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CRYSTAL VIOLET DYE BIOSORPTION AND PHYTOEXTRACTION USING LIVING *SALVINIA NATANS* AND *SALVINIA NATANS* POWDER: A COMPARATIVE STUDY

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ABSTRACT. The main focus of this work was to investigate the biosorption behavior of living and powder *Salvinia natans* on Crystal violet (CV) removal. The effects of process parameters were studied in order to determine the optimum phytoremediation conditions. Adsorption isotherm and kinetic models for both processes were used to analyze the equilibrium data. It was found that Langmuir isotherm and pseudo-second-order kinetics models describe better the CV removal process. Thermodynamic parameters showed that the biosorption and phytoextraction process is endothermic. From the obtained results it can be concluded that *S. natans* powder showed higher bisorption capacity on CV removal compared to the living one.

***Key words**: Salvinia natans, Crystal violet, biosorption, phytoextraction, surface characteristic*

INTRODUCTION

Wastewater discharge containing hazardous dyes poses an important and increasing environmental danger [1]. Dyes remains one of the major constituents of the wastewater produced by many industries, such as textile, paint, varnishes, ink, plastics, pulp and paper, cosmetics, tannery and the dye-producing ones [2, 3, 4]. The discharge of extremely small amounts of dyes even at minimum concentrations can be toxic and difficult to degrade and remove due to their complex stabile structure [5, 6, 7]. Among various dyes, Crystal violet (CV), also known as Basic Violet 3, belongs to the triphenylmethane dyes class. It is a cationic dye used for various purposes among which include: biological stain, dermatological agent, veterinary medicine, poultry feed additive to

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inhibit propagation of mould, intestinal parasites and fungus etc. The presence of CV in the aqueous water system is considered risky to human health [8]. Because it is carcinogenic and non-biodegradable it can persist in a variety of environments. Therefore, CV it has been classified as a recalcitrant molecule since it is poorly metabolized by microbes [7, 9, 10].

Most of the technologies employed for dyes removal from wastewaters are based on physicochemical processes such as dilution, adsorption, coagulation and flocculation, chemical precipitation, oxidation, ion-exchange, reverse osmosis and ultra-filtration [11].

Bioremediation receives a considerable amount of attention as an alternative process to traditional methods in dyes and heavy metal removal from contaminated waters [12]. Bioremediation is a natural process which relies on bacteria, fungi and plants to alter contaminants, as these organisms carry out their normal life functions [13].

Therefore, many researchers use agricultural by-products such as olive tree pruning waste [14], sawdust [15], fir cone [16], olive-stone, olive mill solid, cocoa shells [17], grape stalks [18], in the removal of textile dye effluents [19].

Phytoremediation represents the use of green plants to remove or degrade contamination from soils and surface waters. It has been proposed as a cheap, sustainable, effective, and environment-friendly alternative to conventional remediation technologies. Plants use solar energy (through photosynthesis) to extract chemicals from the soil and to deposit them in the above-ground part of their bodies or to convert them to a less toxic form [20, 21]. Aquatic plant biomass, irrespective whether living or dead represents an abundant, cost-effective biological resource that possesses an immense capacity to accumulate organic dyes [22, 23] and hence is exploited worldwide for developing environment-friendly wastewater treatment technologies. Phytoremediation is often referred as botanical bioremediation or green remediation [24] and defined as the use of green plants to remove pollutants from the environment or to render them harmless. It is considered a new highly-promising technology for the remediation of polluted sites [25] and is currently divided into areas, being one of as phytoextraction.

Plant assays are highly sensitive to many environmental pollutants, including dyes [26] but few cases have been reported in which it is proven that plants have the potential capacity to degrade textile dyes [27]. The potential of some aquatic plants to accumulate dyes has been well demonstrated, supporting their possible use in the phytoextraction of contaminated water. These include: *Nymphaea violacea* [28], *Eleocharis dulcis* [29], *Ceratophyllum demersum* [30], *Myriophyllum spicatum* [31], *Lemna gibba* [32] and *Lemna minor* [33].

Salvinia natans is an ideal testing system that can be used in the water quality studies to monitor aquatic pollutants [34]. Besides the aquatic floating macrophytes, *S. natans* floating fern is a fast growing free-floating aquatic

