Crystal Violet Dye Removal from Aqueous Solutions Using *Elodea Canadensis* as Biofilter

ANAMARIA TOROK[1], BOLDIZSAR NAGY[1], SZENDE TONK[2], ERZSEBET BUTA[3], ROBERT SZEP[4], CORNELIA MAJDIK[1]*, ALEXANDRA GABRIELA NICULAE[5]

[1] Babes Bolyai University, Faculty of Chemistry and Chemical Engineering, 11 Arany Janos Str., 40028, Cluj Napoca, Romania

[2] Sapientia Hungarian University of Transylvania, Faculty of Sciences and Arts, 4 Calea Turzii, 400193, Cluj Napoca, Romania

[3] University of Agricultural Sciences and Veterinary Medicine, Faculty of Horticulture, 3-5 Manastur Str., 400372, Cluj Napoca, Romania

[4]Sapientia Hungarian University of Transylvania, Faculty of Technical and Social Sciences, 1 Piata Libertatii, 530104, Miercurea Ciuc, Romania

[5] University Politehnica of Bucharest, Faculty of Applied Chemistry and Materials Science, 1-7 Gheorghe Polizu Str., 011061, Bucharest, Romania

The phytoremediation of Crystal violet dye was developed using the aquatic plant Elodea canadensis as biofilter. We analyzed the role of functional groups in phytoremediation by aquatic plants using the Fourier transform infrared spectroscopy (FTIR). The aquatic plant's abiotic stress responses were evaluated under exposure of 20 - 90 mg/L initial dye concentration, where a significant decrease of the photosynthetic pigments content suggests the plant's biosynthesis deregulation at higher concentrations. We determined the effect of operational parameters such as contact time, plant quantity initial concentration, initial pH and temperature on the removal efficiency. Data were analysed using the kinetic (pseudo-first- and second-order) and isotherm (Langmuir, Freundlich and Dubinin-Radushkevich) models. Our results show that the phytoremediation process follows the pseudo-second-order kinetic model, meaning that it takes places as chemisorption. The Crystal violet uptake was successfully described using the Freundlich isotherm model as a multilayer adsorption with heterogeneous energetic distribution of active sites.

Keywords: Crystal violet, aquatic plant, phytoremediation, effect of operational parameters, kinetics, isotherm

The removal of pollutants from aqueous solutions and soils is a priority research topic in environmental chemistry. The hazardous pollutants present in wastewaters can be removed by various conventional methods including filtration, ion exchange, chemical precipitation, photocatalytic oxidation and ozonization [1,40]. Considering the current expectations in wastewater treatment field, the adopted technology should be low-cost, eco-friendly, without further formation of harmful by-products and based on using a high efficiency biomaterial for heavy metals and toxic industrial dyes removal [2, 41].

Phytoremediation has gained researchers attention and popularity as an efficient environmental-friendly and *in situ* technology, able to remove different kinds of pollutants [3,4].

In this alternative technology, live plants are used to clean up and adsorb organic or inorganic pollutants and to minimize the pollution impact.

Several terrestrial and aquatic macrophytes have a great ability to remediate polluted environments due to an extremely diverse metabolism which allows them to remove recalcitrant pollutants [5,42]. *Elodea canadensis* is a freshwater submerged macrophyte species from the *Hydrocharitaceae* family with long, flexuous leafy stems, initially rooted in the substrate that provide a good habitat for many aquatic invertebrates or fishes. Also, they have an important role in changing the nutrient levels, regulating oxygen and balance the *p*H of lakes and rivers ecosystem [6, 7]. *Elodea canadensis* can be a useful tool for the removal of different kind of toxic wastes such as organic fungicides [5,8], ibuprofen, diclofenac, naproxen, clofibric acid, triclosan and caffeine [9], atrazine [10] and inorganic contaminants: Fe [11], Ni [12], Cu [13], Mn, Cr, Zn, Cd, and Pb [14].

