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Implementation of integrated adsorption and biological process in wastewater treatment for permanent dye removal and its subsequent decontamination

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ABSTRACT

The use of the microwave-activated sugar beet pulp as an alternative adsorbent material in combination with biological processes has been applied for the colored wastewater treatment. The maximum adsorption capacity of the crystal violet dye has been achieved relatively fast, during 315 s, with no dependence of the pH, when the adsorbent mass was 15 and 0.2 g/L of the initial dye concentration. The adsorption equilibrium data fully complied with the Temkin isotherm. The afterward biological decontamination of the adsorbed dye was related to the biodegradable potential of *Streptomyces microflavus* CKS6 cells. The microorganism revealed strong affinity toward the dye and minimal nutritional requirements for the metabolic activity expressions. Total dye decomposition occurred after 9 h, whereby lignin peroxidase enzyme has been recognized to be mainly involved in the process. According to the phytotoxicity analysis, no harmful residues were found in the decolorized working medium.

Keywords: Crystal violet dye; Sugar beet pulp; Adsorption; Biodegradation; Wastewater treatment; Response surface methodology

1. Introduction

The pollution of water and surrounding soils with dyes is a result of continuous rising of the careless human activities that have been contributed by the rapid industrialization throughout the world. The effects of the toxic industrial chemicals on the ecosystems are a matter of concern and of public health significance [1]. Besides the colored wastewaters damage the esthetic nature of water streams, they may enhance the chances of hazardous impact on the aquatic flora and fauna [2]. Dyes usually have complex aromatic molecular structures, containing metals and other chemicals, which make them more stable and difficult to degrade [2,3]. Crystal violet (CV) is a water soluble, widely used dye for several purposes, mainly to colorize diverse products [3]. The presence of very low concentrations of the dye (less than 1 mg/L) in the effluent is highly visible, undesirable and cannot be easily removed by conventional treatment systems [2]. Among many physical and chemical techniques available, that are limited in use because of being procedurally complicated or economically unfeasible [4–6], the adsorption phenomenon is still being allocated as a promising technology for fast and permanent removal of such a type of pollutants in wastewater, offering

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several attractive advantages [1–3,7]. Furthermore, the adsorption, coupled with biological process, may provide total decomposition of the dye, leaving non-toxic residues, and thus solving the disposal problem of the adsorbent with accumulated dye [8]. The bioremediation of the dye-contaminated effluent is observed to be an effective proposal due to its reputation as a low-cost, environmentally friendly, and publicly acceptable treatment technology [6,9,10]. Biological processes, due to microorganisms' genetic and metabolic activity versatility, have potential to convert or degrade the pollutants into harmless substances, such as water, carbon dioxide and various salts of inorganic nature [10,11]. Currently, an extensive research has been focused to find a cheap and optimal, broad-spectrum and highly efficient dye-degrading microbial biomass [6,10]. In addition, the use of whole cells, rather than isolated enzymes, becomes advantageous, since costs associated with enzyme purification are substantial. Also, the degradation process has been often carried out by a number of enzymes that are working sequentially, instead of the use of one specific enzyme particularly [12].

The sugar beet pulp (SBP), a cellulosic by-product of sugar refining industry, has been nowadays frequently exploited for the investigation of adsorption and removal of different common types of contaminants found in water streams. SBP belongs to the group of the economic adsorbents, locally available in large quantities (more than 14×10^6 t/year in the European Community), serving as a possible alternative to activated carbon [1–3,7,13]. For the most part it contains 65%–80% of polysaccharides, representing roughly 40% of cellulose, 30% of hemicelluloses and 30% of pectin [14]. The pectic substances, particularly, are complex heteropolysaccharides containing galacturonic acid, arabinose, galactose and rhamnose [2,13]. The polysaccharides and their modified products, due to the carboxyl groups on galacturonic acids, most likely, take great responsibility for SBP ability to strongly bind cations, during the adsorption processes [13–15]. Some researchers have particularly emphasized that the carbonization of the SBP, by pyrolysing techniques with various chemicals, may additionally enhance the sorption capacity and the physical stability of the carrier [13,15].

Accordingly, this study has explored the possibility to permanently remove the crystal violet from water solutions by adsorption and immediately perform the dye biodegradation. Primarily, the production of microwave-activated SBP, as an adsorption carrier, has been carried out for the evaluation of binding affinity toward the cationic dye. The most important process-influenced parameters have been investigated, with the aim to achieve fast and efficient contaminant uptake, afterward, the remediation of the accumulated pollutants, by using of the potent dye-degrading microbial biomass (*Streptomyces microflavus* CKS6), has been applied.

2. Experimental

2.1. Sample material and chemicals

SBP was obtained from Nordic Sugar Member of Nordzucker Group Nordic Sugar A/S Copenhagen, Denmark. Crystal violet (tris (4-(dimethylamino) phenyl) methylium chloride) was purchased from Acros organics Co., New Jersey, USA, while HCl, ethanol 96%, and NaOH were purchased from "Zorka Pharma", Šabac, Serbia and "Lachema", Brno, Czech Republic, respectively. yeast extract powder and pancreatic digest of casein for growing bacteria medium were obtained from Biolife Italiana, Milano, Italy, whereas pyrogallol (1,2,3-trihydroxybenzene) was obtained from Sigma-Aldrich, Steinheim, Germany, respectively. Methylene blue and phenol red were purchased from NRK Engineering, Belgrade, Serbia.

