these slants was routinely done every month, incubated for growth at desired temperature and time and later stored at 4°C. Inoculum from the slants was used for the production of stationary phase of growth in liquid culture medium for biosorption experiments. The pH of the culture medium was adjusted to 6.0 with 0.1 M NaOH, sterilized at 120°C for 20 min, inoculated with fungi and kept at $28 \pm 2°C$ and 150 rpm for 168 hours.

Batch biosorption experiments

For biosorption experiments, *T. viride* biomass was inactivated on water bath at 90°C for 1 hour, centrifuged at 10,000 rpm for 10 min and dried in an oven at 80°C for 24 hours. The dead biomass of *T. viride* was prepared for biosorption as shown in a previous paper [2]. No living colonies were found during tests, which confirmed that *T. viride* biomass was dead.

The removal of Cd(II) by dead biomass of *T. viride* was examined by measuring the concentration of heavy metal in batch mode at different pH values (from 3.0 to 7.0), biomass dosages (from 1 to 10 g/L), contact time (0–72 hours) and temperatures (25°C, 40°C and 50°C). The experiments were performed following the procedure described in a previous work [2]. After sampling, the solutions were centrifuged at 13,000 rpm for 10 min, after which the Cd(II) concentration of the supernatant was analyzed.

For desorption studies, 8 g/L dead *T. viride* biomass was contacted with 50 mL Cd(II) solution (50 mg/L) in 150 mL Erlenmeyer flask. After the adsorption experiment, the biomass was separated by centrifugation at 13,000 rpm for 10 min and washed for two times with distilled water to remove residual Cd(II) from the

surface. Then it was transferred to 50 mL desorbing agents: H_2SO_4, HNO_3, NaOH and $CaCl_2$ (0.1 M). The mixtures were shaken at 150 rpm and room temperature ($25 \pm 2°C$) for 24 hours. After desorption, the samples were again centrifuged and analyzed to determine Cd(II) concentration in solution [25].

Batch bioaccumulation experiments

To study the growth and bioaccumulation properties of *T. viride*, 5 mL of stock Cd(II) solution was supplemented to 90 mL culture media and inoculated with 5 mL of *T. viride* spore suspension from slants (7×10^5 CFU/mL) to give final Cd(II) concentrations of 25, 50, 75, 100, 150 and 200 mg/L and a final working volume of 100 mL. All the experiments were carried out in 250 mL Erlenmeyer flasks. The flasks were incubated at $28 \pm 2°C$ for 168 hours at 150 rpm in an orbital shaker. Samples were taken at predetermined time intervals (from 0 to 168 hours) for control (growth medium without heavy metal), 25 and 50 mg/L Cd(II) and at 168 hours for concentrations of 75–200 mg/L Cd(II). The bioaccumulation of Cd(II) by *T. viride* in the pH range 4.0–7.0 was also tested. The pH values were adjusted by the addition of 0.5 M CH_3COOH and 0.1 M NaOH. The growth was monitored by the measurement of the dry weight concentration of biomass (X, g/L) by drying at 105°C for 5 hours. All experiments were performed in duplicate and the data presented are the average values.

Reagents and equipment

All chemicals were of analytical reagent grade and no supplementary purification was performed. We prepared a stock solution (1000 mg/L) by dissolving $Cd(NO_3)_2 \cdot 4H_2O$ (Riedel) in distilled water. Cd(II) concentration was determined by T60 UV-Visible Spectrophotometer, based on the method with xylenol orange, at 575 nm [26]. The pH measurements were performed with a Prolab2000 multiparameter. The batch experiments were carried out in an IKA KS 4000 IC control orbital incubator. All glassware used for experimental purposes was washed in 20% nitric acid and rinsed with distilled water to remove any possible interference by other metals.

FTIR analysis

The infrared spectra of dead and living *T. viride* biomass with and without metal ions were acquired using JASCO FT/IR-4200 Spectrometer. The powdered dried samples before and after the biosorption process were analyzed in the range 3500–600 cm^{-1} with a resolution of 4 cm^{-1} by using the Attenuated Total Reflectance (ATR) technique and placing enough sample to cover the diamond sensor.

Quantification of metal removal

Metal uptake and removal efficiency by dead and living *T. viride* were determined according to Eqns 1–3 [2,27]:

$$q_e = \frac{C_i - C_e}{m} V \qquad (1)$$

$$q_t = \frac{C_i - C_t}{m} V \qquad (2)$$

$$R\,(\%) = \frac{C_i - C_e}{C_i} \times 100 \qquad (3)$$

where q_e represents the amount of metal removed from solution (mg/g) at equilibrium and q_t at time t; $R(\%)$ is the biosorption efficiency; C_i, C_e and C_t are the concentration (mg/L) of metal ions in the initial solution, at the equilibrium and respectively, at time t; V (L) is the volume of the solution; m (g) is the amount of biomass used in the experiment.

The results employing the comparative growth and bioaccumulation of Cd(II) by *T. viride* were expressed as units of dried cell mass, X (g/L).

Desorption efficiency was given by (Eqn 4) [2]:

$$D\,(\%) = \frac{\text{amount of metal ions desorbed}}{\text{amount of metal ions adsorbed}} \times 100 \qquad (4)$$

Biosorption kinetics

In this investigation, the Lagergren first-order model (Eqn 5), Ho pseudo-second order model (Eqn 6) and Elovich equation (Eqn 7) were applied to test the biosorption kinetic data to examine the mechanism involved in Cd(II) biosorption by dead biomass of *T. viride* [28,29].

$$\log(q_e - q_t) = \log q_e - \frac{k_1}{2.303} t \qquad (5)$$

where k_1 is the rate constant of the pseudo-first order equation (min^{-1}). The adsorption rate constants can be determined experimentally by plotting $\log(q_e - q_t)$ versus t.