The wastewaters polluted with organic dyes are difficult to eliminate due to their complex aromatic structure, which are stable to light or heat and are considered carcinogenic and mutagenic for the water bodies and human health in high concentrations [2]. Crystal violet is a triphenylmethane dye, widely used in various industries such as: cotton and silk dyeing, paints and printing ink, histological stain in veterinary medicine or as skin disinfectant in the medical community [15, 16]. However, in high concentrations, the Crystal violet dye can cause eye irritation, painful sensitization to light and it may lead to respiratory and kidney failure [15].

The primary aim of our study was to develop an alternative method for Crystal violet dye removal from aqueous solutions using *E. canadensis* as live biofilter. In order to predict the phytoremediation mechanism, we assessed the effects of the different operating parameters on the removal efficiency. The obtained data were analyzed through isotherm and kinetic models in order to achieve useful information and to characterize the phyto-remediation process. The Fourier transform infrared spectroscopy (FTIR) study was used for the extensive characterization of Crystal violet adsorption. Also, in order to reveal the plants responses to the abiotic stress produced by the Crystal violet dye, the plants photosynthetic pigments (carotenoid, chlorophyll *a* and *b*) were also determined by UV-vis spectroscopy.

* email: majdik@chem.ubbcluj.ro

Experimental part

Plant material and growing conditions

Elodea canadensis with an addition of fertilizer (Complex 3, 0.5 %) was grown in a greenhouse (at University of Agricultural Sciences and Veterinary Medicine in Cluj-Napoca, Romania).

The plants with an age of 60 days were selected for the phytoremediation experiments. The plants were kept under laboratory conditions for an acclimatization period of four days in modified Hoagland nutrient solution at room temperature, with a 14/10 h (light/dark) photoperiod.

The modified Hoagland nutrient solution contained: 1.25 mM KNO_3 1.25 mM $Ca(NO_3)_2$, 0.5 mM $MgSO_4 \cdot 7H_2O$, 0.25 mM KH_2PO_4, 11.6 μM H_3BO_3, 4.5 μM $MnCl_2 \cdot 4H_2O$, 10 μM Fe(III)EDTA, 0.19 μM $ZnSO_4 \cdot 7H_2O$, 0.12 μM $Na_2MoO_4 \cdot 2H_2O$, and 0.08 μM $CuSO_4 \cdot 5H_2O$ (the chemicals were purchased from Merck, Germany). All chemicals used were of analytical grade.

Phytoremediation experiments and dye analysis

The Crystal violet (CV) cationic triphenylmethane dyes were used as organic pollutants in the phytoremediation experiments. The cationic dye (CV λ= 590 nm) was procured from Penta (Czech Republic). The working solutions were prepared by diluting 1 g/L stock solution with nutrient solutions. The solutions concentrations were analyzed with the double beam UV-visible spectrophotometer (UV-Vis: GBC Cintra 202, Australia).

The phytoremediation process efficiency was analyzed for various operational parameters in the following conditions:

(a) Effect of contact time m_{plant} = 4 g (fresh weight), C_i = 20 mg/L (initial CV concentration), V = 0.2 L, at room temperature, 1 to 18 day phytoremediation period;

(b) Effect of initial concentration of CV: C_i = 20 - 90 mg/L, m_{plant} = 4 g (fresh weight), V = 0.2 L, at room temperature, 9 day phytoremediation period;

(c) Effect of plant quantity: m_{plant} = 1 - 5 g (fresh weight), C_i = 30 mg/L, V = 0.2 L, at room temperature, 9 day phytoremediation period;

(d) Effect of initial pH in the range 3.0 to 9.0, m_{plant} = 4 g (fresh weight), C_i = 30 mg/L, V = 0.2 L, at room temperature, 9 day phytoremediation period;

(e) Effect of temperature: t_1 = 10°C, t_2 = 23°C, t_3 = 40°C, m_{plant} = 4 g (fresh weight), C_i = 30 mg/L, V = 0.2 L, 9 day phytoremediation period.