2.2. Preparation of the adsorbent

The dried SBP was primarily powdered and sieved to a particle size of 1.2–2.4 μm. The activated carbon preparation was performed in a microwave oven at 400 W, in a solution of 20% ethanol (the ratio of SBP [g]: ethanol [mL] was 1:6), for 2 min [16]. The mixture was filtered and the solid part was dried in an oven at 105°C overnight. Such a prepared adsorbent, named activated sugar beet pulp (ASBP), was stored in a sealed vial and used for further experimental work.

2.3. Preparation of the dye stock solution

The crystal violet (CV) dye stock solution was prepared by dissolving 0.5 g of the dye in 1 L of distilled water. The working concentrations of the dye in aqueous solutions were varied from 0.05 to 0.25 g/L, by diluting the CV stock solution with distilled water. Initial pH of dye solutions was adjusted to 3–9 using 0.1 M HCl or 0.1 M NaOH. Fresh dilutions of the desired dye concentrations were prepared at the beginning of each experiment.

2.4. Experimental design of the adsorption process

The effects of most influential adsorption process operating parameters (according to preliminary experiments), the adsorbent dose, initial CV concentration, time of agitation and initial pH of the solution, on the adsorption capacity were evaluated by using the Box–Behnken experimental design within the response surface methodology. The value ranges of process variables were decided on the basis of prior examinations and are presented in Table 1. The mathematical relationship between independent variables and the response surface has been approximated by second-order polynomial equation.

Table 1
Experimental ranges and levels of the independent variables in the experimental design

Factors	Range and level		
	–1	0	+1
A: Adsorbent dose (g/L)	5	15	25
B: Initial CV concentration (g/L)	0.05	0.15	0.25
C: Time of agitation (s)	30	315	600
D: pH	3	6	9

2.5. Batch adsorption experiments

2.5.1. Dye adsorption

100 mL of the appropriate dye solution concentration and the adsorbent mass were taken into 250 mL Erlenmeyer flask and placed on a translatory shaker (IKA – KS 4000i control, Staufen, Germany) at 150 rpm agitation speed and room temperature of 25°C. After certain time (according to the experimental design), samples were collected, filtered and analyzed by UV/vis spectrophotometer (Ultrospec 3300 pro, Amersham Biosciences, USA) at 584 nm for residual dye concentration.

The adsorption capacity was computed by using of the following equation:

$$q_t = \frac{\left(C_i - C_e\right)V}{m_{\text{ads}}} \tag{1}$$

where C_i and C_e are the initial and equilibrium dye concentrations (g/L), respectively, V is the solution volume (L) and m_{ads} is the adsorbent dose (g) [16].

2.5.2. Isotherms experiments

Equilibrium experiments were conducted in five different capped 250 mL Erlenmeyer flasks contacting 200 mL of the following dye concentrations: 0.05, 0.1, 0.15, 0.2 and 0.25 g/L with 4 g of the adsorbent. The mixtures were shaken on a translatory shaker (150 rpm) at 25°C during 240 min. Obtained samples were filtered and analyzed spectrophotometrically at 584 nm.

The Langmuir, Freundlich and Temkin models were employed for adsorption isotherm modeling of the experimental data [16,17].

2.5.3. Kinetic experiments

Adsorption kinetic experiments were conducted under the same conditions as previously described procedure for adsorption isotherms. Samples were taken at different predetermined time intervals, filtered and analyzed spectrophotometrically (584 nm).

The controlling mechanism of the adsorption process was investigated by fitting the pseudo-first- and pseudo-second-order kinetic models to the experimental data [16,17].

2.6. FTIR analysis

The functional groups of the ASBP before and after adsorption of CV dye were estimated by Fourier transform infrared (FTIR) spectroscopy (BOMEM MB100, Canada). The measurements were carried out at 25°C in the 400–4,000 cm^{-1} wave number range, with a 4 cm^{-1} resolution. The samples of adsorbents (free and CV adsorbed) were dried for overnight to remove any water retained. For the translucent sample disks preparation, 1 mg of the adsorbent was mixed with 300 mg of KBr [16].

2.7. Adsorbed dye biodegradation

Streptomyces microflavus CKS6 microorganism, employed for the biodegradation experiments, was isolated from forest soil (Kranjska Gora, Slovenia). The strain was identified to nearest species based on morphological characteristics, while sequence of the 16S rRNA encoding gene (gene accession numbers KP715853) was higher than 99%.

The biodegradation experiments were carried out by using of 0.01 g of dried ASBP with adsorbed CV dye and 10% inoculum of *Streptomyces microflavus* CKS6. The fresh bacteria culture was prepared in ISP1 broth medium (pancreatic digest of casein 5.0 g/L; yeast extract 3.0 g/L) [25] and it was used without prior acclimatization. The number of bacterial cells in the prepared culture was 1.5×10^9 CFU/mL. The mixture (ASBP + inoculum) was placed in 25 mL of 10% ISP1 in saline medium (8.5% NaCl) and incubated at 30°C on a translatory shaker, under the aeration of 120 rpm. After the predicted period of time, 2 mL of the aliquot was withdrawn, centrifugated (10,000 × g, 10 min) and measured spectrophotometrically (584 nm), for residual dye concentration determination. The percentage of the solution decolorization was calculated according to Eq. (2):

$$\text{Decolorization}\,(\%) = \frac{A_I - A_o}{A_I} \times 100 \tag{2}$$

where A_I is an initial absorbance and A_o is an observed absorbance.