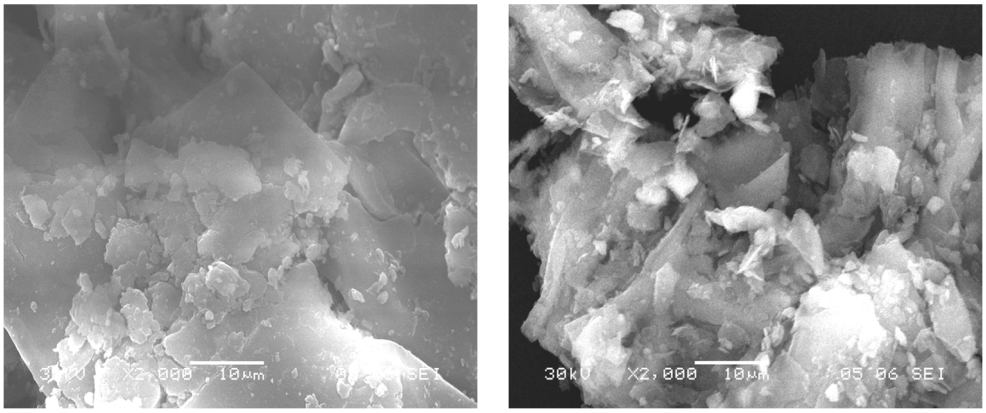
weed and was found to be particularly efficient in metal and pesticide removal from wastewaters [35]. Nevertheless, the use of powders obtained from plants, such as *S. natans* for organic dyes removal could be more advantageous than the use of living plants.

The aim of the present study was to compare the removal capacity and efficiency of Crystal violet dye using the aquatic plant *S. natans*, in both living and powder form. This research focuses on the removal of CV, from a comparative perspective, as well on the characterization of the plant's systems for the biosorption and accumulation capacities and morphological changes for the two processes.

RESULTS AND DISCUSSION

SEM analysis

The SEM micrograph of the adsorbent (after the phytoremediation experiments), *S. natans* and dye loaded adsorbent is presented in Fig. 1a, b. The analysis of the images showed the heterogeneous surface within *S. natans* powder particle where adsorption could occur. The micrographs show that the dye had densely and homogeneous adheres to the surface of carrier, as a result of either natural entrapment into the pores [36], due to physical adsorption by electrostatic force or due to covalent binding between the dye molecules and adsorbent.



(a) (b)

Fig. 1. SEM micrographs of *S. natans*
a) control plant and b) after the phytoremediation process.

Effects of the initial CV concentration

The biosorption of CV was carried out at different initial dye concentrations ranging from 20 to 90 mg/L contacting 0.4 g *S. natans* powder obtained from 5 g fresh biomass, pH initial = 5.4, 150 rpm stirring rate at room temperature 23ºC, with 240 min of contact time (until equilibrium was reached). Experiments with living biomass were carried out at the same initial dye concentrations ranging from 20 to 90 mg/L containing 5g fresh biomass, at room temperature 23ºC, $pH_{initial}$ = 5.4 and 200 mL synthetic dye solution.

The results are presented in (Fig. 2). In both cases, the biosorption capacity augments with the increasing of the initial concentration. The increase in biosorption capacity occurs due to the higher adsorption rate and the utilization of all the available active sites for biosorption at higher CV concentration.

According to the obtained results, it was concluded that the aquatic plants' highest removal efficiency was attained at the smallest initial concentration and that the removal capacity of aquatic plants depended strongly on the initial dye concentrations. Also the plant phytoextraction capacity is influenced by the plant's surface active sites and by the plant's uptake abilities and saturation.

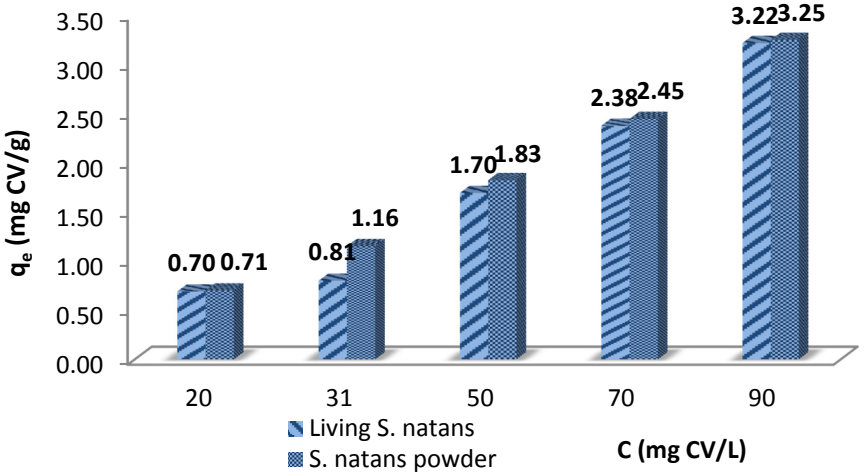


Fig. 2. Influence of the initial dye concentration over the biosorption capacity for CV phytoextraction and biosorption; C_i = 20-90 mg/L, 0.4 g biomass, 23ºC, pH 5.4, 150 rpm (biosorption).