The phytoremediation process was characterized by the determination of *E. canadensis* phytoremediation efficiency (E, %) and capacity (mg/g), calculated by the eq. (1) and (2), respectively.

$$Efficiency = \frac{C_i - C_f}{C_i} \cdot 100 \quad (1)$$

$$q_{max} = \frac{(C_i - C_f) \cdot V}{m} \quad (2)$$

where: C_i and C_f represent the initial and final concentration of CV (mg/L) in the aqueous solution, q_{max} (mg/g) represents the amount of dye adsorbed on plant weight unit; V (L) stands for the volume of dye aqueous solution and m (g) for plant quantity.

The Fourier transformed infrared (FTIR) analysis

Crystal violet dye was controlled and used (after phytoremediation), when the dried aquatic plant samples were subjected to the Fourier transformed infrared spectroscopy (FTIR) analysis. The dried samples were prepared encapsulating 1.2 mg of finely grounded plant particles in 300 mg of KBr. Infrared spectra were obtained using a JASCO 615 (Japan) FTIR spectrometer 4000-500 cm^{-1} (resolution, 2 cm^{-1}) and the data were processed using ORIGIN PRO 8.5 software.

Photosynthetic pigments analysis

Fresh leaf tissues were homogenized with ethanol and the extracts were centrifuged for 10 min. at 5000 rpm.

The chlorophylls (chlorophyll *a* and *b)* and total carotenoid (*x+c* = xanthophylls and carotenes) pigments quantitative determination was evaluated spectrophotometrically at maximum absorbance of 664 (A_{664}), 648 (A_{648}) and 470 (A_{470}) nm, method described previously by Lichtenthaler and Buschman [17].

Statistical analysis

Each experiment was repeated three times. The experimental data were statistically analyzed using *t*-test. The phytoremediation efficiency values obtained for the tree experiments had a very small variation around the mean value (i.e. less than 5%).

Results and discussions

FTIR analysis

The FTIR spectra of *E. canadensis* control plant (a), CV control dye (b) and the *E. canadensis* after the phytoremediation experiments with CV (c) are shown in figure 1. The FTIR analyses give valuable information about the characteristic functional groups present on the plant surfaces, which have an important role in the phytoremediation process of the dye molecules. The control plant and treated plant spectra showed no changes in the range of 3500 - 2000 cm^{-1}, which corresponds to the stretching vibrations of –O-H, -N-H and –C-H groups.

After the phytoremediation experiments with CV dye, th[illegible]ce

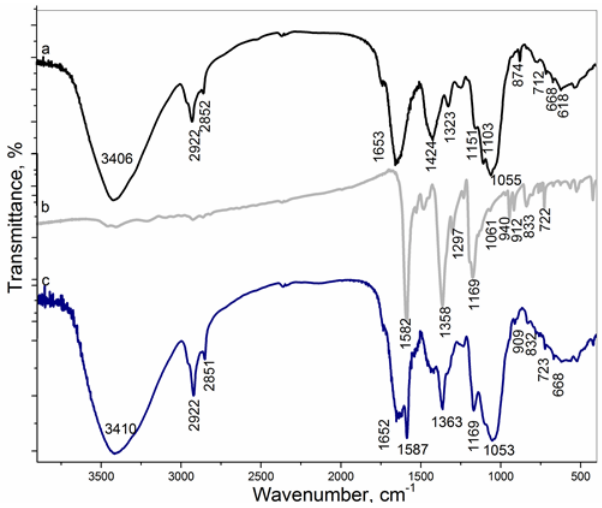

Fig. 1. FTIR spectra analysis of *E. canadensis* control plant (a), CV dye (b) and after the phytoremediation experiments of CV dye (c)

of three new peaks in the range of 2000 - 1000 cm^{-1}. The newly appeared peaks at 1587 cm^{-1}, 1363 cm^{-1} and 1169 cm^{-1} are attributed to the C=C stretching of the benzene ring, due to the -C-H bond deformation in the methyl group and also to the -C-N group vibrations.

The bands identified in < 900 cm^{-1} region exhibited minor changes, which corresponds to phosphate and sulfonate functional groups and to the bending modes of aromatic compounds.