All of the evaluated experimental conditions were chosen according to the preliminary experiments, taking into account the possibility of growth and bacterial enzymatic activity within the evaluated ranges.

2.8. Determination of microorganism's enzyme profile and enzyme activity

The peroxidase, lignin peroxidase and manganese peroxidase enzymes assays were employed for the characterization of the extracellular enzyme profile of the new CKS6 strain.

2.8.1. Peroxidase assay

The evaluation of the *Streptomyces microflavus* CKS6 peroxidase has been performed by the using of pyrogallol as a substrate, following the procedure of Šekuljica et al. [18]. The enzyme sample was prepared in the cuvette with CKS6 inoculum and pyrogallol (0.013 M) in potassium phosphate buffer (pH 7.0, 0.1 M), whereby the blank sample contained only buffer and the control sample contained buffer instead of the inoculum. The change of color from yellow (pyrogallol) to dark brown (purpurogallin) was monitored spectrophotometrically at 420 nm [19,20].

2.8.2. Lignin peroxidase assay

The qualitative lignin peroxidase assay has been performed by using methylene blue as a substrate. The CKS6 inoculum (without dialysis) was mixed with a methylene blue (1 mM), sodium tartrate buffer (0.5 M, pH 4) and H_2O_2 (4.5 mM) in the ratio of 22:1:3:1 (v:v:v:v), respectively, in the test tube, whereas the blank sample was prepared with distilled water, instead of the inoculum. The

color change (from blue to green) in the presence of lignin peroxidase has been monitored and compared with a blank sample [21].

The lignin peroxidase activity was done by using of the same dye, following the procedure of Bholay et al. [22] with slight modifications. 2 mL of the enzyme aliquot samples, before and after biodegradation, were centrifuged (10,000 × g, 10 min) and 1.1 mL of the supernatant was mixed with 300 μL of sodium tartarate buffer (pH 4.0, 0.5 M) and 50 μL of methylene blue (1.2 mM) in the cuvette. The reaction had been started by adding of H_2O_2 (50 μL, 2.7 mM), afterward the mixture was incubated (20 min, 30°C) and measured spectrophotometrically at 650 nm. The results were interpreted as the percentage of decolorization of the methylene blue dye by the enzyme (conversion of the dye into Azure C) as compared with the control sample (distilled water instead of enzyme sample) and calculated as (A_{650} for control sample – A_{650} for enzyme sample/A_{650} for control sample) × 100 [21,22].

2.8.3. Manganese peroxidase assay

In the manganese peroxidase assay, which is based on the phenol red oxidation reaction, sodium succinate buffer (1 mL, 250 mM, pH 4.5) was mixed with phenol red (700 μL, 0.71 mM), $MnSO_4$ (400 μL, 1.25 mM), sodium lactate buffer (1 mL, 250 mM, pH 4.5), gelatin (1 mL, 0.5%) and enzyme supernatant sample (500 μL) in the test tube. In the case of manganese independent peroxidase, the assay mixture was prepared without adding of $MnSO_4$. The control sample was prepared by using of distilled water instead of the enzyme sample. By adding of H_2O_2 (400 μL, 0.62 mM), the reaction has been started. During the incubation period at 30°C, 1 mL of the assay mixture was taken every minute up to 4 min and added into NaOH (400 μL, 5 M) to stop the reaction, afterward it was measured spectrophotometrically at 610 nm. Manganese-dependent peroxidase activity was calculated by following the procedure of Arora and Gill [23] whereby one unit of enzyme activity was equivalent as an absorbance increase of 0.1 units per mL of the enzyme sample.

2.9. Phytotoxicity study

Phytotoxicity analysis of the initial CV dye solution and its decolorization products after the biological treatment with CKS6 was performed in order to assess their toxicity. The samples were dissolved in an agar and poured into Petri dishes. The test was carried out in three replicates and the dilutions were done in a ratio of 1:4 (v:v). The *Triticum aestivum* seeds were utilized for this purpose, while pure agar medium was used as a control. After 4 d of seeds germination, all test results were recorded and expressed by length of shoot and root and relative seed germination (%) index (RSGI) [24,25]. The percentage of RSGI is calculated as follows:

$$\text{RSGI}(\%) = \frac{\text{SG}_S}{\text{SG}_C} \times 100 \quad (3)$$

where SG_S and SG_C are seeds germination in samples and seeds germination in control (cm), respectively.

3. Results and discussion

3.1. FTIR spectra study

The FTIR vibrational spectra of the ASBP before (spectrum A) and after CV dye adsorption (spectrum B) were shown in Fig. 1.

The ASBP surface has been characterized by the presence of the broad peak region between 3,100 and 3,600 cm^{-1}, that has been attributed to the vibrations of hydrogen-bonded hydroxyl groups ν(O–H). Several prominent peaks appeared at 1,747.3 cm^{-1}, corresponding to the stretching vibration of carbonyl functional group in ketones and aldehydes [2], 1,637.4 cm^{-1} (asymmetric carboxylate peak) [26] and 1,384.7 cm^{-1}. The fingerprint region includes the bands of the sugar ring (C_5O–) between 950 and 1,200 cm^{-1} [27,28], which affected the stretching of the C–O bond of the C–OC and C–OH from 1,024 to 1,107 cm^{-1}.

