Integral approach of sorption coupled with biodegradation for treatment of azo dye using *Pseudomonas* sp.: batch, toxicity, and artificial neural network

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Abstract

The present study investigated the removal of azo dye (crystal violet) by adsorption (using a low-cost adsorbent fly ash), biodegradation (using bacterial species, *Pseudomonas* sp.), and an integrated approach of sorption coupled with biodegradation (using fly ash immobilized with *Pseudomonas* sp.) on a comparative scale. To ascertain immobilization of bacteria on fly ash, immobilized bacterial cells were characterized by energy-dispersive X-ray spectroscopy, scanning electron microscopy, Fourier-transform-infrared spectroscopy, and fluorescence microscopy. Batch studies were conducted for optimization of the process parameters for ensuring maximum dye removal. The optimum pH, temperature, and initial dye concentration for the highest percentage of dye removal were found to be pH 7, 37 °C, and 50 mg/L in all the three cases. Under optimized conditions, the highest percentage of dye removal was found to be 89.24, 79.64, and 99.04% for biodegradation, sorption, and integrated approach of sorption and biodegradation, respectively. Finally, phytotoxicity studies carried out with the treated water on *Cicer arietinum* seeds also carried proved that these processes and the adsorbent did not exert any toxic effects on the seeds. Artificial neural network modeling revealed a close interaction between theoretically predicted and experimentally obtained results and with an error of around 1.1%. Thus, this novel, environmentally sustainable and economically viable technique may be applied for effective removal of crystal violet from industrial wastewater.

Keywords Crystal violet · *Pseudomonas* sp. · Biodegradation · Sorption · Fly ash · Immobilization · Toxicity · ANN

Introduction

Increase in industrialization has encouraged wide-scale production of toxic chemicals including a variety of dyes which are present in almost all the types of effluents. Dyes are perceptible by naked eyes even in trace concentrations and are also considered unsuitable for human consumption (Kodam et al. 2005). The presence of dyes in water bodies prevent sunrays to pass through the water-layer-hindering photosynthesis of the aquatic organisms present therein (Hogan et al.

2010; Das et al. 2015a). Discharge of dye mixed industrial waste waters in aquatic ecosystem leads to their accumulation in living organisms through food chain, which in turn causes many ecotoxicological effects and public health hazards (Ghosh and Saha 2013; Chowdhury and Saha 2013). Dye compounds also have the ability to increase the chemical and biochemical oxygen demand of the water body they are discharged into (Srinivasan et al. 2014). Dyes are also reported to be carcinogenic in nature and cause dysfunction of kidney, liver, brain, reproductive system, and central nervous system (Ghosh and Saha 2012; Ahmad 2009).

Among the various commercially available dyes, azo dyes are most widely used in different industries such as textile, cosmetics, food, leather tanning, printing, paper, pulp, etc. (Chowdhury et al. 2011) owing to its convenient and cost-effective synthesis in comparison with natural dyes (Aksakal and Ucin 2010). Among the azo dyes available, crystal violet (CV) has been used extensively in pharmaceutical and textile industries.

Nowadays, nearly, 10–15% of all dyes discharged with various waste products are found to accumulate in

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neighboring ecosystems (Ghosh and Saha 2013). Conventional methods applied for elimination of azo dyes like CV compounds from industrial waste waters include ion exchange, reverse osmosis, membrane filtration, chemical precipitation, etc. (Fernando et al. 2013). However, these methods face limitations like incomplete removal of dye compounds, high reagent and energy requirement, generation of toxic byproducts, expensive operating costs, and laborious processes (Martins et al. 1999). In this context, dye degradation by different microorganisms has been found to be a more efficient and advantageous process. Biodegradation is usually considered to be cost effective as it requires minimal or no pre-treatment at all, is eco friendly, and cleaner in nature with minimum sludge production (Mondal 2008).

Several groups of bacteria such as *Pseudomonas aeruginosa*, *Dietzia* sp., *Bacillus subtilis*, *Halomonas* sp., etc. have been previously reported for their capability of decolorizing azo dyes (Prasad 2014; Saha et al. 2012). They have developed their enzyme systems for decolourizing the dye compounds. *Pseudomonas* sp. has been reported to degrade the varieties of azo dyes such as C. I. Basic Yellow 28, Reactive Red 22, Methyl Yellow 87, Direct Blue 6, Acid Violet 7, etc. (Daneshvar et al. 2006; Erkurt et al. 2010).