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} t \qquad (6)$$

where k_2 is the rate constant of pseudo-second order adsorption (g/mg min). If pseudo-second order kinetic is applicable, the plot t/q_t against t of Eqn 6 should give a linear relationship, from which q_e and k_2, can be calculated from the slope and the intercept of the straight line resulting from the graphical representation of Eqn 6.

$$\frac{1}{q_t} = \frac{\ln(\alpha\beta)}{\beta} + \frac{\ln t}{\beta} q \qquad (7)$$

where α is the initial adsorption rate (mg/g min) and β is the desorption constant (mg/g min) during one experiment. The constants α and β can be calculated if a plot of q_t versus $\ln t$ is linearly correlated, according to Eqn 7.

Biosorption isotherms

Langmuir, Freundlich, Temkin and Dubinin–Radushkevich (D–R) isotherm models in the linearized forms were selected to fit the experimental data.

The Langmuir isotherm can be linearized as Eqn 8 [23]:

$$\frac{C_e}{q_e} = \frac{1}{q_m} C_e + \frac{1}{K_L q_m} \qquad (8)$$

where K_L (L/g) is a constant related to the adsorption/desorption energy and q_m (mg/g) is the maximum sorption upon complete saturation of the biomass surface.

To determine whether the batch sorption system is viable, we used the adimensional parameter R_L (Eqn 9), denoted as a separation factor, and which can be computed from Langmuir isotherm, as an indicator of the nature of the sorption process [30].

$$R_L = \frac{1}{1 + K_L C_i} \qquad (9)$$

where $R_L > 1$: unfavorable conditions for sorption; $R_L = 1$: linear isotherm; $R_L = 0$: irreversible isotherm; $0 < R_L < 1$: favorable isotherm.

The Freundlich isotherm model can be linearized in the form of Eqn 10 [31]:

$$\log q = \log k_F + \frac{1}{n}\log C_e \tag{10}$$

where k_F (mg^{1-n} g^{-1} L^n) is the sorption capacity when equilibrium concentration of metal ion, $C_e = 1$ and n represents the degree of dependence of sorption on the equilibrium concentration.

Temkin isotherm can be expressed in the linear form as Eqn 11 [31]:

$$q_e = B \ln K_T + B \ln C_e \tag{11}$$

where K_T is the equilibrium binding constant corresponding to the maximum binding energy and constant B is related to the heat of adsorption.

The D–R isotherm is described in the linear form by Eqn 12 [32]:

$$\ln q = \ln q_m - \beta \varepsilon^2 \tag{12}$$

where q and q_m are defined above and ε is the Polanyi potential, expressed by Eqn 13:

$$\varepsilon = RT \ln\left(1 + \frac{1}{C}\right) \tag{13}$$

where R is ideal gas constant (8.3145 J/mol K) and T is the absolute temperature (K). q_m is denoted as the saturation limit and may characterize the total specific volume of sorbent micropores. β is the activity coefficient (mol^2/kJ^2) related to the adsorption mean free energy, E (the free energy change required during the transfer of 1 mole of solute from solution to the solid surface, Eqn 14).

$$E = \frac{1}{\sqrt{2\beta}} \tag{14}$$

The magnitude of E (kJ/mol) allows the estimation of the type of biosorption reactions. If E is in the range of 8–16 kJ/mol, biosorption is governed by chemical ion-exchange. When $E < 8$ kJ/mol, physical forces may be determinant in metal ion uptake. If $E > 16$ kJ/mol, biosorption may be dominated by particle diffusion [2,32].

Thermodynamic parameters

Thermodynamic parameters were evaluated using Eqns 15 and 16:

$$\Delta G^0 = -RT \ln K_d \tag{15}$$

$$\ln K_d = \frac{\Delta S^0}{R} - \frac{\Delta H^0}{RT} \tag{16}$$

where ΔG^0, ΔH^0, ΔS^0 and T are the Gibbs free energy, enthalpy, entropy and the temperature in Kelvin, respectively. The slope and intercept of $\ln K_d$ vs. $1/T$ plots give the values of enthalpy (ΔH^0) and entropy (ΔS^0), respectively.

The distribution coefficient (K_d) is calculated considering the concentration of heavy metal in suspension (C_i) and that of heavy metal in supernatant (C_e) after centrifugation, according to Eqn 17:

$$K_d = \frac{C_i - C_e}{C_e}\frac{V}{m} \tag{17}$$

where V is the volume of the solution (L) and m is the mass of dead biomass (g) [31].

SVM–AGA methodology

One alternative approach for system predicting is the technique of SVM based on the structural risk minimization (SRM) principle. On the basis of this principle, SVM achieves an optimum network structure by striking a right balance between the quality of the approximation of the given data and the complexity of the approximating function. The SVM reveals the underlying statistical relationships among variables corrupted by random error [33]. This technique was chosen in this paper based on the fact that it alleviates the main drawback of parametric regression referring to the mismatch of assumed model structure and the actual data. In nonparametric regression, a priori knowledge about the process (chemical and physical laws that govern it) is not required. It addition, SVM is recommended for its high generalization capability, that meaning the chance to obtain accurate models. Another advantage of the method is the small number of parameters that the user has to choose: type of kernel with its parameters and a cost parameter, C, which defines the balance between tolerance for training errors and generalization capability. There are three types of kernel functions commonly used in SVM implementations: linear, polynomial and radial basis function (RBF) kernels.

SVM applied here for modeling purpose correlates the biosorption process efficiency (output) with pH, biomass dosage, initial concentration of metal solution, contact time and temperature (inputs).

The RBF kernel has been selected for the modeling of the data, as is the most commonly used one by SVM practitioners and it has been reported to provide the best results. The equation which characterizes the RBF kernel function is given bellow (Eqn 18):

$$K(x_i, x_j) = \exp(-\gamma\|x_i - x_j\|^2), \quad \gamma > 0 \tag{18}$$

where x_i and x_j are training vectors and γ is kernel parameter. The cost parameter, C, is added, being the only free parameter of SVM.