After CV dye adsorption, several changes have been observed (spectrum B), these are reflected in the intensification, shifting or disappearance of certain bands (Fig. 1). More precisely, the peak at 2,925.8 cm^{-1} (spectrum A), that may correspond to C–H in the hydrocarbon chains, was shifted to 2921.9 cm^{-1} in the spectrum B, probably, by involving of the methyl groups (–CH_3) during the adsorption process [16]. Additionally, peaks at 1,024.1 and 1,056.9 cm^{-1} were shifted to 1,031.8 and 1,066.6 cm^{-1}, respectively. These absorption spectra shifted in the presence of hydrogen bonding as a result of the hydroxyl groups [7]. The intensification of region between 669.25 and 617.17 cm^{-1} was due to out-of-plane deformation mode of C–H for different substituted benzene rings [16]. The shifting of certain bands from the initial position decreases the number of specific surface available functional groups, which should be probably due to the covering with the dye particles [28].

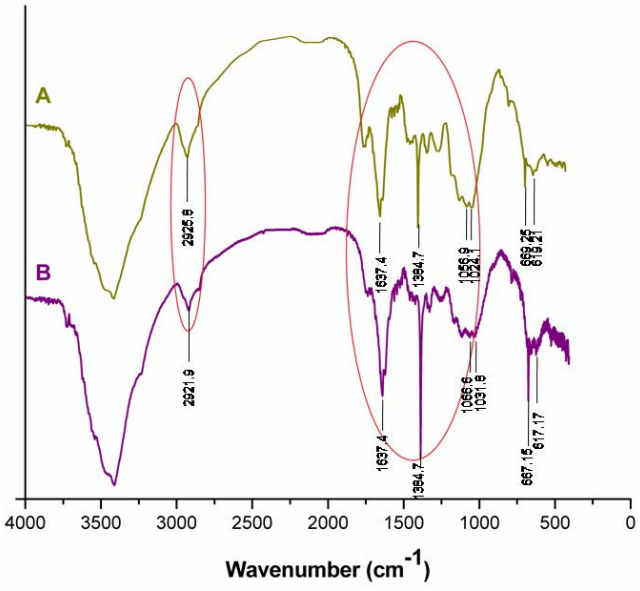

Fig. 1. FTIR spectra of the ASBP before (A) and after (B) adsorption of CV dye.

Table 2
ANOVA for the model response fitted to polynomial equation

Response	R^2	F-value	p-value prob > F	CV*	AP**
Adsorption capacity (mg/g)	0.9939	163.84	<0.0001[a]	8.5205	55.46
Lack of fit			4.1822	0.0902[b]	

*Coefficient of variation (%).
**Adequate precision.
[a]Significant factor.
[b]Not significant factor.

Table 3
ANOVA for the model parameters fitted to polynomial equation

Parameter	prob > F*
A	<0.0001[a]
B	<0.0001[a]
C	<0.0001[a]
D	<0.5566
AB	<0.0001[a]
AC	<0.0009[a]
AD	<0.0095[a]
BC	<0.0001[a]
BD	<0.1040
CD	<0.4634
A^2	<0.0001[a]
B^2	<0.9308
C^2	<0.0269[a]
D^2	<0.0352[a]

A: Adsorbent dose (g/L).
B: Initial CV concentration (g/L).
C: Time of agitation (s).
D: pH.
[a]Significant factor (prob > F < 0.05).

3.2. Fitting the adsorption process experimental design

The experimental design offered a total of 29 experiments, within 24 were factorial and 5 were replication at the central points. For the four examined factors (Table 1), the Box–Behnken model revealed that the quadratic model was the most suitable one for the approximation of the current investigated system. The ANOVA results for the model response and parameters are presented in Tables 2 and 3, respectively.

The model response was found to be significant (Table 2). According to R-squared values, there was a good correlation between the actual and the predicted values. For the purpose of the regression analysis, the model was modified (reduced by rejection of non-significant factors, Table 3), afterward the best fitted model equation (Eq. (4)) was obtained as:

$$\text{Adsorption capacity (mg/g)} = 7.32 - 5.83A + 5.51B + 2.27C - 6.18AB - 1.47AC + 1.05AD + 2.13BC + 3.44A^2 - 0.68C^2 \quad (4)$$

3.3. Effects of the process variables on the adsorption capacity

3.3.1. pH

The adsorption of CV dye onto ASBP at 25°C and varying pH range (pH 3–9) showed an approximately consistent adsorption capacity of the dye, despite to pH alterations (Fig. 2a). It was obvious that neither basic nor acidic environment affect the surface structural changes on ASBP binding sites, and its adsorption ability toward the dye molecules, probably because of the potential to create the balance between the protonated and deprotonated forms within wider pH range alterations. Such a behavior is considered as an advantage in environments where CV may be absorbed by ASBP at any pH, considering, it is one of the most critical parameters in the contaminated water treatment. Similar to this work, Harifi-Mood and Hadavand-Mirzaie [3] found that the effect of initial pH on the removal of the Basic violet 16 was almost negligible. On the contrary, other researchers found that adsorption of Remazol Black B [2] and Gemazol turquoise blue-G [29] onto SBP was favored in very acidic conditions, under pH 1 and 2, respectively, while exclusively basic reaction environment enhanced the adsorption of methylene blue and safranin dyes [7,14].