Like biodegradation, adsorption is also another widely investigated method for wastewater treatment. Fly ash, chitosan, graphene oxide nanoplatelets, etc. have been previously reported as effective, inexpensive adsorbents for efficient wastewater treatment (Chowdhury et al. 2011; Chowdhury and Saha 2011; Liu et al. 2015; Goswami et al. 2017). Of all other adsorbents reported so far, fly ash is produced in abundance as a by-product of pulverized coal combustion in thermal power plants. Recycling of fly ash for wastewater treatment may be considered as an environmentally as well as economically viable approach for a sustainable management of a hazardous industrial waste (Shaobin et al. 2004).

Combination of biodegradation and adsorption may augment the efficiency of both processes for better treatment of dye-rich wastewaters. This integrated system increases the dye degradation presumably by keeping dye molecules bioavailable for microbial attack. It also induces better availability of immobilized bacterial cell surface area frequently accessible for dye particles (Chen et al. 2003). Immobilization of microbial cells on organic or inorganic supports can occur both naturally and artificially. Among the different artificial processes investigated so far, physical adsorption, ionic binding, covalent binding, chemical cross-linking, lattice entrapment, and membrane entrapment have received significant scientific attention.

In this study, CV removal was carried out by adsorption using fly ash, by biodegradation using *Pseudomonas* sp. and by an integrated approach which employs adsorption coupled with biodegradation. The process was optimized using

artificial neural network (ANN) analysis. The phytotoxicity analysis of the treated solution was studied with *Cicer arietinum* seeds to elucidate whether the treated water was suitable for discharge into the environment (Senthilkumar et al. 2013).

Materials and methods

Dye and chemicals

Crystal violet (CV; MF: $C_{25}N_3H_{30}Cl$; molar mass: 407.979 g/mol; MP: 205 °C) of commercial grade was obtained from Merck, India. Luria–Bertani broth, nutrient agar, dipotassium hydrogen phosphate (K_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4), ferrous sulphate heptahydrate ($FeSO_4 \cdot 7H_2O$), magnesium sulphate heptahydrate ($MgSO_4 \cdot 7H_2O$), calcium chloride ($CaCl_2$), and calcium hydroxide ($Ca(OH)_2$) were purchased from Merck, India. Sodium borates, EDTA, formaldehyde, and Triton X were obtained from Sigma Aldrich, India. Ethidium bromide was purchased from Invitrogen, India and acridine orange from Himedia, India. Pure cultures of *Pseudomonas* sp. (MTCC 11808) were commercially procured from Microbial-Type Culture Collection, Chandigarh.

Fly-ash (adsorbent) collection and treatment

Fly ash was collected from thermal power station present in Kolkata, West Bengal, India. The collected fly ash was washed with double distilled water, dried at 70 °C for 24 h, and sieved to eliminate any undesired particle. Then, it was further treated with 10% calcium hydroxide for 3 h, filtered, and dried at 70 °C in a hot air oven for 24 h. Finally, the resulting treated fly ash was utilized for further studies.

Immobilization

Freshly prepared Luria–Bertani broth was inoculated with pure cultures of *Pseudomonas* sp. and placed in an incubator (REMI CIS-24 PLUS, India) overnight at 27 ± 2 °C with mild agitation. 5 mL of resulting culture was further added to the 95 mL of autoclaved water and incubated for 24 h. At the end of 24 h, 5 g of $Ca(OH)_2$ -treated fly ash was added to this culture and kept in an incubator for 24 h at 37 °C temperature and 120 rpm agitation speed. The mixture was then centrifuged (REMI PR-24, India) at 5000 rpm for 10 min and stored at 4 °C for further studies.

Characterization of fly ash

The adsorbents were characterized by energy-dispersive X-ray spectroscopy (EDX; ZEISS EVO-MA 10, Germany),

scanning electron microscopy (SEM; ZEISS EVO-MA 10, Germany), and Fourier-transform-infrared spectroscopy (FTIR; PerkinElmer, Waltham, United States).