The simulation experiments in this paper were performed using the SVR algorithm (SVM for regression) provided in LIBSVM library [34]. The package offers two variants of the algorithm, but the ε-SVR variant is chosen, where ε is a parameter of the loss function with values in [0,inf]. The SVM model was associated with a GA as solving instrument of the optimization procedure. GA was implemented with adaptive parameters (adaptive genetic algorithm, AGA), that is, adaptive crossover and mutation rates, resulting in an improvement of its performance. The SVM–AGA method was further developed for determining the optimum working conditions, which lead to the maximum process efficiency. This optimization procedure is as follows:

- A new chromosome is initialized or evolved, with five real-valued genes, corresponding to pH, biomass dosage, initial concentration of metal solution, contact time and temperature.
- The corresponding fitness function is computed by applying these five values as inputs to the SVM model; the fitness function is the process efficiency (output of the model).
- The chromosome is further processed by the evolutionary algorithm operators.

Because of the interpolation capabilities of the model, the optimization method could search in a flexible way the solution space.

Results and discussion

Biosorption of Cd(II) by dead biomass of T. viride
Effect of initial pH on Cd(II) removal

Metal binding sites on the cell surface and the chemistry of metal in solution are influenced by the pH of the solution. To study the effect of pH on Cd(II) biosorption by dead fungal biomass, uptake and biosorption efficiency of Cd(II) (100 mg/L) on the biosorbent as a function of pH was examined in the pH ranges of 3.0–7.0 with a contact time of 72 hours, at room temperature (25°C) and using 8 g/L biosorbent dosage. The working pH range above 7.0 was avoided due to possibility of metal precipitation on biomass surface, making the sorption studies impossible. Metals biouptake and the ecotoxicological effects in aqueous solutions depend strongly on their speciation. The speciation diagram indicates that Cd^{2+} is the only dominant specie up to pH 10.0 [35]. The biosorption capacity of T. viride biomass was strongly affected by the initial pH of aqueous metal solution; it was found to increase with the pH, exhibiting a maximum of 7.31 mg/g at pH 6.0. The removal efficiency at pH 6.0 was 57.92% (Fig. 1a). A further increase in pH adversely affected the biosorption capacity of T. viride. This is due to the cell wall ligands, which at low pH were closely associated with the hydronium ions H_3O^+ and, due to some repulsive forces, which restricted the approach of metal cations. Increasing pH favors the negative charge of ligands (carboxyl, phosphate, amino groups), which subsequently attract the positively charged metal ions, and promotes their biosorption on cells surface [25]. A similar effect of pH has also been observed when other kinds of biomasses were employed as biosorbents for Cd(II) removal from aqueous solutions [36]. Under these considerations, the further biosorption experiments for Cd(II) removal from aqueous solution by dead T. viride biomass for were carried out at pH 6.0.

Effect of biomass dosage on Cd(II) removal

For the determination of the optimum biomass dosage, different amounts of dead T. viride in the range 1–10 g/L were suspended in 50 mL Cd(II) solution of 100 mg/L, at pH 6.0, for 72 hours and at 25°C. Figure 1b shows the effect of biomass dosage on sorption capacity and the removal efficiency of Cd(II) by fungal biomass. Biomass dosage significantly influenced Cd(II) sorption. At 1 g/L T. viride, the removal efficiency was found to be 7.45% and greatly increased to 57.92% with further increasing the biosorbent concentration to 8 g/L. When the biosorbent dosage was increased to 10 g/L, the removal efficiency further increased to 58.2%, but at this biosorbent dosage the sorption capacity decreased to 6.12 mg/g as shown in Fig. 1b. At low biosorbent dosages, the sorption on the surface is saturated faster showing a higher uptake (q_e) value. Any increase in biosorbent dose makes available more active sites, but the metal uptake will decrease. This behavior could be the consequence of biosorbent available area aggregation or overlapping, and also to a possible increase of the diffusional route, causing a poorer Cd(II) uptake [25]. Thus, in the next experiments, the optimum biosorbent dosage for Cd(II) removal was selected as 8 g/L dead T. viride.

Effect of contact time and temperature on Cd(II) removal

Figure 2 shows the comparative data of the effect of contact time and temperature on biosorption capacity of T. viride biomass at
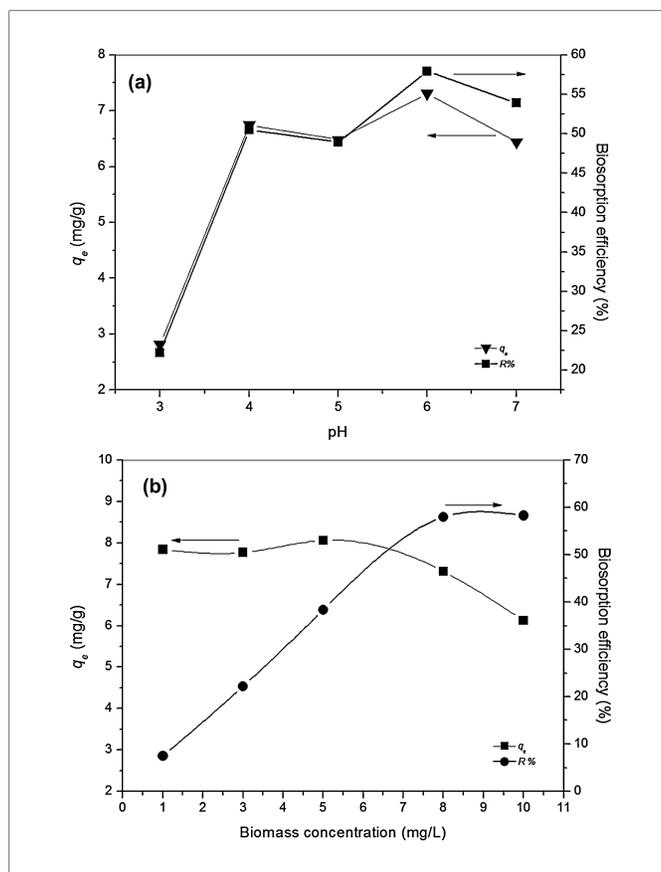
FIGURE 1

Effect of solution pH (a) and biomass dosage (b) on Cd(II) biosorption by dead T. viride (Cd(II) concentration: 100 mg/L; temperature: 25°C; contact time: 72 hours).