3.3.2. Adsorbent dose

To optimize the removal of the CV from the aqueous solutions, the adsorbent (ASBP) dose was varied from 5 to 25 g/L, whereas its considerable impact on the adsorption capacity has been observed (Fig. 2b). The adsorption capacity increases with the decrease in the amount of adsorbent and the simultaneous increase of the dye concentration in the system, thus the maximum value of 29.39 mg/g has been achieved by the presence of 5 g/L of the ASBP. It may be observed that the increased adsorption capacity was not due to the increase in total surface area of adsorbent, despite the enlarged availability of more adsorption sites, but obviously some other mechanism has occurred. Probably, adsorbent aggregation and the mutual particle interaction affected the dissimulation of the active surface sites that remained unsaturated during the process. Also, the consumption of less amounts of the adsorbent is favorable from the economic standpoint, by the resource saving. The same phenomena have been observed for the previously reported adsorption of other two cationic dyes, methylene blue and safranin, onto SBP [7]. Additionally, Li et al. [30] also reported that active carbon derived from SBP showed similar properties as an unmodified adsorbent. With the increase of the adsorbent concentration, the percentage of methylene blue dye removal

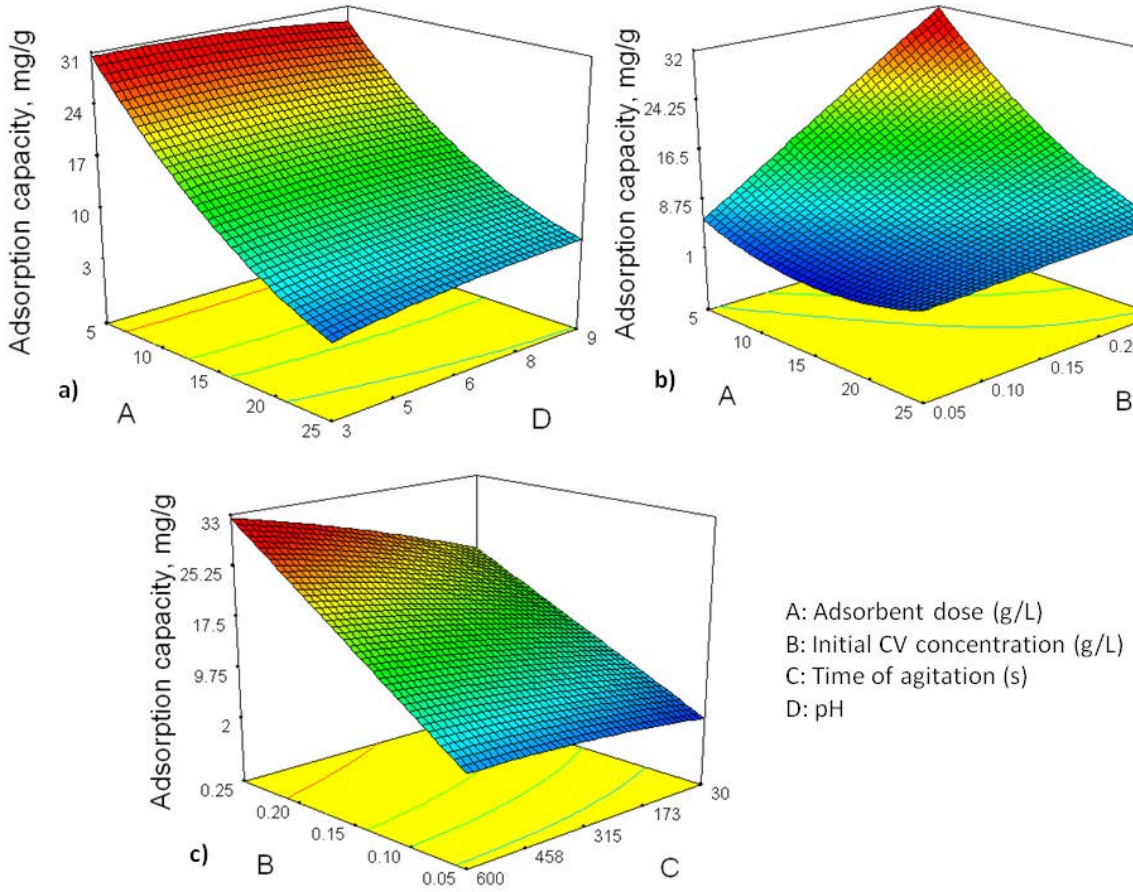

Fig. 2. Contour plot of combined effects of: adsorbent dose and pH (*AD*, a), adsorbent dose and initial CV concentration (*AB*, b) and initial CV concentration and time of agitation (*BC*, c) on the adsorption capacity.

also increases, but the amount of adsorbed dye per unit mass of the adsorbent decreases considerably [30].

3.3.3. Initial dye concentration

The increase in the initial dye concentration within the investigated range causes the increase in the adsorption capacity. This effect has been favored in the system where the agitation time has been prolonged up to 600 s, wherein less amounts of the adsorbent dose have been employed (Fig. 2c). The initial sorbate concentration provides an important driving force to overcome all mass transfer resistance of dye cations between the aqueous and solid phases [2,14,29]. The increases of loading capacity of the adsorbent with increasing of the initial dye concentration may also be due to higher interaction between the dye and the adsorbent [2,29]. The same phenomena have been found for the adsorption of Basic violet 16 [3], Remazol Black B [2], Methylene blue [14] and Gemazol turquoise blue-G dye [29] onto carbon prepared from SBP.