Batch studies

Pure cultures of *Pseudomonas* sp. (grown in Luria–Bertani broth), Ca(OH)_2 treated fly ash and *Pseudomonas* sp. immobilized Ca(OH)_2 -treated fly ash were used separately for the dye-removal experiments. CV solutions were prepared with minimal media composed of K_2HPO_4 , KH_2PO_4 , $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and CaCl_2 . Dye-removal efficiency was determined over 15 days in case of biodegradation and 7 days for adsorption in an incubator with constant agitation. During this study, aliquots of treated dye solutions were collected aseptically once every 24 h and centrifuged at 10,000 rpm for 15 min. The supernatant was collected in each case and its absorbance was recorded using UV/VIS spectrophotometer (PerkinElmer Lambda 25, Waltham, United States) at 580 nm.

The % of dye removal was calculated using the formula:

$$\% \text{ removal} = \frac{(\text{initial dye concentration} - \text{final dye concentration})}{\text{initial dye concentration}} \times 100. \quad (1)$$

Several batch studies were conducted over different ranges of selected experimental parameters [temp 25–45 °C; pH 1–11; initial dye conc. 5–100 mg/L; inoculum volume 10–100 mL/L (for biodegradation studies); and adsorbent dose 10–60 mg/L (for adsorption and integrated adsorption–biodegradation studies)] by keeping other parameters constant.

Fluorescence study

0.7 mL aqueous suspension of immobilized Ca(OH)_2 treated fly ash was incubated with 0.2 mL of staining buffer (pH 9.2) containing sodium borate (100 mM), EDTA (60 mM), Triton X-100 (0.05%) and formaldehyde (0.05%), and 0.1 mL of dye mixture (100 µg/mL) containing ethidium bromide and acridine orange in a 1:1 ratio at room temperature for a minute. Afterwards, 10 µL of incubated sample was observed using hanging drop method under the fluorescence microscope (LEICA, DM LB, Houston, USA) at 500–530 nm for acridine orange and at 510–595 nm for ethidium bromide (Mansour et al. 1984).

Toxicity study

Comparative analysis of toxic effects of untreated and treated effluents was determined with *Cicer arietinum*

seeds. A total of three experimental groups were prepared consisting of control (sterile distilled water), untreated wastewater, and biotreated wastewater. The rate of germination of this type of seeds for 5 days was observed.

Phytotoxicity was estimated in terms of germination of seeds, root–shoot length, and percentage of phytotoxicity calculated as per the following equation (Goswami et al. 2013):

$$\begin{aligned} \text{Percentage of phytotoxicity} \\ = \frac{(\text{radicle length in control} - \text{radicle length in test})}{\text{radicle length in control}} \times 100. \end{aligned} \quad (2)$$

Toxic-stress-induced alterations in biochemical parameters were determined from estimation of protein (Folin–Lowry method) (Banerjee et al. 2017) and carbohydrate contents of the treated seeds (Anthrone method) (Banerjee et al. 2017).

ANN analysis

ANN technique is a recent technology implemented for optimization of process parameters using a biological 1 neural schema (Chowdhury and Saha 2013). In this study, feed forward back propagation (BP) neural network with a linear transfer function was used to study the removal of azo dye using integral technique. MATLAB (Version 7) was chosen to develop the ANN model program. In this study, four parameters including temperature (K), pH of solution, time (min), and concentration of dye (mg/L) were chosen as independent variables and dye removal (%) was considered as dependent variable. Best neural network was generated by a trial and error method after optimization of maximum number of epochs (using).

Statistical analysis

All the experiments were conducted thrice to ensure the accuracy, repeatability, and reproducibility of the experimental parameters. All the data were reported as mean value $\pm$ standard deviation (Roy et al. 2016). Data analysis was carried out using Origin software (Version 9.1; Origin Lab Corporation, USA). Statistical analysis of ANN optimization was carried out using MATLAB (Version 7, USA).

Results and discussion

Characterization of fly ash

Energy-dispersive X-ray spectroscopy (EDX) analysis

From EDX spectrum, raw fly ash and calcium hydroxide treated fly ash were analyzed and the compositions are given in Table 1. The EDX spectrum of fly ash is shown in Fig. 1. The major elements present in fly ash were oxygen, silicon, aluminium with some sodium, chlorine, and others. Percentage of calcium increased significantly in the treated fly ash indicating effective treatment of raw fly-ash particles. $\text{Ca}(\text{OH})_2$ treatment of fly ash reportedly increases surface area of fly ash by rendering it a better porous structure (Chowdhury et al. 2011). Previous studies have also reported improved adsorption of dyes using calcium-treated fly ash (Peterson and Rochelle 1988).