In conclusion, it can be mentioned that the CV dye spectra contain (fig. 1b) three specific intense peaks at the range of 2000 - 1000 cm^{-1}, which can be considered as the CV dye fingerprints zone. The appearance of three CV

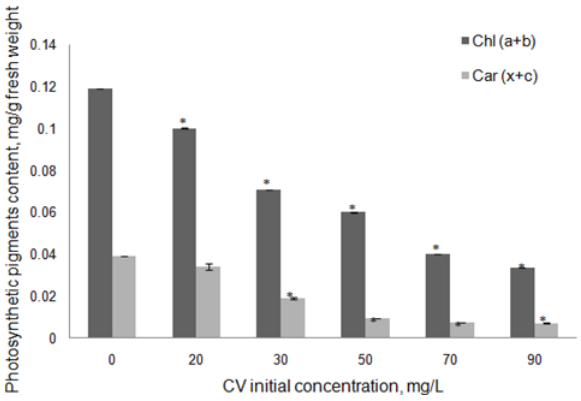

Fig. 2. Contents of total chlorophyll: $a+b$ [Chl($a+b$)] and total carotenoids: $x+c$ [Car$(x+c)$] (mg/g fresh weight) in control *E. canadensis* and exposed to various initial concentration of CV dye (mean ± SD, n = 3, * indicates the significantly difference at $P < 0.05$)

peaks (1587 cm^{-1}, 1363 cm^{-1}, and 1169 cm^{-1}) was identified in the treated plant spectrum, which clearly indicates the CV dye uptake by the *E. canadensis*.

Our results are in concordance with other reports based on the removal of triphenylmethane dyes using different kind of plants and biosorbents [18-21].

Photosynthetic pigments

The aquatic plants photosynthetic pigments were determined after nine days (ideal phytoremediation period, determined from the experiments of effect of contact time) of exposure at initial concentration of CV in the range of 20 - 90 mg/L. The result indicates that the exposed plants chlorophyll (chlorophyll *a* and *b*) content is significantly decreased after the CV treatment (fig. 2).

The highest decrease can be observed at 70 and 90 mg/L initial concentrations, whereas the decline of chlorophyll content compared to the control plant is 66 and 72 %, respectively. The *E. canadensis* carotenoids ($x+c$) content were also significantly affected after the phytoremediation of CV dye at initial concentration higher than 30 mg/L.

Therefore, it can be noticed that the exposure in high concentrations of CV dye, induce biosynthesis deregulation, wherein the plants cannot resist to the induced abiotic stresses. This hypothesis is confirmed by the photosynthetic pigments content decrease.

The effect of operational parameters

The phytoremediation process of CV by *E. canadensis* is dependent by several operational parameters such as: plant quantity, contact time, initial concentration, pH and temperature. The phytoremediation efficiency (E, %) depending on different operational parameters is presented in figure 3.