3.4. Equilibrium isotherms for the CV dye adsorption

The assessment of fitting of the experimental data with the Langmuir, Freundlich and/or Temkin isotherm model has been performed. The linearized forms of these models were given as the following equations, respectively:

$$\frac{1}{q_e}=\left(\frac{1}{K_L q_m}\right)\frac{1}{C_e}+\frac{1}{q_m} \tag{5}$$

$$\log q_e=\log K_F+\frac{1}{n}\left(\log C_e\right) \tag{6}$$

$$q_e=k_{1T}\ln k_{2T}+k_{1T}\ln C_e \tag{7}$$

where q_e (mg/g) is the amount of dye adsorbed at equilibrium and C_e (g/L) is the equilibrium concentration. q_m (mg/g) and K_L (L/mg) are Langmuir constants which were calculated from the plot of $1/q_e$ vs. $1/C_e$ (Eq. (5)), and were related to adsorption capacity and energy of adsorption, respectively [14]. The Freundlich constants, K_F and n, that can be calculated from the plot of log (q_e) vs. log (C_e) (Eq. (6)), represent indicators of adsorption capacity and adsorption intensity, respectively [1,31]. Isotherm constants, k_{1T} and k_{2T} (L/mg), are related to the heat of adsorption and the binding constant corresponding to the maximum binding energy, respectively,

and may be calculated from the plot of log (q_e) vs. log (C_e) (Eq. (7)). k_{1T} is equaled to the relation of RT/b, whereas R represents the gas constant (8.314 J/mol K), T (K) is the absolute temperature and b is Temkin energy constant (J/mol) [17].

The results of the adsorption isotherm modeling and corresponding equilibrium constants are presented on Fig. 3 and Table 4.

The Temkin isotherm model fully complies with the experimental curve (Fig. 3), demonstrating higher corresponding regression coefficients (R^2) in comparison with the other two models, thus may be applicable over the entire solute concentration range of the CV adsorption onto ASBP. The Temkin model is based on the assumption that the increase of the adsorbent covering linearly decreases the heat of adsorption of all the molecules in the layer due to adsorbate–adsorbate interactions. According to the obtained results, the assumption of the monolayer adsorption existence onto the adsorbent, which has homogeneous surface and a finite number of available sites, as regarded to the Langmuir isotherm theory [14], may be excluded. It is unlikely that the active sites onto the ASBP surface are identical, nor that negligible interaction between adsorbed molecules is present. The actual system may be rather similar to the Freundlich model, that is valid for a multilayer sorption model on a surface with mutual interactions between adsorbed chemical particles [1,31]. The previously literature reports were diverse, indicating that the adsorption of some cationic dyes onto untreated and modified SBP was in accordance with Langmuir adsorption isotherms [7,14]. On the other hand, the adsorption of the Basic violet 16 onto untreated SBP may be well approximated with both of the equilibrium isotherm models, Langmuir and Freundlich [3].

3.5. Kinetic modeling of CV dye adsorption

The effect of time on the adsorption capacity, during CV dye adsorption onto ASBP, observed at different initial dye concentrations is presented in Fig. 4.

According to the obtained kinetic results (Fig. 4), the adsorption capacities increase along with the rise of the reaction time up to 45 min [32,33]. After this point, the amount of adsorbed dye molecules onto the ASBP was in a state of dynamic equilibrium with the amount of dye desorbed from the adsorbent surface [34,35]. The initial dye concentration affects the degree of the adsorption capacity, but does not influence the equilibrium attainment (Fig. 3). However, adsorption capacities were slightly lower when the initial CV concentration was 0.2 g/L in comparison with 0.15 g/L, probably due to non-uniform distribution of the adsorbate and adsorbent in the reaction mixture.

A kinetic analysis of adsorption, based on the pseudo-first and pseudo-second-order models, was further employed to fit experimental data, and their linearized forms are represented by the following equations, respectively:

$$\log(q_e - q_t) = \log q_e - \frac{k_1}{2.303}t \quad (8)$$

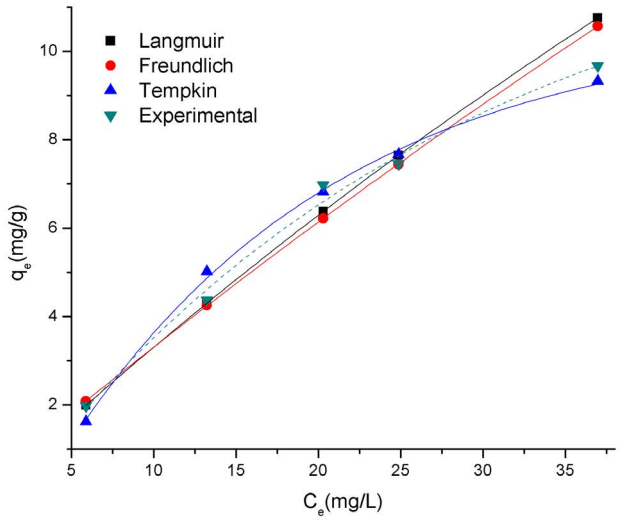

Fig. 3. Adsorption isotherms of the CV dye adsorption onto ASBP at 25°C.