25 mg/L (Fig. 2a), 50 mg/L (Fig. 2b) and 100 mg/L (Fig. 2c). In this plot, it is apparent that there are three stages in the sorption kinetics for all three cadmium concentrations. The first stage of biosorption is faster, and takes place in the first 2–3 hours for all metal concentrations used. During the first 48 hours the initial process of external mass transfer was very slow and is termed as the second stage of sorption. The third stage of sorption (48–72 hours) was found to be clearly separated by a plateau depending on the availability of metal ions in the solution for sorption [37]. The 3rd stage of sorption was saturated around 2.7–3.2 mg/g for 25 mg/L Cd(II) and 25–50°C, at 4.55–6.1 mg/g for 50 mg/L Cd(II) and 25–50°C and at 7.3–7.8 mg/g for 100 mg/L Cd(II) and 25–50°C. By contrast, the biosorption efficiency of T. viride increased with rise in temperature from 83% to 99.99% for 25 mg/L, from 69.1% to 88.5% for 50 mg/L and from 57.92% to 63.55% for 100 mg/L, respectively, during 72 hours of contact time. These results indicated the endothermic nature of Cd(II) biosorption onto dead T. viride biomass. Similar endothermic nature of the biosorption process was also reported for other metal-biomass systems [31].

The sorption capacity of T. viride could boost with the temperature, due to the generation of a larger number of active sites on the sorbent surface available for sorption, increase of porosity and enlargement of the total volume of pores. In addition, a reduction in the thickness of the boundary layer surrounding the sorbent can be registered with temperature increasing. As a result, a decrease of
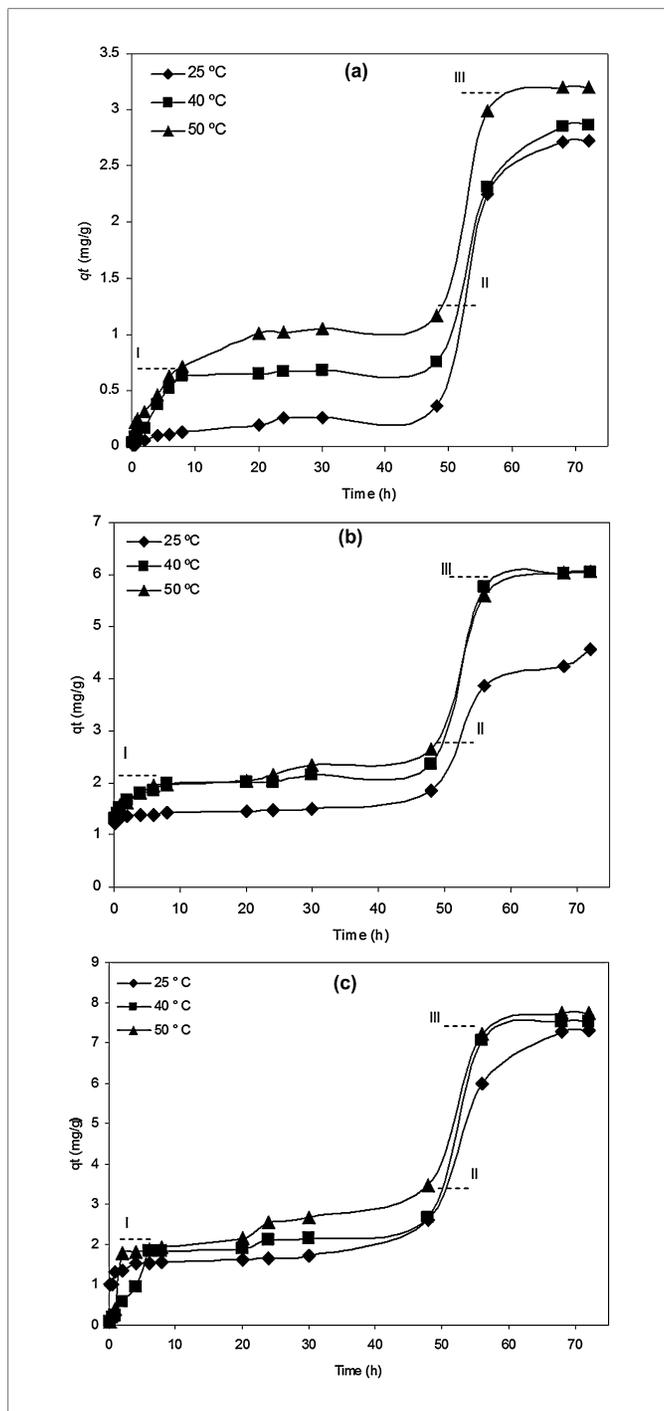
FIGURE 2

Effect of contact time and temperature on Cd(II) biosorption by dead *T. viride*: **(a)** 25 mg/L Cd(II); **(b)** 50 mg/L Cd(II); **(c)** 100 mg/L Cd(II) (pH 6; biomass dosage: 8 g/L; contact time: 72 hours).

mass transfer resistance in the boundary layer could occur [32]. At low temperature values, the adsorption sites with lower active energy were first occupied, and the other sites with higher active energy were occupied with the increase of temperature [31].

Biosorption kinetics, isotherms and thermodynamic parameters
Table 1 lists the results of kinetic parameters for different Cd(II) concentrations and temperatures. It is clear from these results that the correlation coefficient values (R^2) for the pseudo-second order

model applied for Cd(II) biosorption by dead *T. viride* are very high (0.92–0.99), suggesting that this model fits well with the experimental data. The pseudo-second order model is in agreement with chemisorption being the rate controlling step [28].