Table 1 Comparative studies on compositions and properties of raw fly ash and treated fly ash

Elements	% of weight	
	Raw fly ash	Treated fly ash
O	56.76	59.17
Ca	1.17	35.42
K	2.19	1.12
Si	14.73	0.64
Ti	1.47	0.18

Scanning electron microscopy (SEM)

The images of immobilized bacterial cells, raw fly ash, and treated fly ash under SEM are shown in Fig. 2.

From SEM images (Fig. 2a–d), it was observed that *Pseudomonas* sp. cells were immobilized on fly ash. From Fig. 2c, d, it was clearly seen that the rod shaped, thin bacterial cells immobilized on treated fly ash, while in Fig. 2a, b, there was no rod-shaped species on fly ash which was observed. Therefore, it can be said that after immobilization of *Pseudomonas* sp. on fly ash, the bacterial strain was found to be attached on to the fly-ash particles by physical entrapment. In this approach, bacterial cells were held or entrapped within suitable matrix such as fly ash (Datta et al. 2013).

Fourier-transform-infrared (FTIR) spectroscopy

The FTIR spectra of raw fly ash (RF), treated fly ash (TF), and bacteria immobilized on treated fly ash (IF) are shown in Fig. 3. The stretching bands of Si–O–Al recorded between 1200 and 600 cm^{-1} indicated vibrations of the Si–O and Al–O bands present in raw fly ash. The prominent band at 1040 cm^{-1} also suggested the presence of Si–O stretching. In $\text{Ca}(\text{OH})_2$ -treated fly ash, similar peaks were found in the range of 1200–600 cm^{-1} , indicating the stretching bands of Si–O–Al and vibrations of Si–O and Al–O bonds. A broad peak at 1038 cm^{-1} was also attributed to Si–O stretching. The peak at 1416 cm^{-1} indicated the presence of –OH groups. The peak at 3643 cm^{-1} was attributed to –OH absorption bands, suggesting the presence of $\text{Ca}(\text{OH})_2$ in treated fly ash (Sengupta et al. 2015). In case of immobilized bacterial cells, similar band stretching as the previous two

Fig. 1 Energy-dispersive X-ray spectroscopy image of raw (a) and treated fly ash (b)

Fig. 2 Scanning electron microscopy image of **a** treated fly ash; **b** bacterial cells; **c, d** embedded bacteria on fly-ash particles

was found which may be indicated that the bacterial cells immobilized on fly ash by physical entrapment.

Batch studies

Effect of temperature

From Fig. 4, it was observed that the percentage of dye removal by adsorption, and also by combine approach was increased with a corresponding increased in temperature up to at 37 °C, because porosity as well as total pore volume of the adsorbent was increased, while temperature increased (Khan et al. 2012). Time required for the maximum dye decolourization using fly-ash adsorbent (~7 days) was less than that of biodegradation (~15 days) approach. On the other hand, the percentage of maximum dye removal using adsorption method was slightly less than biodegradation method. As temperature increased beyond 37 °C, removal efficiency decreased in all cases. The reason may be that at higher temperature, the dye molecules adsorbed on the

fly-ash surface may came out and desorption occurred. In case of organism, at higher temperature, bacterial cells may damaged, and therefore, the efficiency of removal reduced as temperature increased. In case of coupled approach, percentage of dye removal was higher than these two individual approaches and required time for maximum dye removal was also less than other two processes. In case of biodegradation, the percentage of dye removal was 76.82% after 15 days, and for adsorption, the percentage of dye removal was 73.33% after 7 days.

Effect of pH

From Fig. 5, it was seen that the percentage of dye removal by biodegradation, adsorption, and also by combine approach was found to increase with a parallel rise in solution pH (pH 1–7) and maximum at pH 7. With further increase in solution pH (beyond pH 7), no significant change in dye removal was recorded. Hence, it may be suggested that positive surface charge attained by the adsorbent at

Fig. 3 FTIR spectra of raw fly ash, treated fly ash, and immobilized bacterial cells on fly ash

Fig. 4 Effect of temperature on biodegradation, sorption, and combination of biodegradation and adsorption of dye

lower hindered the binding of cationic dye molecules. With an increase in solution pH, the adsorbent surface gradually attained negative charge resulting in improved removal of CV (Khan et al. 2013).