The effect of pH

The pH has been identified as one of the most important parameter, that effect on dye biosorption. The pH of the aqueous medium is an important factor, which affects directly the living system's biological and

biochemical functions and it is directly related to the competition between protons and dye ions to active sites on the biosorbent surface. The effect of the solution pH onto CV biosorption and phytoextraction was studied within the range of pH 3.0 - 10. The highest removal efficiency for both processes was determined at the initial pH value of 5.4 and can be observed in (Fig. 3). At lower pH, the H^+ ions compete effectively with dye cations, showing a decrease in the dye removal efficiency. At higher pH, the plants surface becomes negatively charged, and this can intensify electrostatic force of attraction of the CV cations, increasing the phytoremediation efficiency.

The aquatic plants can tolerate a wide range of initial pH from acidic to alkaline. Their dye removal efficiency was notable in various cases and the hydrophytes possess characteristic properties to equilibrate the pH, which may present the plants responses to the induced water stress. These results can be explained by the plants metabolic reactions involving consumption, production or transfer of protons during the processes [37].

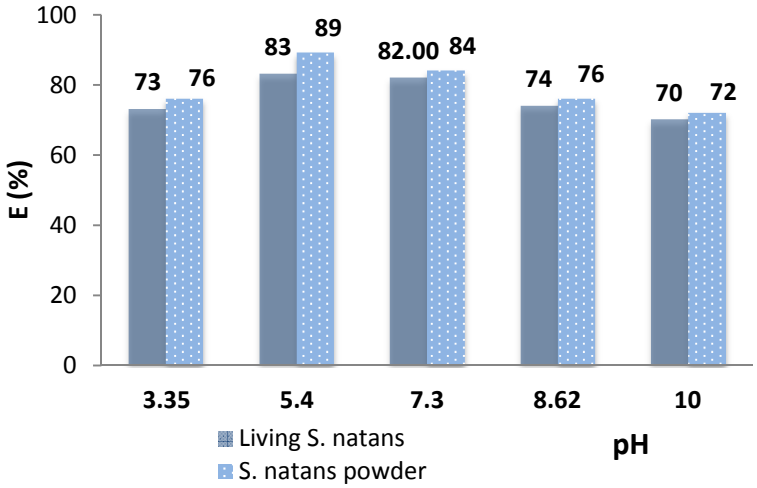


Fig. 3. The effect of initial pH values on the removal efficiency for CV phytoextraction and biosorption; C_i = 50 mg/L, 0.4 g biomass, 23ºC, 150 rpm (biosorption).

The effect of temperature

During the biosorption processes, temperature has a major effect on the plants' biochemical processes affecting the enzyme activity, the translocation of nutrients and photosynthesis [38]. The effect of temperature on the phytoremediation (for phytoextraction and biosorption, respectively) efficiency of CV dye was tested at the range of 10 - 35ºC. Results showed that the removal efficiency of dye CV increases with an increase in temperature from 10 to 35ºC

(Fig. 4), for both processes. The observed increase in biosorption of CV with rise in temperature is indicative of the fact that the biosorption process is endothermic in nature. The increase in the temperature of the system affects the solubility and particularly the chemical potential of the dye, which is known to be a controlling factor in the process of adsorption. A temperature increase minimizes the solubility of dye, hence adsorption accentuates. An increasing number of molecules may acquire sufficient energy to undergo an interaction with the active site at the surface [37].

The results indicated that the two types of phytoremediation processes have the ability to remove CV dye from the aqueous solutions.

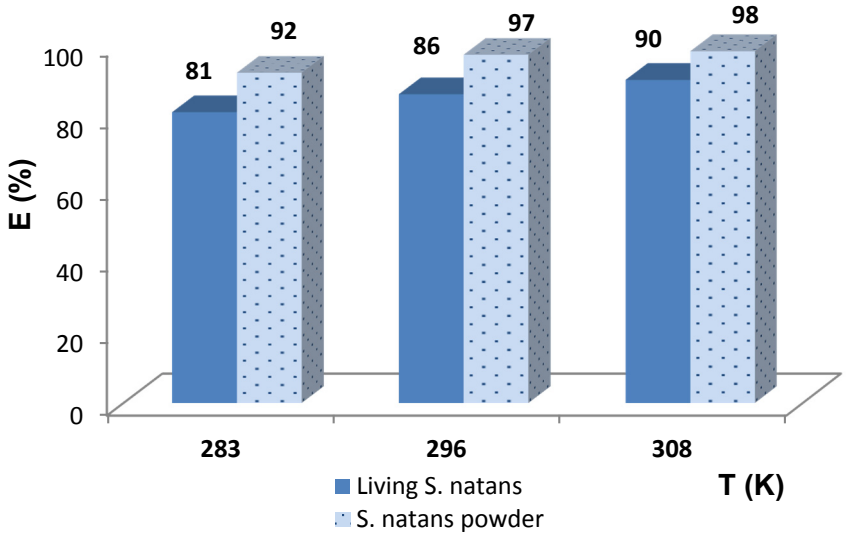


Fig. 4. Temperature influence over the removal efficiency of CV on living *S. natans* and *S. natans* powder; C_i = 50 mg/L, 0.4 g biomass, pH 5.4, 150 rpm (biosorption).