Effect of contact time and initial dye concentration

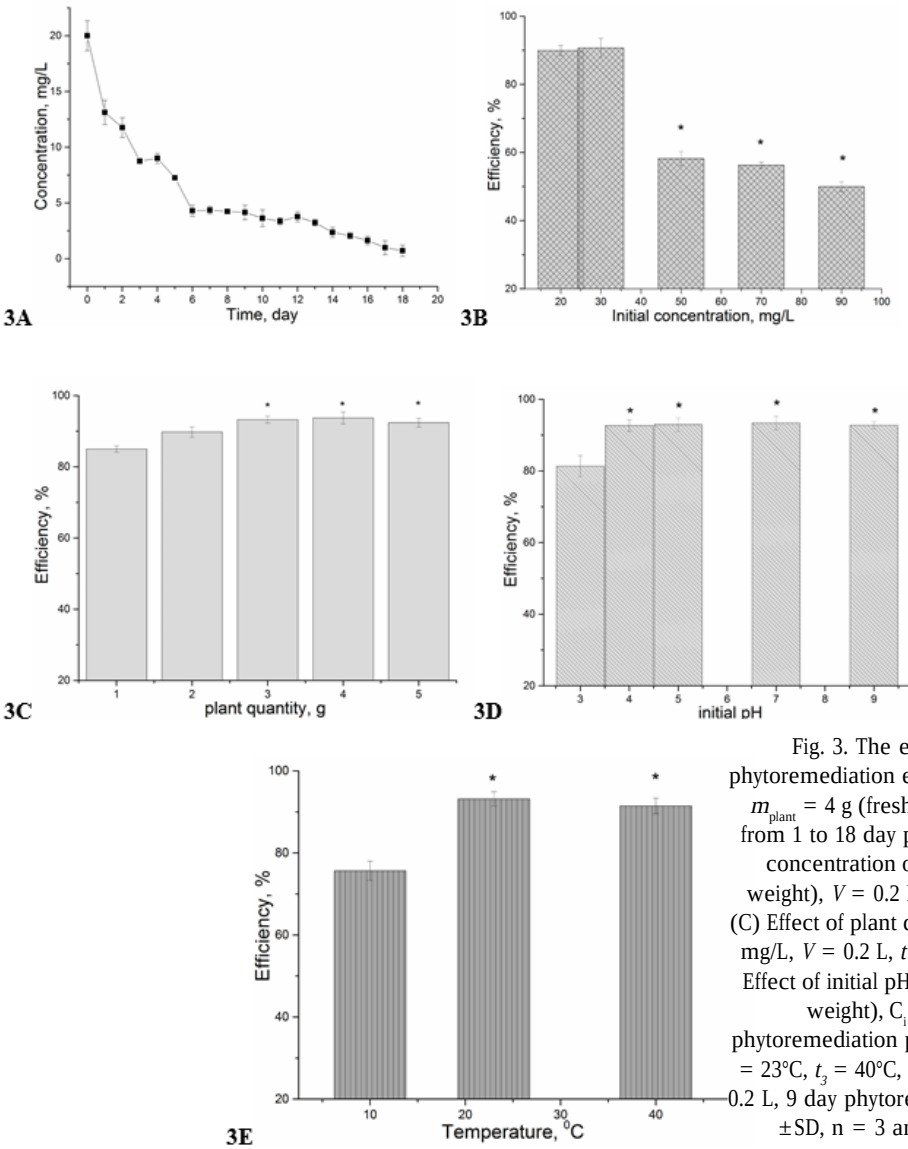

Fig. 3. The effect of operational parameters on the phytoremediation efficiency of CV dye (A) Effect of contact time m_{plant} = 4 g (fresh weight), C_i = 20 mg/L, V = 0.2 L, t = 23°C, from 1 to 18 day phytoremediation period; (B) Effect of initial concentration of CV: C_i = 20 - 90 mg/L, m_{plant} = 4 g (fresh weight), V = 0.2 L, t = 23°C, 9 day phytoremediation period, (C) Effect of plant quantity m_{plant} = 1 - 5 g (fresh weight), C_i = 30 mg/L, V = 0.2 L, t = 23°C, 9 day phytoremediation period; (D) Effect of initial pH from 3.0 to 9.0 initial pH, m_{plant} = 4 g (fresh weight), C_i = 30 mg/L, V = 0.2 L, t = 23°C, 9 day phytoremediation period; (E) Effect of temperature t_1 = 10°C, t_2 = 23°C, t_3 = 40°C, m_{plant} = 4 g (fresh weight), C_i = 30 mg/L, V = 0.2 L, 9 day phytoremediation period. (The values are the mean ±SD, n = 3 and the asterisk indicates the significantly differences at $P < 0.05$)

The efficiency of CV dye phytoremediation in function of contact time was analyzed for 18 days, until the final equilibrium was reached. We have noted that during the removal process, high quantities of CV were absorbed in the first six to10 days of experiment (fig. 3A). Thus, to study the effect of different operational parameters, an ideal period of nine days was chosen for further experiments.

The initial dye concentration is an important factor in the phytoremediation process. In order to evaluate the effect of CV dye concentrations on removal efficiency, *E. canadensis* was exposed to five different concentrations in the range of 20 - 90 mg/L (fig. 3B). The highest phytoremediation efficiency was recorded for 20 and 30 mg/L initial CV dye concentrations.