The biosorption equilibrium investigations were conducted in aqueous solutions with pH 6.0, Cd(II) concentration of 25–500 mg/L, for 72 hours and temperature ranging from 25 to 50°C. The sorption equilibrium was described by sorption isotherms whose constant values depend on the surface properties. Sorption isotherm studies offer valuable information on the pathways of adsorption, reactions and on the capacity of the sorbent [2,30–32]. From Fig. 3a, it can be seen that the sorption capacity sharply enhances with the increase of equilibrium concentration; then, it grows up slightly, approaching the maximum.

The parameters corresponding to different isotherm models for the biosorption of Cd(II) on *T. viride* are presented in Table 2. It is obvious from the R^2 values (>0.99) that Langmuir model is suitable for describing the batch mode biosorption of heavy metal onto the biomass of dead fungi.

The Langmuir model estimates the sorption capacity of the sorbent used by considering sorbate uptake as occurring on a homogeneous surface. A monolayer sorption process is suggested, without any interaction among the molecules adsorbed on the sorbent surface. Moreover, the Langmuir model presumes homogeneous adsorption energies onto the surface, without any transmigration of the adsorbate [38]. The values of separation factor, R_L, between 0 and 1 indicated favorable biosorption conditions (Fig. 3b). The maximum monolayer biosorption capacities (q_m) of the biosorbent for Cd(II) in batch mode are 8.86 mg/g, 10.16 mg/g and 10.95 mg/g at 25, 40 and 50°C, respectively. The increasing values of q_m with temperature indicated the endothermic nature of the process (Table 2).

The application of D–R model allowed the calculation of the mean free energy E (kJ/mol) of sorption per molecule of sorbate. In our study, the value of E at 25°C is less than 16 kJ/mol (15.82 kJ/mol), whereas at 40°C and 50°C it is above 16 kJ/mol (19.32 kJ/mol and 19.75 kJ/mol, respectively). This behavior suggests that at low temperature chemical ion-exchange is the dominant process involved in Cd(II) biosorption by dead *T. viride* and with increase in temperature particle diffusion mechanism could play an important role.

The thermodynamic data are listed in Table 3. The positive variation of enthalpy (ΔH^0) with temperature indicates an endothermic process of Cd(II) removal from solution, while the positive variation of Gibbs free energy (ΔG^0) reveals a non-spontaneous process. The positive values of entropy (ΔS^0) reflect the affinity of *T. viride* toward Cd(II) ions in aqueous solution and may suggest some structural changes of the adsorbent [39].

*Desorption of Cd(II) from **T. viride** biomass*
Four different agents (0.1 M) were tested for desorption of Cd(II) from dead *T. viride* biomass in two cycles, H_2SO_4, HNO_3, NaOH and $CaCl_2$ (Fig. 4). Maximum desorption of Cd(II) occurred with HNO_3 followed in decreasing order by H_2SO_4, $CaCl_2$ and HNO_3. To assess the reusability of the biomass in consecutive cycles, a second cycle was performed, involving all eluants. Although in the first and second cycle NaOH had a small influence in the desorption of Cd(II) from the biomass with a desorption efficiency of 15.3%, it

TABLE 1

Kinetic parameters for Cd(II) biosorption by dead *T. viride*

Initial concentration (mg/L)	Kinetics	Parameters	Temperature		
			25°C	40°C	50°C
25	Pseudo-first-order	q_e (mg g^{-1})	2.6826	2.6248	2.8711
		k_1 (min^{-1})	4.75×10^{-5}	9.14×10^{-5}	15.19×10^{-4}
		R^2	0.9510	0.6256	0.8113
	Pseudo-second-order	q_e (mg g^{-1})	0.3186	0.8196	1.2226
		k_2 (g mg^{-1} min^{-1})	0.0056	0.0021	0.0027
		h (mg g^{-1} min^{-1})	0.0005	0.0026	0.0041
		R^2	0.9265	0.9709	0.9854
	Elovich equation	α (mg g^{-1} min^{-1})	0.6796	0.0091	0.0142
		β (mg g^{-1} min^{-1})	17.3340	6.7426	4.7194
		R^2	0.8305	0.9138	0.9464
50	Pseudo-first-order	q_e (mg g^{-1})	3.2628	4.4477	4.4712
		k_1 (min^{-1})	5.54×10^{-5}	7.34×10^{-5}	9.64×10^{-4}
		R^2	0.8528	0.7695	0.8979
	Pseudo-second-order	q_e (mg g^{-1})	1.4986	2.1239	2.2709
		k_2 (g mg^{-1} min^{-1})	0.0481	0.0150	0.0097
		h (mg g^{-1} min^{-1})	0.1079	0.0677	0.0504
		R^2	0.9996	0.9974	0.9938
	Elovich equation	α (mg g^{-1} min^{-1})	39932.49	22.4997	10.0771
		β (mg g^{-1} min^{-1})	13.1873	5.7339	5.0150
		R^2	0.6543	0.9557	0.8869
100	Pseudo-first-order	q_e (mg g^{-1})	6.0686	6.8509	6.7704
		k_1 (min^{-1})	7.25×10^{-5}	13.96×10^{-5}	16.99×10^{-4}
		R^2	0.7953	0.7245	0.7618
	Pseudo-second-order	q_e (mg g^{-1})	1.7135	2.6225	2.9376
		k_2 (g mg^{-1} min^{-1})	0.0206	0.0010	0.0013
		h (mg g^{-1} min^{-1})	0.0606	0.0073	0.0115
		R^2	0.9987	0.9614	0.9673
	Elovich equation	α (mg g^{-1} min^{-1})	1.6627	0.0250	0.0435
		β (mg g^{-1} min^{-1})	5.0289	2.0386	1.7827
		R^2	0.6874	0.8766	0.8991

increased the sorption in the second cycle from 69.4% (achieved in the first cycle) to 78.3%. This consequence suggests that a pretreatment of the biomass would enhance the sorption capacity of *T. viride* toward cadmium ions.

The rest of the desorbing agents decreased the sorption efficiency with 50.2–64.2%, indicating that the use of desorbing agents is not useful for the reusability of the biomass and other considerations have to be taken into account.