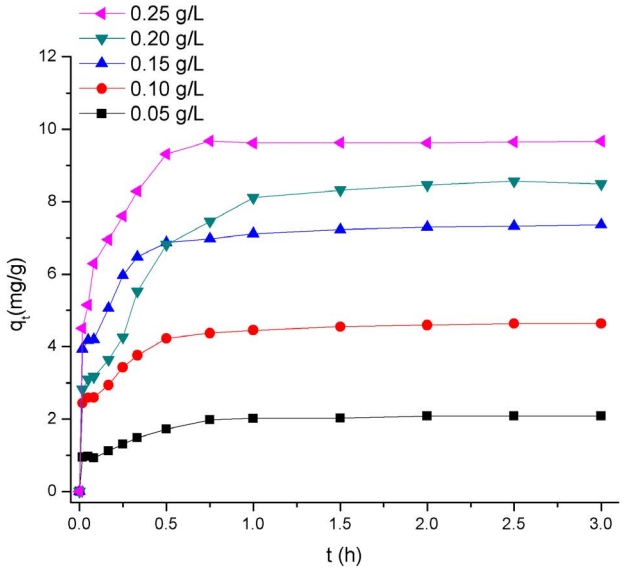

Fig. 4. Effect of time on the adsorption capacity of the ASBP.

Table 4
Equilibrium parameters of the CV dye adsorption onto ASBP at 25°C

Langmuir			Freundlich			Temkin		
R_L^2	K_L (L/mg)	q_m (mg/g)	R_F^2	n	K_F (mg/g)(L/g)^n	R_T^2	k_{1T}	k_{2T} (L/mg)
0.9434	0.0104	33.378	0.9584	1.0265	0.1219	0.9724	4.8991	0.2503

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} t \quad (9)$$

where q_e (mg/g) is the amount of the dye adsorbed onto ASBP surface at equilibrium and q_t (mg/g) is the amount of the dye adsorbed at time t. The rate constants of the pseudo-first-order and pseudo-second-order adsorption kinetics are k_1 (1/min) and k_2 (g/mg min), respectively [29]. The corresponding kinetic parameters calculated from kinetic linear plots were obtained at five different initial dye concentrations and were reported in Table 5. According to the obtained values of the regression coefficients, as well as the agreement between experimental and predicted q_e values, the adsorption of CV dye onto the ASBP was found to follow the pseudo-second-order kinetic model. With regard to the principle of the pseudo-second-order kinetics, the rate limiting step of the adsorption process may be chemical sorption [14].

In accordance with the earlier literature reports, the adsorption kinetics of the cationic dyes onto SBP (unmodified and modified) was mostly based on the model of pseudo-second-order [1,7,14].

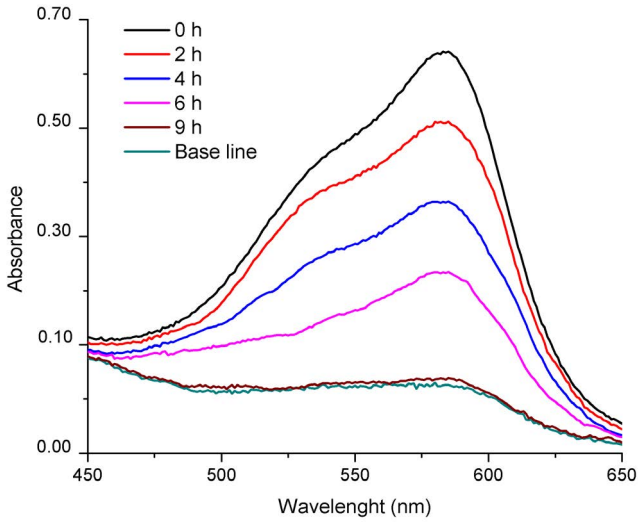

Fig. 5. Time course of the CV dye biodegradation by *Streptomyces microflavus* CKS6.

3.6. Bacterial biodegradation of the adsorbed CV dye

For the biodegradation performance, the dye was primarily adsorbed under the optimal conditions obtained from the previous optimization process. By utilizing 15 g/L of the ASBP, 0.2 g/L of the initial CV concentration, during the agitation time of 315 s, under pH 6, the adsorption capacity resulted in 12.02 mg/g and was quite close to the predicted value (11.96 mg/g), revealing the suitability of the RSM model employed and its applicability in optimizing the actual adsorption conditions.

At the beginning of the biodegradation process, a slight working medium coloration occurred, due to the dye particles desorption from the adsorbent surface, as a result of a solute concentration gradient between the liquid and solid phase, which strived to be split. Immediately after, the CV dye adsorption by CKS6 from the medium was evident, by making the cell surface deeply colored and, consecutively, leaving the working solution colorless. Simultaneously, the adsorbent surface occupation by the CKS6 took place, caused by the microorganism affinity toward the dye molecules. The literature offered a limited information about the mechanism behind the interactions between bacterial cells and dyes. The decolorization with microorganisms (whether with live or dead cells) can be due through several complex mechanisms: surface adsorption, ion exchange, complexation (coordination), chelation and micro-precipitation [36]. The microbial cell wall comprises of polysaccharides, proteins and lipids and varied functional groups may be involved in the interactions with dye molecules. The working medium decolorization has been authenticated all the time during the process by the UV/Vis spectrum (Fig. 5), revealing that all peaks decrease approximately in proportion to each other, until the major absorbance peak disappeared completely, probably as a consequence of dye molecule break down. Up to the process completion, which occurred after 9 h, all of the microbial cells, ASBP and working solution remained entirely colorless. In addition, no extra absorbance peaks appeared in decolorized medium, indicating that CKS6 has completely degraded the dye molecules, and probably utilized them for its own cells' growth. Similar pattern has been detected when CV was exposed to *Citrobacter* sp. and *Streptomyces fulvissimus* CKS7 degradation activity [25,37].