For other concentrations, no significant decrease in efficiency was noticed. These results can be attributed to the physical contact between the dye molecules and root surface of the plant, allowing the mass transfer resistance to be decreased (due to the enhanced diffusion) with the increase in dye concentration (the results revealed an increase of the plant uptake capacity from 2.06 to 28.62 mg/g for C_i = 20 mg/L and C_i = 90 mg/L) [22, 23]. These results may confirm the relationship that exists between *E. canadensis* removal efficiency and initial CV dye concentrations. Our results are in good agreement with the report of Torbati et al., who analyzed the removal of Basic Red 46 using *Nasturtium officinale* [24].

The effect of plant weight

In order to study the effect of plant weight over the CV phytoremediation efficiency, various plant quantities (1 - 5 g) were tested. The results clearly indicate (fig. 3C) that the highest dye removal was achieved for 4 g fresh weight plant. The increase in the removal efficiency can be attributed to the higher surface area and more active sites available for the phytosorption process. It can be also mentioned that the difference between removal efficiency at 4 respectively 5 g of plant quantity (fresh weight) was not considerable, thus, 4 g plant was performed as ideal plant weight for further phytoremediation experiments. These observations are in concordance with Vafaei et al., who analyzed the phytoremediation of an anionic dye, Acid Blue 92 using *Hydrocotyle vulgaris* [25].

The effect of initial pH

Different initial pH values were tested during the phytoremediation experiments. It was found that the removal efficiency increase along with the initial pH increase in the aqueous medium (fig. 3D). The phytoremediation efficiency was significantly higher in four cases, the highest dye removal was observed at the initial *p*H value of 7.0. Generally, the *p*H of the medium has a major influence in the plants growth regulation and could also affect the mobility and availability of ions, and the uptake of some nutrients [25,26]. In our experiments, minor differences were observed in the removal efficiency of the aquatic plants exposed to initial pH in the range 4.0 to 9.0.

We concluded that the CV removal efficiency of *E. canadensis* is favorable in a wide range of *p*H from acidic to alkaline.

The effect of temperature

The effect of temperature on the phytoremediation efficiency of CV dye was tested in the temperature range of 10 - 40°C, at an initial dye concentration of 30 mg/L. As Ffigure 3E shows, the removal of CV dye increase with increasing the temperature from 10 to 40°C. In the case of submerged macrophytes, at low temperatures, the composition of plasma membrane lipids is changed, fact which alters the plant membrane fluidity leading to lower membrane permeability and lower metal uptake [27]. Therefore, this hypothesis is in good agreement with our results, whereas at 10°C the *E. canadensis* phytoremediation efficiency was found to be much lower. Our observations are compatible with the results of other literature reports with different kind of plants [23, 28].

Phytoremediation process characterization

In order to investigate the phytoremediation mechanism of CV dye, Lagergren pseudo-first-order and Ho and McKay pseudo-second-order kinetic models were used to fit the experimental data [29,30]. The pseudo-first-order kinetic can be described by the formula below

$$ln(q_e - q_t) = ln\, q_e - k_1 t \quad (3)$$

where: q_e is the amount of dye adsorbed at equilibrium (mg/g), q_t is the amount of dye adsorbed at time t (mg/g), k_1 is the adsorption rate constant of (1/day).

The pseudo-second-order kinetic model can be described using the following equation:

$$\frac{1}{q_e - q_t} = \frac{1}{q_e} + k_2 t \quad (4)$$

where: k_2 is the pseudo-second-order rate constant of phytoremediation capacities, q_e and q_t are phytoremediation capacities at equilibrium and at time t.

If the pseudo-second-order kinetic model is applicable, the plot of equation rearranged gives a straight line whose slope is equal to k_2.

The interpretation was based on the experimental data analysis obtained after the CV dye phytoremediation experiments, in the range of 20 - 90 mg/L initial concentrations.

The pseudo-first-order kinetic model assumes that the adsorption may take place as one adsorbate molecule onto one active site on the adsorbent surface. The pseudo-second order kinetic models hypothesis describes the adsorbent as is adsorbed on two active sites which also involves the electrons exchange or sharing between adsorbate and adsorbent [31, 43].