Biosorption of Cd(II) by living biomass of *T. viride*
Effect of initial pH on Cd(II) removal
Taking into consideration the previously mentioned importance of the pH in metal biosorption and bioaccumulation processes, experiments were undertaken at different pH values of the metal solutions in broth.

In the experiments, the initial pH values in the range 4.0–7.0 were adjusted before the addition of the viable *T. viride*. The growth and removal efficiency of *T. viride* is shown in Fig. 5a. *T. viride* was able to bioaccumulate 100% of 50 mg/L Cd(II) at pH 6.0. Moreover, the growth of the microorganism in the presence of Cd(II) was estimated at 13.37 g/L. These values were similar to the growth of the microorganism in the absence of Cd(II), indicating that at this point (pH 6.0 and 50 mg/L Cd(II)) *T. viride* could tolerate the metal ion.

For lower pH values (4.0–5.0), a decrease in the metal uptake capacity and growth of *T. viride* were noticed. Only 20–40% Cd(II)

was bioaccumulated by the living microorganism. The low bioaccumulation capacity at this low pH values is attributed to the competition of hydrogen ion with metal ion on the sorption site. Thus, at lower pH, negative charge intensity on the site is diminished as a result of binding sites protonation, resulting in the reduction or inhibition of metal ion binding [40].

Growth of *T. viride* in the presence of Cd(II)
We investigated the growth of *T. viride* and Cd(II) bioaccumulation as a function of initial metal ion concentration. Figure 5b shows the growth profiles of *T. viride* in a medium without heavy metal (control) and for a growth medium containing 25 and 50 mg/L Cd(II), during 168 hours.

The control culture of *T. viride* contained 1.3 g/L biomass in the first day of cultivation, which increased to 13.03 g/L after 80 hours. The addition of Cd(II) into the *T. viride* enriched medium resulted in a slower growth of the microorganism, in the time interval of 60–144 hours, but then biomass reached 14.56 and 13.37 g/L after 168 hours, when the medium contained 25 and 50 mg/L metal ions, respectively. *T. viride* was able to remove 100% Cd(II) from solutions containing 25 and 50 mg/L Cd(II). Our results also indicated that *T. viride* could tolerate cadmium concentrations up to 200 mg/L (Table 4). The removal efficiency of Cd(II) bioaccumulation efficiency decreased from 100% to 21.27%, with any increase in metal concentration from 25 to 200 mg/L. Also the growth of *T. viride* was affected by the presence
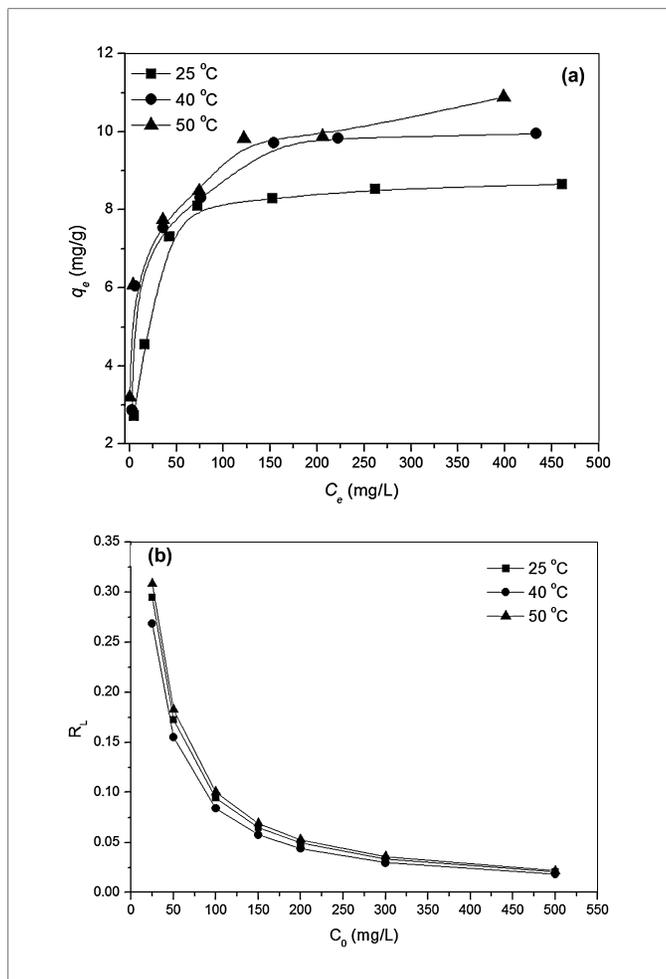
FIGURE 3

Equilibrium characteristics for Cd(II) biosorption by dead *T. viride*: **(a)** equilibrium isotherm for Cd(II) biosorption by dead *T. viride* (pH 6; biomass dosage: 8 g/L; contact time: 72 hours); **(b)** separation factor profile for Cd(II) biosorption by dead *T. viride* at various temperatures.

of 200 mg/L Cd(II), reaching to 6.99 g/L biomass. This behavior can be met in the cases of other metals and microorganisms. Arunakumara et al. [41] reported that the growth of *Spirulina platensis* was inhibited by Pb^{2+} at high concentrations. El-Helow et al. [42] determined that a *Bacillus thuringiensis* culture was able to remove 2.05–6.8 mg of cadmium per gram of biomass, respectively.

Increasing concentrations of the metal ion in solution resulted in a reduction of the bioaccumulation efficiency. Table 4 shows that in the presence of 25, 50, 75, 100, 150 and 200 mg/L in 168 hours, *T. viride* culture was able to accumulate 1.54, 3.48, 4.02, 5.89, 7.85 and 4.17 $mg_{Cd}/g_{biomass}$, respectively.

These values indicate that living *T. viride* is more effective for Cd(II) removal when compared with the performance reported for other microbial biomasses.

Mechanism of Cd(II) removal by dead and living biomass of *T. viride*

The interaction mechanism between the metal ions and dead and living cells of *T. viride* was investigated by using Fourier Transform Infrared (FTIR) spectroscopy. Functional groups present on the cell surface were identified by FTIR spectroscopy as each group has a unique energy adsorption band [43].