Biosorption and phytoextraction kinetics

The prediction of the biosorption rates gives important information for designing batch biosorption systems. Lagergren's pseudo-first-order and Ho's pseudo-second-order model, were applied on the experimental data to clarify the biosorption and phytoextraction kinetics of CV onto *S. natans* biomass [39,40]. The linear form of the pseudo-first-order (1) and pseudo-second-order (2) rate equation is given as:

$$\ln(q_e - q_t) = \ln q_e - k_1 t \quad (1)$$

and

$$\frac{dq_t}{dt} = k_2 \left(q_e - q_t\right)^2 \quad (2)$$

where q_t and q_e (mg/g) are the amounts of the adsorbed dye ions at equilibrium time (mg/g) and t (min), respectively and k_1 and k_2 is the rate constant (min^{-1}).

The R^2 and $q_{e,exp}$ values presented in (Table 1) indicated that the biosorption mechanisms and the phytoextracion process of CV onto *S. natans* biomass does not follow the pseudo-first-order kinetic model. It can be also seen that the experimental biosorption capacities are not in good agreement with the calculated ones. Therefore, the pseudo-first-order model is not suitable for modelling the CV biosorption and phytoextracion processes onto *S. natans*.

The rate constant for the pseudo-second-order model (k_2), R^2 and q_e values are given in (Table 2). The R^2 values are found very high (in range of 0.992-0.999 for CV biosorption and 0.9838-0.992 for phytoextraction, respectively). In addition, the theoretical $q_{e,cal}$ values were closer to the experimental $q_{e,exp}$ values. In view of these results, it can be said that the pseudo-second-order kinetic model provided a good correlation for the biosorption and phytoextraction of CV onto *S. natans* in contrast to the pseudo-first-order model [41].

Table 1. Pseudo-first-order and pseudo-second-order rate constants, calculated and experimental q_e values for CV removal using living *S. natans* and *S. natans* powder at different initial concentrations; C_i = 20-90 mg/L, 0.4 g (biosorption)/ 5 g (phytoextraction) biomass, 23ºC, pH 5.4

C (mg/L)	q_e (exp) (mg/g)	Pseudo-first-order			Pseudo-second-order		
		k_1 (1/min)	q_e (calc) (mg/g)	R^2	k_2 (g/mg·min)	q_e (calc) (mg/g)	R^2
Living *Salvinia natans*							
20	0.70	5.96×10^{-2}	0.65	0.986	1.06×10^{-2}	0.81	0.994
31	0.81	2.25×10^{-2}	0.54	0.886	8.88×10^{-2}	1.87	0.972
50	1.70	5.76×10^{-2}	1.14	0.859	1.18×10^{-2}	1.81	0.983
70	2.39	6.22×10^{-2}	2.18	0.931	2.57×10^{-2}	2.82	0.985
90	3.22	6.96×10^{-2}	3.32	0.978	2.60×10^{-2}	3.69	0.991
***Salvinia natans* powder**							
20	0.71	1.02×10^{-3}	0.87	0.905	4.29×10^{-2}	0.71	0.990
31	1.16	0.06×10^{-3}	0.42	0.609	1.03×10^{-2}	1.09	0.988
50	1.83	1.29×10^{-2}	0.68	0.853	0.07×10^{-3}	1.83	0.997
70	2.45	1.70×10^{-2}	1.39	0.910	0.02×10^{-3}	2.59	0.999
90	3.25	1.38×10^{-2}	2.90	0.943	0.06×10^{-4}	3.74	0.983

Biosorption and phytoextraction isotherm models

Equilibrium adsorption isotherms have fundamental importance for the adsorption process design since they indicate how dyes are partitioned between the adsorbent surface and liquid phases at equilibrium as a function of

the dye concentration [42]. In this study, the biosorption and the phytoextraction experimental data were investigated using four equilibrium models, namely the Langmuir, Freundlich Temkin and Dubinin–Radushkevich (D–R) [43-46].

The Langmuir model assumes that a monomolecular layer is formed when biosorption and phytoextraction takes place without any interaction between the adsorbed molecules.

The Langmuir isotherm linear equation is expressed as follows:

$$\frac{1}{q_e} = \frac{1}{q_m b} \times \frac{1}{C_e} + \frac{1}{q_m} \tag{3}$$

where q_e is the solid-phase adsorbate concentration at equilibrium (mg/g), q_{max} is the maximum adsorption capacity corresponding to the monolayer adsorption capacity (mg/g), C_e is the concentration of CV solution at equilibrium (mg/L), and b is the adsorption equilibrium constant that is related to the apparent energy of adsorption.