Considering the enzyme profile of the CKS6 (Tables 6 and S1), one may postulate that lignin peroxidase acts a major role in the triphenylmethane dye (Crystal violet) degradation. Extracellular peroxidases have been previously shown to be able to catalyze a wide variety of structurally diverse organic compounds, including polymeric dyes from the textile industry, that are among the most difficult to degrade [9,38]. Lignin peroxidase acts by catalyzing the benzylic oxidation, carbon–carbon bond cleavage, hydroxylation, phenol dimerization and O-demethylation [39]. Also, lignin peroxidase does not require the presence of manganese peroxidase to start the degradation of these dyes [40,41].

The lignin-degrading enzymes of the CKS6 (Table 6) expressed a several-fold increased activities after the biodegradation of the CV, in comparison with the sample where the dye was absent, revealing their considerable affinity toward the dye. A similar observation of the enzyme activity and it's induction by the dye has been reported by Kumar and Mongolla [42].

The ASBP, remained after biodegradation process, was separated from the reaction mixture by centrifugation, dried and subjected to the desorption process with the 70% ethanol solution, in order to estimate whether the dye residues still existed on the adsorbent surface. The results showed that no dye was found in the eluate, indicating that the adsorbent has been recovered and possibly may be further employed in the next wastewater treatment cycle.

Table 5
Adsorption kinetics parameters of the CV dye adsorption onto ASBP

		Pseudo-first-order model			Pseudo-second-order model		
IC[a] g/L	$q_{e,exp}$[b] mg/g	k_1 1/h	$q_{e,mod}$[c] mg/g	R^2	k_2 g/mg h	$q_{e,mod}$[c] mg/g	R^2
0.05	2.09	1.7505	0.98	0.9359	5.0954	2.16	0.9975
0.10	4.66	1.7205	1.59	0.9565	3.7097	4.72	0.9992
0.15	7.37	1.8261	2.20	0.9304	2.9989	7.45	0.9996
0.20	8.58	1.7034	4.45	0.6401	0.5019	9.12	0.9739
0.25	9.68	1.8835	1.67	0.6255	2.7209	9.81	0.9995

[a]Initial concentration of crystal violet dye.
[b]$q_{e,experimental.}$
[c]$q_{e,modeling.}$

Table 6
Lignin-degrading enzyme activities of *Streptomyces microflavus* CKS6 culture media before and after the dye biodegradation

Sample	Enzyme activity	
	Lignin peroxidase, %	Mangan-dependent peroxidase, U/L
Before biodegradation	1.47 ± 0.18	0.86 ± 0.24
After biodegradation	11.24 ± 0.48	3.03 ± 0.31

Table 7
Phytotoxicity comparison of the CV dye and produced metabolites (after the biodecolorization) in relation to *Triticum aestivum* seeds

Parameters	CAM[a]	CV (5 mg/L)	Medium after biodegradation
RSGI (%)	100	80	95
S^b	3.08 ± 0.05	1.01 ± 0.26	3.11 ± 0.24
R^c	1.22 ± 0.21	0.54 ± 0.32	1.31 ± 0.28

[a]Control agar medium.
[b]Length of shoot (cm).
[c]Length of root (cm).

3.7. Phytotoxicity of the dye-degrading products

The phytotoxicity assesment of discolored working solution (after the bacterial decolorization of the CV adsorbed onto the ASBP), containing dye-degrading products, has been performed with the environmental safety aspect of this research. The relative sensitivity toward the initial dye solution and decolorization products in relation to 10 seeds of *Triticum aestivum* were evaluated and obtained results are presented in Table 7.

A remarkable growth in the shoot and root length in all of the produced metabolite samples, accomplished with the good germination rate has been observed, relative to the untreated dye. A slightly higher values of all of the evaluated phytotoxicity parameters have been noted even regarding to the control agar medium, which contributes to the importance of this investigation. All results refer to the non-toxic nature of the CV-biodecolorized medium by the use of *Streptomyces microflavus* CKS6 microbial culture and their further potential application, such as the use for the fertilization purposes in the agriculture sector.

4. Conclusions

The implementation of integrated adsorption and biological processes in the wastewater treatment ensures permanent contaminant removal and its simultaneous transformation into harmless forms. According to the previous state of art, the novelty of this research is reflected in unique construction of an adequate process for the complete treatment of colored wastewater. Furthermore, the proposed procedure is simple, economic, relatively fast and in accordance with the principles of resources saving and environmental protection. Up to now, the use of physical, chemical or biological treatments individually is mainly limited by the insufficient reliability of process equipment and the unwanted quality of purified effluents. In addition, a high capital and operating costs and the problem of the by-products handling (such as sludge) frequently appeared. Therefore, the proposed procedure may contribute to a more advanced and more effective solution for solving the actual problems.

The utilization of the agro-industrial waste materials together with the microorganism with the distinct biodegradation potential and minimal nutritional requirements for the metabolic activity expressions, represent safe and economically viable solution for industrial effluents treatment. Considering the microorganism's enzyme profile, one may postulate its direct and safe disposal into the environment, by contributing to the soil and water remediation. After the completed process, the adsorption material remained regenerated and may be further employed in the following wastewater treatment or be implemented in the animal feed formulations.

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Supplementary information

Table S1
Enzyme profile of strain *Streptomyces microflavus* CKS6

Enzyme	Substrate	Presence
Peroxidase	Pyrogallol	+
Lignin peroxidase	Methylene blue	+
Manganese independent/dependent peroxidase	Phenol red	–/+