For the detection of active sites involved in biosorption process, FTIR spectra of the unloaded and metal-loaded biomass of dead and living *T. viride* were analyzed. The IR spectra (Fig. 6) are highly complex, reflecting the complex nature of the biomass. Three prominent peaks, in the unloaded *T. viride* biomass were noticed at 3248.50, 1632.44 and 1013.40 cm^{-1}. The peak 3248.5 cm^{-1} was stronger due to amine group (NH stretching) and hydroxyl group vibrations (OH stretching) which was reported to occur at 3550–3230 cm^{-1}. The peak at 2931.2 cm^{-1} is defined by C–H stretching vibrations which occur at 3000–2700 cm^{-1}. The peaks between 1900 and 1550 cm^{-1} are indicative for carbonyl (C=O) stretching vibration, while that corresponding to 1632.4 cm^{-1} and 1372.1 cm^{-1} are supposed to be caused by C=O stretching mode of carboxyl group conjugated to a –NH deformation mode resulting in –CO–NH–, indicative of amide bond formation [44,45]. A characteristic peak was detected at 1013.4 cm^{-1}, representing the

TABLE 2

Isotherm parameters for Cd(II) biosorption by dead *T. viride* at different temperatures

	Temperature	q_m (mg/g)	K_L (L/mg)	R^2
Langmuir isotherm	25°C	8.86	0.0958	0.9997
	40°C	10.16	0.1089	0.9990
	50°C	10.95	0.0895	0.9948
	Temperature	**n**	**K_F (mg/g(L/mg)$^{1/n}$)**	**R^2**
Freundlich isotherm	25°C	5.8944	3.3998	0.6598
	40°C	2.2833	6.5632	0.9425
	50°C	2.6760	7.1586	0.8968
	Temperature	**K_T (L/mol)**	**B**	**R^2**
Temkin isotherm	25°C	1.00013	251.3×10^6	0.7218
	40°C	1.00014	289.7×10^6	0.9491
	50°C	1.00014	288.9×10^6	0.9489
	Temperature	**q_m (mg/g)**	**β**	**R^2**
D–R isotherm	25°C	13.82	1.99×10^{-9}	0.7307
	40°C	13.21	1.33×10^{-9}	0.9637
	50°C	13.57	1.28×10^{-9}	0.9528

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TABLE 3

Thermodynamic data of Cd(II) biosorption by dead *T. viride* at different initial concentrations

C_i (mol/L)	ΔH^0 (kJ/mol)	ΔS^0 (J/mol K)	ΔG^0 (kJ/mol)		
			298.15 K	313.15 K	323.15 K
4.44×10^{-4}	56.12	178.19	2.99	0.31	−1.46
8.89×10^{-4}	11.61	7.79	4.32	4.15	4.03
17.79×10^{-4}	12.14	16.30	7.28	7.03	6.87
26.68×10^{-4}	12.69	14.32	8.42	8.20	8.06
44.48×10^{-4}	11.87	6.71	9.89	9.76	9.70
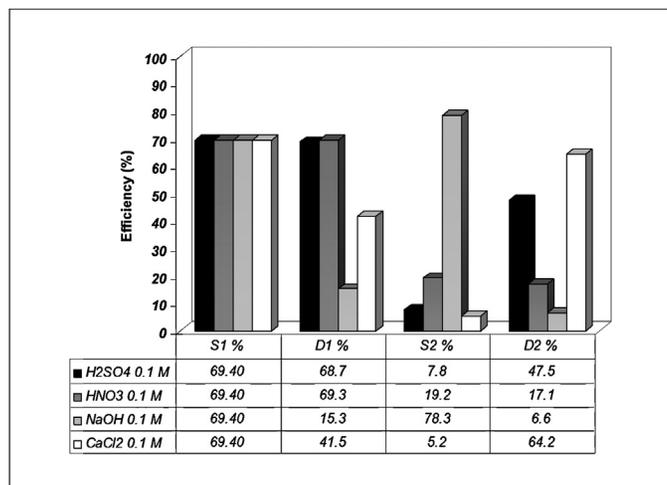
FIGURE 4

Sorption–desorption profiles of Cd(II) by dead *T. viride* biomass (Cd(II) concentration: 100 mg/L; biomass dosage: 8 g/L; temperature: 25°C).

P–OH stretching vibration of phosphate groups. The FTIR spectrum of *T. viride* loaded with Cd(II) showed shifts of several peaks when compared with unloaded biomass; these shifts are not meaningful, which implies that heavy metal removal by the microorganism is predominately made by electrostatic interactions. When Cd(II) ion is retained on the biomass, the peaks corresponding to 2853.24–2854.21 cm^{-1} (C–H stretching vibrations) and 1741.40–1744.46 cm^{-1} (C=O stretching vibration) become more prominent; these mean that when Cd(II) interacts with dead and living *T. viride* the structure becomes more 'relaxed'. The characteristic peak detected at 1013.4 cm^{-1} and representing the P–OH stretching vibration is significantly reduced; this means that the phosphate groups are definitely implied in the removal process of Cd(II) from aqueous solutions by dead and living *T. viride*.

These results are confirmed by the experimental data, very well fitted by the Langmuir model, which suggests that the uptake occurs on a homogeneous surface by monolayer sorption without interactions among the adsorbed molecules. The values of mean free energy, *E* (kJ/mol), indicate that chemical ion-exchange is the dominant process involved in Cd(II) biosorption at 25°C and with an increase in temperature particle diffusion mechanism could have taken an important role.