Freundlich isotherm is an empirical equation based on a heterogeneous adsorption due to the diversity of adsorption sites or diverse nature of the adsorbed metal ions and dyes, free or hydrolysed species. The Freundlich isotherm linear equation is expressed as:

$$\log q_e = \log K_F + \frac{1}{n} \times \log C_e \tag{4}$$

where k is related to adsorption capacity and n is related to intensity of adsorption. The lnq_e versus lnC_e plot allows the determination of the Freundlich constants.

The monolayer saturation capacity of CV q_m was calculated to be 12.74 mg/g, while the Langmuir constant, which is related to adsorption energy, was determined to be 0.226 mg/L. Freundlich isotherm constants were also calculated (Table 2). The linearity of the two plots, expressed by R^2, can give information about the fitting between the experimental data and the isotherm model. The one closest to linearity could be considered as describing better the adsorption equilibrium in a certain system.

When comparing the determination coefficient for these two models it can be concluded that the experimental data were most decided by Langmuir model $R^2 = 0.972$ for biosorption and $R^2 = 0.958$ for phytoextraction.

The Dubinin–Radushkevich isotherm model was also applied to the equilibrium data, in order to determine the nature of the biosorption processes as physical or chemical. The D–R sorption isotherm is more general than the Langmuir isotherm as its derivation is not based on ideal assumptions such as equipotent of the sorption sites, absence of stoic hindrance between sorbed and incoming particles and surface homogeneity on microscopic level.

$$\ln q_e = \ln q_m - \beta\varepsilon^2 \tag{5}$$

where, q_e is the mole amount of metal ions an dyes adsorbed on per unit weight of biomass (mol/g), q_m is the maximum biosorption capacity (mol/g), β is the activity coefficient related to biosorption mean free energy (mol^2/J^2) and ε is the Polanyi potential, where, R is the universal gas constant (8.314 J/mol K) and T is the absolute temperature (K).
Free energy E per molecule, of adsorbate, which helps to distinguish between the physical and chemical adsorption of metal ions is given below:

$$E = \frac{1}{\sqrt{-2\beta}} \tag{6}$$

The isotherm constants q_m and β were obtained from the intercept and the slope of the plot $\ln q_e$ *vs*. ε^2. If E values are between 8 and 16 kJ mol^{-1}, the biosorption process is chemical and if E < 8 mol^{-1} the biosorption process is physical [47]. The Dubinin-Radushkevich isotherm expresses the adsorption mechanism with a Gaussian energy distribution onto a heterogeneous surface. The Dubinin-Radushkevich isotherm parameters are shown in the (Table 2). In our case, the value of the mean free energy for both processes was 5 mol^{-1} indicating a physisorption process.

Temkin isotherm equation contains a factor that takes into account the adsorbent-adsorbate interactions. It is based on the fact that the heat of adsorption of all the molecules in the layer decreases linearly with the coverage of molecules due to the adsorbate-adsorbent repulsions and the adsorption of cadmium ions uniformly realised on the surface. In addition, it also assumes that the fall in the heat of adsorption is linear rather than logarithmic, as implied in the Freundlich isotherm. The equation of this model is given below:

$$Q_e = B\ln\times A_t + B\ln\times C_e \tag{7}$$

$$B = \frac{RT}{bT} \tag{8}$$

where A_T is the Temkin isotherm equilibrium constant (g/L), b_T is Temkin isotherm constant and B is a constant related to the heat of adsorption (J/mol). From the q_e *vs*. $\ln C_e$ plot, A_T and B constants were determined. Taking into consideration the calculated value of the constant related to heat of sorption, which has a value smaller than 20 kJ mol^{-1}, we concluded that according to this isotherm, the sorption process takes place as physisorption (Table 2).

Table 2. Langmuir, Freundlich, Dubinin-Radushkevich and Temkin calculated coefficients using linear regression analysis for CV removal using living *S. natans* and *S. natans* powder; C_i = 20-90 mg/L, 0.4 g (biosorption)/ 5 g (phytoextraction) biomass, 296 K, 23ºC, pH 5.4

	Langmuir			Freundlich			Dubinin-Radushkevich			Temkin		
	K_L (L/mg)	q_{max} (mg/g)	R^2	n	K_f $(mg^{(1-1/n)}L^{1/n}/g)$	R^2	β (mol^2/kJ^2)	E (kJ/mol)	R^2	A_T (L/g)	B (J/mol)	R^2
Living *S. natans*	0.417	2.07	0.958	1.15	3.04	0.930	2×10^{-6}	5	0.914	2.50	3×10^{-6}	0.886
***S. natans* powder**	0.226	12.74	0.972	1.03	3.13	0.967	2×10^{-6}	5	0.870	2.50	4×10^{-6}	0.862

The biosorption and phytoextraction thermodynamics

The thermodynamic parameters, the change in free energy change (ΔG^o), enthalpy (ΔH^o) and entropy (ΔS^o) of the biosorption process –were evaluated for the biosorption of CV onto *S. natans* powder at different temperatures 10–35ºC.