The percentage of maximum dye removal using adsorption method was less than biodegradation method. In case

Fig. 5 Effect of pH on biodegradation, adsorption, and combination of biodegradation and sorption of dye

of coupled approach, percentage of dye removal was higher than these two individual approaches and required time for maximum dye removal was also less than other two processes. This integrated approach induces the dye degradation possibly by keeping dye particles bioavailable for microbial attack. It may also induce frequent availability of immobilized bacterial cell surface area accessible for dye molecules.

Effect of initial dye concentration

From Fig. 6, it has been evident that the maximum percentage of dye removal by biodegradation, adsorption, and also by combine approach was achieved with an initial dye concentration of 50 mg/L. It was observed that *Pseudomonas* sp. could efficiently use CV as a present as sole carbon source in minimal media. The reduced percentage of dye removal observed at lower dye concentrations may have had occurred due to lesser number of viable bacterial cells present due to insufficient availability of carbon source (dye). However, at very high initial dye concentration, dye induced, toxicity may be considered responsible for unavailability of viable bacterial cells which in turn resulted in lower percentage of dye removal (Das et al. 2015b). In case of coupled approach, percentage dye removal was higher than two individual approaches and required time for maximum dye removal was also less than other two processes. This coupled approach increases dye degradation probably by keeping dye molecules bioavailable for microbial invasion.

Fig. 6 Effect of initial dye concentration on biodegradation, adsorption, and combination of biodegradation and sorption of dye

Effect of inoculum volume or adsorbent dose

From Fig. 7, it was observed that the percentage of dye removal by biodegradation, adsorption, and also by combine approach was highest at inoculum volume of 100 mL for each litre of dye solution in biodegradation studies and at dose of 0.04 g/L. As the adsorbent dose increased, a parallel rise in the percentage of dye removal was also observed. This may have had occurred due to an increase in available adsorbent surface area which in turn provided more active sites for dye uptake (Mondal 2008). Further increase in adsorbent dosage was found to cause a decline in percentage dye removal. Saturation of adsorption sites or aggregation of adsorbent particles may be held responsible for the decline in percentage dye removal taking place with higher adsorbent dosages.

Fluorescence microscopy

As evident from Fig. 8, the presence of living bacterial cells on fly-ash particles was significantly higher in comparison with dead cells. The characteristics of different fluorescent dyes are listed in Table 2. On the basis of the result obtained in this study, it may be concluded that most of the immobilized bacterial cells were viable in nature rendering successful integration of biodegradation with adsorption. It may

Fig. 7 Effect of inoculum volume on degradation, fly-ash dose on adsorption, and immobilized sample dose on combination of biodegradation and sorption approach

Fig. 8 Living and dead cells embedded on fly ash. The cells (living) having green fluorescence are indicated by red arrow and the cell (dead) having red fluorescence are denoted by green arrow. Fly ash designated by blue arrow

also be assumed that equal contribution of both processes applied in combination was responsible efficient removal of azo dye from aqueous solutions (Stiefel et al. 2015).

Toxicity analysis

Differences in seeds germination could be connected with change of some biochemical processes. All the results of physical and biochemical parameters related to phytotoxicity analyses after 5 days are given in Table 3.

The treatment of *Cicer arietinum* seeds with untreated dye solutions suggested a significant decrease in germination (%) with respect to that of the control, and thus, the treatment with untreated CV solution (concentration of 50 mg/L) showed a significant increase in percentage of phytotoxicity in comparison with seeds treated as control. On the other hand, percentage of germination in *Cicer arietinum* treated with biotreated wastewater was significantly higher than those seeds treated with untreated CV solutions and were similar to control levels. Seeds exposed to biotreated wastewater exhibited the lowest values of phytotoxicity for *Cicer arietinum* seeds (Bhattacharya et al. 2014).