Optimization results

The performance of SVM is heavily dependent on the model's parameters; consequently, their selection is an important
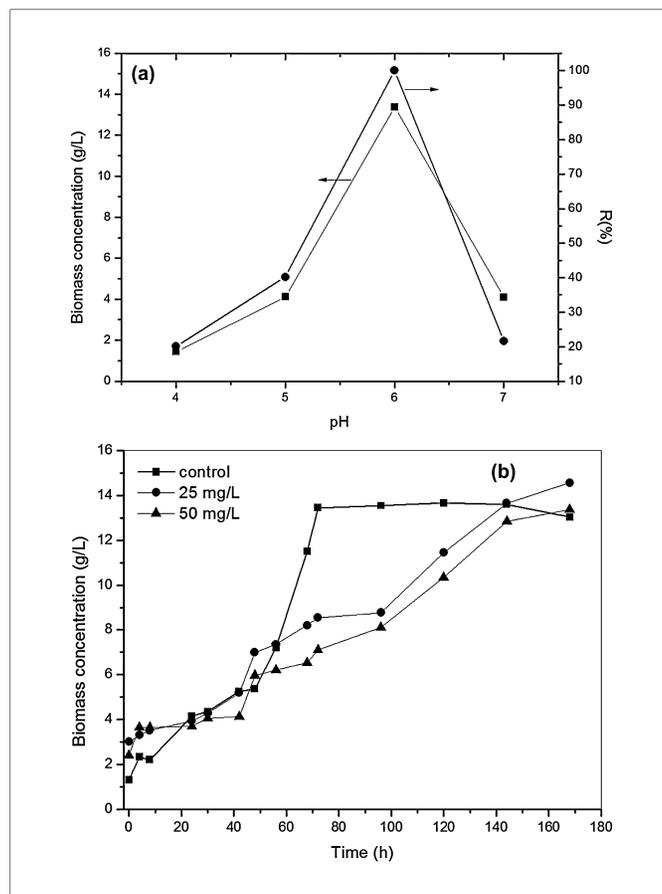
FIGURE 5

Behavior of *T. viride* and Cd(II) as partners during metal immobilization by living biomass: **(a)** effect of solution pH on Cd(II) removal and growth of *T. viride* biomass; **(b)** growth of *T. viride* at various initial Cd(II) concentrations.

TABLE 4

Effect of initial Cd(II) concentration on metal uptake (q_e), metal removal efficiency ($R\%$) and growth of *T. viride* (X, g/L)

C_i (mg/L)	*T. viride*		
	q_e (mg/g)	R (%)	X (g/L)
Control	–	–	13.03
25	1.54	100	14.56
50	3.48	100	13.37
75	4.02	69.87	12.95
100	5.89	76.63	11.82
150	7.85	72.94	11.05
200	4.17	21.27	6.99

TABLE 5

Optimization results for Cd(II) removal by *T. viride*

No.	Population dimension	Number of generations	Tournament dimension	pH	Biomass dosage	Time	Temp.	Initial conc.	Efficiency
1	20	20	2	5.85	8.41	3380	48	36.69	90.45
2	50	20	2	5.63	7.87	3867	46.5	30.60	94.98
3	100	100	2	6.00	8.01	3848	46.5	28.14	98.33
4	100	100	4	6.00	8.00	3833	46.5	26.11	98.91
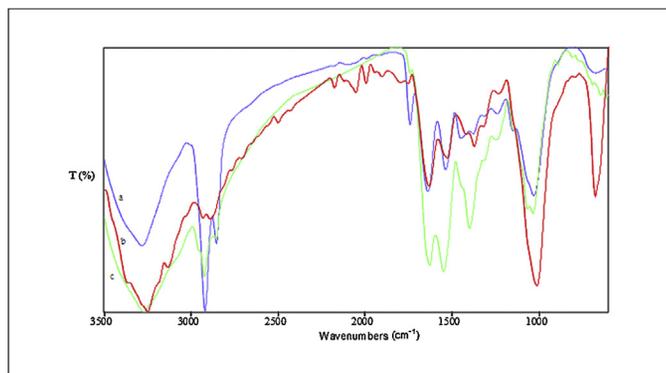
FIGURE 6

FTIR spectra of **(a)** dead *T. viride* + Cd(II); **(b)** living *T. viride* + Cd(II); **(c)** *T. viride*.

requirement. The SVR model parameters, C and γ, were selected by performing cross-validation on the data. The best performance was obtained with $C = 64$ and $\gamma = 1$, leading in the testing phase to MSE (mean squared error) = 0.85 and $R^2 = 0.919$. These errors are considered acceptable to continue with the addition of SVM model in the optimization procedure.

An important step in using GA as optimization solving method is the determination of the best values for its control parameters: number of generations, dimension of initial population, crossover and mutation probabilities. The last two are obtained automatically, based on adaptive feature implemented in GA, but tournament dimension is a supplementary parameter, corresponding to the chosen selection method (tournament selection).

Table 5 contains the best optimization results, meaning optimum values for the operating variables (pH, biomass dosage, contact time, temperature and initial concentration) which lead to a maximum efficiency of the process. For the crossover and mutation rates, the values determined in an autoadaptive manner

are 0.95 and 0.05, respectively. As a result, we found that the optimal operational values for 98.91% Cd(II) removal were: pH 6.0, contact time: 3833 min, biosorbent dosage: 8 g/L, metallic ion concentration: 26.11 mg/L, and temperature: 46.5°C.

Conclusions

This study brings the novelty of biosorption and bioaccumulation of Cd(II) by a new-isolated *T. viride* strain from a soil in Iasi city, Romania. Both biosorption and bioaccumulation were studied in batch system. The process is strongly influenced by pH, biomass dosage, contact time and temperature. Biosorption can be described very well by the Langmuir model, with high determination coefficients for all studied temperatures. The kinetics showed three distinct stages, characterized by a pseudo-second-order rate equation. The uptake capacity of dead and living *T. viride* appears to be similar, but living *T. viride* revealed higher removal efficiencies for all Cd(II) concentrations studied. The methodology developed in this article, based on support vector machines and genetic algorithm, was applied for finding the optimal working conditions which lead to the maximum removal efficiency of 98.91% Cd(II). The results are accurate and useful for experimental practice, being able to replace or, at least, to plan experiments that are resources consuming.

Conflict of interest

The authors have declared no conflict of interest.

Acknowledgements

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