Thermodynamic parameters were calculated using the following equations [48]:

$$\ln K_d = \frac{\Delta H^o}{RT} + \frac{\Delta S^o}{R} \quad (9)$$

$$\Delta G^o = RT \ln K_d \quad (10)$$

where ΔH^o, ΔS^o, ΔG^o, and T are the enthalpy, entropy, Gibbs free energy, and absolute temperature and R the universal gas constant. The ΔH^o and ΔS^o parameters were found from the slope and intercept of the plots of $\ln K_D$ against 1/T. Experimental results were used to calculate the thermodynamic parameters which are presented in (Table 3).

The negative values of ΔG^o indicated the feasibility and spontaneity of CV biosorption and phytoextraction using S. *natans*. The decrease in ΔG^o values shows a decline in the feasibility of biosorption as temperature is increased. The negative ΔS^o value means a decrease in the randomness at the solid/solution interface during the biosorption process. The positive value of ΔH^o indicated the endothermic nature of the biosorption of CV onto *S. natans* [49]. The endothermic process shows that the diffusion from bulk solution to adsorbent surface may require energy to overcome interaction of dissolved ions with solvation molecules [15].

Table 3. Termodynamic parameters for the removal of CV dye on living *S. natans* and *S. natans* powder at various temperatures; C_i = 50 mg/L, 0.4 g (biosorption)/ 5 g (phytoextraction) biomass, pH 5.4

	Δ*S*°	**Δ*H*°**	**Δ*G*°, (kJ/mol)**		
	(kJ/K·mol)	**(kJ/mol)**	**283 K**	**296 K**	**308 K**
Living *S. natans*	-0.12×10^{-2}	0.012	-3.29	-3.45	-3.58
***S. natans* powder**	-0.09×10^{-2}	0.010	-2.81	-2.94	-3.06

CONCLUSIONS

In this study, the use of *S. natans*, in both living and powder form, was tested for the removal of CV from aqueous solutions. The removal capacity depends on the initial dye concentration, biomass quantity, initial pH and temperature. Findings suggest similarities between the two processes for *S. natans,* in both living and powder form. Our results demonstrate that for both processes the sorption on the plant surface is determinant. Equilibrium models (Langmuir and Freundlich isotherm), kinetics (pseudo-first- and pseudo-second-order) and thermodynamics of the considered biosorption process were discussed in detail. According to the Dubinin-Radushkevich and Temkin, the adsorbtion of CV on living *S.natans* and *S. natans* powder was physical in nature. Equilibrium was best described by the Langmuir isotherm, while the kinetic of the process was best described by the pseudo-second-order model for both. Thermodynamic parameters showed that the CV biosorption and phytoextraction processes on *S. natans* are endothermic.

Using the plant in powder form had some advantages. One of them was that no large quantity solutions were required. The other one would be related to the fact that no living plants were necessary.

EXPERIMENTAL SECTION

Living *Salvinia natans*

*S. natans L. (*family of *Salviniaceae)* a free-floating aquatic fern, was the plant chosen for the phytoextraction process. *S. natans* was growth in a hydroponic greenhouse system (at University of Agricultural Sciences and Veterinary Medicine in Cluj-Napoca, Romania), with an addition of fertilizer (Complex 3, 0.5 %). The plants that aged 30 days were selected for the phytoremediation experiments.

Salvinia natans powder

Prior to its utilization, S. natans was washed several times with deionized water and dried at 80°C for 48 hours. The dried samples were grinded and sieved, 200 and 400 μm mesh size were further used in all experiments. The biomass was washed again with 0.01 M HCl to remove any soluble biomolecules that might have caused interference, and then cleaned with sterile distilled water. The samples were filtered and dried at 80°C for 48 h.

Chemicals

The CV was used as pollutant in the phytoremediation experiments. The cationic CV dye (Tris (4-(dimethylamino) phenyl) methylium chloride, chemical formula = $C_{25}N_3H_{30}Cl$, molecular weight = 407.9788 g/mol. Wavelength maximum (λmax = 590 nm). All chemicals and reagents used in the study were of analytical grade (purity ≥ 99 %) and supplied by Merck (Germany).

Phytoextraction experiments with living *Salvinia natans*

The phytoremediation experiments were carried out in controlled conditions (at room temperature 21-23ºC, illuminated with a lamp with the 14/10 h light/dark photoperiod), in 250 mL glass beakers containing 200 mL synthetic wastewater and 5 g fresh aquatic plants along with the macro- and micronutrients [50]. Before experiments, the plants were kept in laboratory conditions for an acclimatization period of 4 days in a modified Hoagland nutrient solution with the following chemical composition: 1 mM KNO_3; 1 mM $Ca(NO_3)_2 2.4H_2O$; 1 mM $NH_4H_2PO_4$; 1 mM $MgSO_4 \times 7H_2O$; 25 mMKCl; 12.5 mM H_3BO_3; 1 mM $MnSO_4 \times H_2O$; 1 mM $ZnSO_4 \times 7H_2O$; 0.25 mM $CuSO_4 \times 5H_2O$; 0.25 mM H_2MoO_4 (85% MoO_3) with Fe(III) citrate.