During seed germination, the amount of protein and insoluble carbohydrate in seeds of control and biotreated wastewater were much higher than that obtained in seeds exposed to untreated CV solutions. The decrease in protein concentration in seeds exposed to untreated CV solutions may have had occurred due to toxic-stress-induced inhibition

Table 2 Fluorescence study

Dye	Excitation maxima (nm)	Emission maxima (nm)	Colours under microscope	Viability of cells
Acridine orange	500	530	Green	Living cells
Ethidium bromide	510	595	Red	Dead cells

Table 3 Phytotoxicity assays in *Cicer arietinum* seeds

Parameters	Control (distilled water)		Wastewater treated with immobilized bacteria		Untreated dye effluent	
Growth parameters						
% of phytotoxicity [(length of radicle in control – radicle length of length of radicle in control in test)/length of radicle in control] × 100	5.82		3.56		88.22	
Biochemical parameters						
Protein estimation (amount of protein in mg.)	108.44 (initial)	180.47 (after 5 days)	109.52 (initial)	191.22 (after 5 days)	109.43 (initial)	72.53 (after 5 days)
Carbohydrate (insoluble) estimation (in mg)	119.09 (initial)	210.73 (after 5 days)	120.54 (initial)	211.73 (after 5 days)	120.96 (initial)	116.73 (after 5 days)

of enzymes that can degrade proteins in polypeptides. The decrease in carbohydrate contents of seeds treated in untreated dye solution may have resulted from the inactivity of amylase enzyme, capable of degrading storage polysaccharides during germination periods. Thus, results obtained in this toxicity assay suggested that CV contaminated water treated by coupled biodegradation and adsorption approach did not pose any toxic effects to the environment and was hence suitable for discharge.

Neural network training

The regression plot of the trained network is shown in Fig. 9a, b. The trained network showed a correlation

coefficient of 0.989 for fly ash and 0.964 for immobilized micro-organism with fly ash (for Levenberg–Marquardt BP algorithm) (Chowdhury and Saha 2013). A high correlation coefficient using this algorithm signified the reliability of the model with the experimental data. Table 4 shows that the ‘Levenberg–Marquardt back propagation (Trainlm)’ algorithm with ‘poslin’ transfer function gave better results in both cases in comparison with other tested BP algorithms. From Supplementary Fig. 1, it was observed that the difference between the experimental results and simulated result (from ANN) was very less (within the range of -1.1%) signified that the ANN model better represents the experimental results in both cases.

Fig. 9 Regression plots (a, b) of the trained network

Table 4 Neural network training

Artificial neural network modeling

Algorithm selected for hidden layer	Selected functions			Correlation coefficient (R) for biodegradation in combination with sorption	Correlation coefficient (R) for sorption
	Output layer	Transfer 1	Transfer 2		
Levenberg–Marquardt back propagation	Trainlm	Poslin	Purelin	0.964	0.989
Resilient back propagation	Trainrp	Poslin	Purelin	0.933	0.985
Conjugate gradient back propagation with Polak–Ribiere updates	Traincgp	Poslin	Purelin	0.842	0.984
Scaled conjugate gradient back propagation	Trainscg	Poslin	Purelin	0.842	0.941
Gradient descent with momentum and adaptive learning rate back propagation	Traingdx	Poslin	Purelin	0.82	0.926

Conclusions

As evident from the results reported in this study, it may be concluded that *Pseudomonas* sp. immobilized on fly ash can remove the azo dye (CV) more efficiently than both the individual approach of biodegradation and adsorption. The efficiency of the process was found to depend upon various parameters like temperature, pH, initial dye concentration, and inoculum volume or adsorbent dose. An experimental temperature of 37 °C; solution pH 7; initial dye concentration of 50 mg/L; and initial inoculum volume of 100 mL for each litre of dye solution (combined sample dose, 0.04 g/L) was found optimum for maximum CV removal of 89.24, 79.64, and 99.04% for the bacterial species, adsorbent, and immobilized bacteria onto treated fly ash, respectively. Thus, the integrated approach of sorption coupled with biodegradation by immobilization technique resulted in the highest percentage of dye removal. Finally, phytotoxicity studies on *Cicer arietinum* seeds were also carried out which proved using fly ash as adsorbent did not exert any toxic effects and could be considered for environmental discharge. ANN results showed that theoretical predicted results were in good accord with those obtained experimentally. Thus, a novel, economically, and environmentally viable technique was used for effective removal of CV from industrial wastewater (Das et al. 2015b).

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

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