The pseudo-first- and pseudo-second-order kinetic model constants, the calculated values of k_1, k_2, $q_{e,calc}$ and $q_{e,exp}$ with their correlation coefficients (R^2) are presented in Table 1. It can be noticed that the correlation coefficient values obtained for the pseudo-second-order kinetic model are higher than the ones obtained for the pseudo-first order. Moreover, the calculated values and experimental data are matching very well. These findings confirm that the CV dye phytoremediation process can be best described by the pseudo-second-order kinetic model. Our results are in good agreement with the Saeed et al., which used grapefruit peels as adsorbent for CV removal [32].

Determination of equilibrium parameters is an essential step in the design of the phytoremediation system [33]. In the present study, three isotherm models: Langmuir, Freundlich and Dubinin-Radushkevich were utilized to fit the experimental data (data obtained from CV phytoremediation in the range of 20 – 90 mg/L initial concentrations).

The Langmuir adsorption isotherm model is known as a monolayer adsorption of an adsorbate onto the solid adsorbent surface, uniform sorption energy with no transmigration of phytosorption in the plane of the surface [34,35, 43]. The maximum phytosorption capacity

Table 1

KINETIC MODELS PARAMETERS FOR THE PHYTOREMEDIATION OF CV BY *E. Canadensis* AT DIFFERENT INITIAL CV CONCENTRATIONS (q_e IS THE AMOUNT OF ADSORBATE ADSORBED IN EXPERIMENTS AND CALCULATED RESPECTIVELY, k_1 AND k_2 IS THE RATE CONSTANT OF PSEUDO-FIRST- AND PSEUDO-SECOND-ORDER ADSORPTION)

C_i/ mg L^{-1}	$q_{e,exp}$/ mg g^{-1}	Pseudo-first-order			Pseudo-second-order		
		k_1/ day^{-1}	$q_{e,calc}$/ mg g^{-1}	R^2	k_2/ g mg-day^{-1}	$q_{e,calc}$/ mg g^{-1}	R^2
20	0.897	2.65×10^{-2}	0.8527	0.862	4.837×10^{-2}	0.9014	0.9648
30	1.3245	2.14×10^{-2}	1.0411	0.9748	3.561×10^{-2}	1.4409	0.9818
50	1.5105	2.13×10^{-2}	1.5089	0.7899	2.73×10^{-2}	1.6795	0.9015
70	2.328	2.11×10^{-2}	1.7631	0.9223	$1.97 \times 10^{-}$	2.4361	0.9868
90	2.714	3.56×10^{-2}	3.6638	0.9372	1.35×10^{-2}	2.912	0.9751

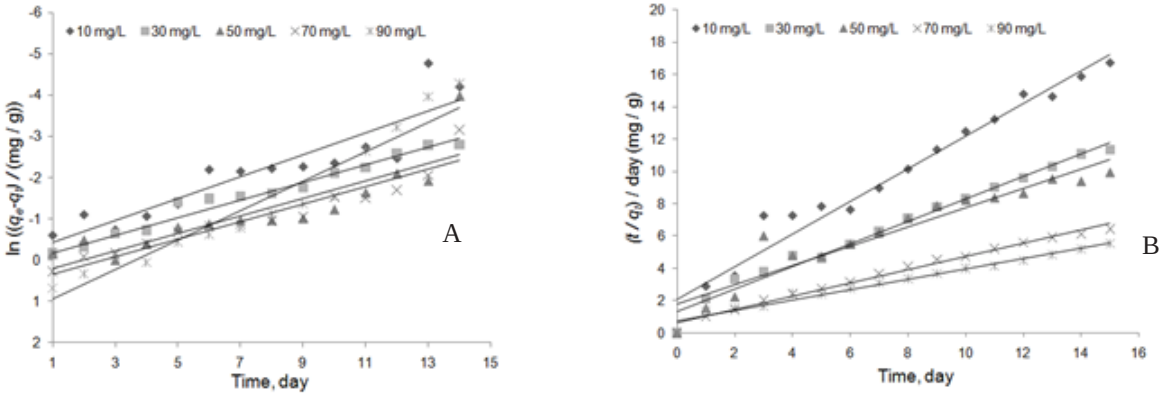

Fig. 4. Plot of the pseudo-first-order (A) and pseudo-second-order kinetic models (B) for phytoremediation of Cv dye by *E. canadensis* plant (m_{plant} = 4 g (fresh weight), C_i = 20 – 90 mg/L, V = 0.2 L, t = 23°C).