Biosorption experiments with *Salvinia natans* powder

The biosorption experiments were performed in batch condition, contacting 0.4 g powder obtained from 5 g fresh plant with 200 mL of CV at different initial concentrations (20 - 90 mg/L), under stirring (150 rpm), at room temperature 23ºC ± 2ºC (296±2 K). In order to establish the evolution of the removal process, samples of 500 μL were collected at different time intervals up to 240 min. The collected samples at predetermined time intervals were centrifuged (10 min) and the dye concentration in the aqueous phase was determined on a daily basis. The concentrations were determined using the double beam UV-visible spectrophotometer (GBC Cintra 202, Australia).

Characterizations of the process

For both processes, phytoextraction and biosorption, the same parameters and conditions were studied: a) effect of plant quantity: m_{plant} =1-5 g b) effect of initial concentration of CV: C_i =20 - 90 mg/L, c) effect of initial pH = 3.0-10; d) effect of temperature: t_1 = 10ºC, t_2 = 23ºC, t_3 = 35ºC (283-308 K).

The pH of the solution was initially adjusted by adding a small amount of 0.1 M HCl or 0.1 M NaOH solutions and then measured using a pH meter.

In order to evaluate the amount of CV retained per unit mass of biomass, the biosorption and phytoextraction capacity were calculated using the following equations [51]:

$$E,(\%) = \frac{C_i - C_f}{C_i} \times 100 \quad (11)$$

$$q_{\max}(mg/g) = \frac{(C_i - C_f)V}{m} \quad (12)$$

where E, (%) represents the removal efficiency, C_i and C_f the initial and final concentrations of CV (mg/L) in the aqueous solution, q_{max} (mg/g) represents the amount of CV retained onto unit weight of plant, V (L) means the volume of dye aqueous solution and m (g) the plant quantity. The experiments were conducted simultaneously both for living and powder-form plant, following the same parameters.

Scanning electron microscopy (SEM)

Scanning electron microscopy was utilized for characterizing surface microstructures, and fundamental physical properties of different adsorbents. The surface morphology of *S. natans* was determined using a scanning electron microscope JEOL JSM 5510 LV (Japan).

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REFERENCES

1. H.A. Mekkawy, M.O. Ali, and A.M. El-Zawahry, *Toxicol Lett*, **1998**, *95*, 155–161.
2. E.A. Clarke and R. Anliker, Organic dyes and pigments, New York, **2005**, p 181–215.
3. G. Mishra and M.A. Tripathy, *Colourage*, **1993**, 40, 35–38.
4. A.O. Akeem, and G. Mustafa, *Toxicol Environ Chem*, **2014**, 96, 837-848.
5. N.M Mahmoodi, *J. Chem. Eng. Data*, **2011**, *56*, 2802–2811.

A. Arunagiri, P. Prabisha, and R. Kalaichelvi, *J Thermodynam* Article ID, **2014**, 670186.

6. J. Mittal, A. Malviya, A.D. Kaur, and V.K.Gupta, *J Colloid Interface Sci*, **2010**, *343*, 463–473.
7. C. Chen, J.T. Kuo, H.A. Yang, and Y.C. Chung, *Chemosphere*, **2013**, *92*, 695–701.
8. R. Ahmad, J Hazard Mater, **2009**, 171, 767–773.
9. S. Khan, A. Arunarani, and P. Chandran, *Clean – Soil Air Water*, **2015**, *43*, 67–72.
10. I.M. Banat, P. Nigam, D. Singh, and R. Marchant, *Bioresour Techno*, **1996**, *58*, 217–227.
11. S. Ahluwalia and D. Goyal, *Bioresour Technol*, **2007**, *98*, 2243–2257.
12. D. Sud, G. Mahajan, and M.P. Kaur, *Bioresour Technol*, **2008**, *99*, 6017-6027.
13. G. Blazques, L. Martin, T. Guadalupe and M. Calero, *Chem Eng J*, **2011**, 170-177.
14. B. Nagy, A. Maicaneanu, C. Indolean, S. Burca, L. Silaghi-Dumitrescu, and C. Majdik, *Acta Chim Slov*, **2013**, *60*, 263- 273.
15. M. Momcilovic, M. Purenovic, A. Bojic, A. Zarubica, and M. Randelovic, *Desalination*, **2011**, *276*, 53-59
16. N. Meunier, J. Laroulandie, J.F. Blais, and R.D. Tyagi *Bioresour Technol*, **2003**, *90*, 255–263.
17. B.H. Hameed, and M.I. El-Khaiary *J Hazard Mater*, **2008**, *162*, 305-311.
18. E.G. Mueller, *Mol Biology*, **1996**, *7*, 1805-1813
I. Raskin, and D. Ensley, New York,"Phytoremediation of toxic metals, **2000** p.12-33.
19. P.L. Gratão, M.N.V. Prasad, P.F. Cardoso, P.J. Lea, and R.A. Azevedo, *J Plant Physiol*, **2005**, *17* 5, 53-64.
A. Marques, O. Anto´Nio, S. Rangel, and M.L. Paula, *Environ Sci Techno.l*, **2009**, *39*, 622–654.
20. R.A. Usman, R. Alkredaa, and M.I. Al-Wabel, *Ecotox Environ Safe*. **2014**, *97*, 263–270.
21. R.L. Chaney, K.M. Malik, Y.M. Li, S.L. Brown, E.P. Brewer, and J.S. Angle *Curr Opin Biotechnol*. **1997**, *8*, 279–284.
22. D.E. Salt and U. Kramer, Phytoremediaton of Toxic Metals, Berlin, **1999** p. 231–246.
23. W.C. Wang and Km. Freemark, *Ecotox Environ Saf*, **1995**, *30*, 289–301.
24. S. Aubert and J.P. Schwitzguebel *Wat Res*, **2004**, *381*, 3569–3575.
25. H.B.L. Pettersson, H.A. Johnston, and A.S. Murray, *J Environ Radioac*, **1993**, *19*, 85–108.
26. R.A. Overall and D.L. Parry, *Environ Pollut*, **2004**, *132*, 307–320.
27. U.N. Rai, S. Sinha, R.D. Tripathi, and P. Chandra, *Ecol Eng,* **1995**, *5* ,5-12.
28. O. Keskinkan, *Asian J Chem*, **2005**, *17*, 1507-1515.
29. M. Mkandawire and E.G. Dudel, *Sci Total Environ*, **2005**, *336*, 81–90.
A. Török, E. Buta, L. Silaghi-Dumitrescu, C. Indolean, C. Majdik, and S. Tonk, *Acta Chim Slov*, **2015**, *62*, 452–461.
30. S. Radic, D. Stipanicev, P. Cvjetko, R.M. Marijanovic´, S. Sirac, B. Pevalek, and M. Pavlica, *Ecotox Environ Saf*, **2011**, *74*, 182–187.
31. G. Sánchez-Galván, O. Monroy, G. Gómez, and E.J. Olguín, *Water Air and Soil Pollu,* **2008**, *194*, 77–90.
32. G. Annadurai, L.Y. Ling, and J.F. Lee, *J Hazard Mater*, **2008**, *152*, 337-346.
33. D. Zhao, K.R. Reddy, V.G. Kakani, and V.R. Reddy, *Eur J Agron*, **2005**, *22*, 391-403.

34. R. Bligny, E. Gout, W. Kaiser, U. Heber, and D. Walker, R. Douce, *Biochim Biophys Acta (BBA) – Bioenergetics*, **1997**, *1320*, 142-152.
35. B. Nagy, C. Mânzatu, A. Török, C. Indolean, A. Măicăneanu, S. Tonk, and C. Majdik, *Rev Roum Chim*, **2015**, *60*, 257-264.
36. Y.S. Ho and G. McKay, *Process Biochem*, **1999**, *34*, 451–465.
37. Ho YS, McKay G, and Wase DAJ, *Ads Sci Technol*, **2000**, *18*, 639–650.
38. M.E. Argun, S.C. Dursun, and M. Ozdemir Karata, *J Hazard Mater*, **2007**, *141*, 77-85.
I. Langmuir, *J Am Chem Soc*, **1918**, *40,* pp 1361-1367.
39. H.M.F. Freundlich, Über die Adsorption in Lösungen, **1906**, pp 385-470.
40. M.J. Temkin and V. Pyzhev, *Acta Physiochim*, **1940**, *12*, 217-222.
41. M.M. Dubinin, E.D. Zaverina, and L.V. Radushkevich, *Zhurnal Fizicheskoi Khimii*, **1947**, *21*, 1351–1362.
42. E. Gîlca, A. Maicaneanu, P. Ilea, *Cent. Eur J. Chem*, **2014**, *12*, 821-828.
43. A.U. Itodo, and H.U. Itodo*, J Life Sci*, **2010**, *7*, 31–39.
44. L.K. Fraji, D.M. Hayer, and T.C. Werner, *J Chem Educ*, **1992**, *69*, 424–427.
45. E. Eren, O. Cubuk, H. Ciftci, B. Eren, and B. Caglar, *Desalination*, **2010**, *252*, 88-96.
46. L. Taiz and E. Zeiger, *Plant Physiology*, **2002**, *35*, 68-72.
47. T. Akar, S. Celik, and S.T. Akar, *Chem Eng J*, **2010**, *160*, 466–472.