Table 2

ISOTHERM CONSTANTS FOR CV PHYTOREMEDIATION BY *E. canadensis*; C_i = 20 - 90 mg/L CV dye, 4 g PLANT

Langmuir				Freundlich			Dubinin-Radushkevich		
K_L/ L mg^{-1}	R_L	q_{max}/ mg g^{-1}	R^2	n	K_F/ $mg^{(1-1/n)}$ $L^{1/n}$ g^{-1}	R^2	E/ kJ mol^{-1}	β/ 10^{-5} mol^2 J^{-2}	R^2
0.140	0.263 (20 mg/L) 0.192 (30 mg/L) 0.125 (50 mg/L) 0.092 (70 mg/L) 0.073 (90 mg/L)	4.325	0.913	0.422	0.340	0.928	12.9	$3 \cdot 10^{-5}$	0.925

calculated from the Langmuir isotherm q_{max} and the K_L constant which is related to the free energy of phytosorption, are listed in table 2. According to Hall et al., there were calculated the essential features of the Langmuir isotherm dimensionless separation factor (R_L) [36]. The value of the separation factor indicates either the adsorption isotherm to be unfavorable ($R_L > 1$), favorable ($0 < R_L > 1$), linear ($R_L = 1$) or irreversible ($R_L = 0$). The calculated R_L values at different CV dye concentrations are in the range of 0.263 to 0.073 in all experimental systems (table 2), which confirms the favorable uptake process of CV by *E. canadensis*.

The Freundlich model proposed a multilayer adsorption with heterogeneous energetic distribution of active sites and interactions between dye molecules and plant [37,38]. The plots of ln_{qe} versus C_e for the CV phytoremediation by *E. canadensis* allows the determination of the isotherm constants K_F and n, which are presented in table 2. Comparing the two isotherm models correlation coefficients (R^2), it can be concluded that, Freundlich model exhibits a slightly better fit to the equilibrium data.

The Dubinin-Raduschkevich isotherm model predicts that the sorption process takes place through the free energy (E = kJ/mol) and predict two kinds of removal: (a) if the free energy values are between 8 - 16 kJ/mol, the removal process takes place as chemisorption and/or (b) if the values are E < 8 kJ/mol, the process occurs as physisorption [39,40]. The calculated E values in our study was found to be 12.9 kJ/mol (table 2), which reflects the fact that CV dye phytoremediation with *E. canadensis* takes places as chemisorption.

Conclusions

In this paper, we investigated the phytoremediation efficiency of *E. canadensis* for CV dye removal from aqueous solutions. The maximum phytoremediation efficiency was obtained under the optimized conditions derived from the study of various operational parameters inffluence. The optimal conditions were found to be: 4 g fresh weight plant, 30 mg/L initial concentration of CV dye, room temperature and initial pH of 7.0 and they allowed us to obtain the highest efficiency. The aquatic plants responded to the induced abiotic stress through the decrease of the photosynthetic pigments content at various initial dye concentrations.

In order to characterize the phytoremediation process, kinetic and isotherm models were used. The experimental data were best fitted with the pseudo-second-order kinetics, which confirms that the phytoremediation of CV dye take places probably *via* surface exchange reaction, as chemosorption. Taking into consideration the results obtained from isotherm analysis, it can be concluded that the equilibrium data match very well the Freundlich isotherm, fact that confirms the multilayer CV dye phytosorption by *E. canadensis*. The Dubinin-Radushkevich isotherm model predicts that the phytoremediation process is of chemical nature.

The result of this study shows the good potential of the *E. canadensis* aquatic plant on the removal of CV dye from aqueous solutions, which propels it as a promising biofilter in future wastewater treatment applications.

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