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Simultaneous Removal of Hazardous Contaminants Using Polyvinyl Alcohol Coated *Phanerochaete chrysosporium*

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ABSTRACT

Currently, the water pollution has become menace across the globe due to anthropogenic activities. The present study was designed to overcome the problem of water pollution wherein free cells of *Phanerochaete chrysosporium* were coated with polyvinyl alcohol and activated for the removal of heavy metals, azo dyes and phenolic compounds. Individual metal removal study suggested that activated PVA@PC has the capacity to remove Pb (98.5%), Al (91.24%), As (82.78%), Cd (64.5%) and Cr (53.56%) metals within 24 h. Activated PVA@PC proved to be efficient in removing different reactive dyes and phenolic compounds within 24 h. Simulated water effluent was prepared to observe simultaneous removal of hazardous contaminants. Studies revealed that activated PVA@PC is efficient to remove these contaminants within 36 h and remains efficient till 5 cycles. Microbial toxicity drastically reduced in activated PVA@PC than simulated effluent, depicting the fungi used in the present study can be a potent option for the waste water treatment plant.

Highlights

- PVA coated *P. chrysosporium* was used to remove metals, azo dye and phenolics.
- Efficacy of PVA@PC for the treatment of simulated effluent water was studied.
- Reusability of PVA@PC was tested for five subsequent cycles.
- Microbial toxicity of fungal treated and untreated samples were evaluated.

Keywords: Azo dyes, Degradation, Heavy metals, *Phanerochaete chrysosporium*, Phenolics

In today's contemporary milieu, water contamination has turned out to be one of the major challenges around the globe (Awasthi *et al.* 2017; Rudakiya 2018). Rocketing the growth of industries in the field of chemical, nuclear, electronic, petroleum sector prompted an exponential ascent in the pollution levels. The effluent water generally consists of heavy metals, dyes, carcinogenic compounds, endocrine disrupting chemicals and many other chemical impurities (Khani *et al.* 2012; Bhattacharya *et al.* 2015; Saha *et al.* 2017). Industries release these harmful chemicals in the effluent water and discharge it into the rivers and oceans (Keharia and Madamwar 2003; Rudakiya and Pawar 2014). The major challenge is to remove the toxic compounds from industrial waste water and to degrade the toxic compounds into less toxic form. Traditionally, industrial effluent is treated using methods like nano filtration, ultrafiltration, membrane separation etc. (Gahlout *et al.* 2017). These techniques are efficient but very selective and expensive for industrial waste water treatment. It is very important to develop a cost effective and efficient solution, which can supplant the ordinary strategies for industrial effluent treatment (Camargo *et al.* 2016; Raper *et al.* 2017).

Bioremediation is a waste management technique that employs microorganisms to break down hazardous contaminants into a less toxic or nontoxic substances (Rudakiya and Pawar 2013a,b). Among all microbes, white rot fungi are the unique organisms

that have potential to mineralize recalcitrant plant polymers (Kapoor and Viraraghavan 1995; Christian *et al.* 2005). These fungi produce lignocellulolytic enzymes, free radicals and organic acids that allow them to degrade hazardous compounds, to mineralize small organics (e.g. phenol, bisphenol A etc.) and to reduce the toxicity of metals by converting them into another form (Chhaya and Gupte 2013; Rudakiya and Gupte 2017). *Phanerochaete chrysosporium* is a model white rot fungus that has been extensively used for the removal of toxic organics, heavy metals and azo dyes (Li *et al.* 2015; Hailei *et al.* 2016; Bosco *et al.* 2017).

The present study was designed with the aim to evaluate the hazardous contaminant removal efficacy of *P. chrysosporium* under *in vitro* condition. Pellets were immobilized using polyvinyl alcohol and activated to enhance the performance of *P. chrysosporium.* Batch and continuous degradation experiments were also conducted for individual contaminant and simulated effluent. Toxicity profiles of control and degraded contaminants were also evaluated using soil and agricultural important bacteria.

MATERIALS AND METHODS

Chemicals and fungal strain

Al_2O_3, $Cd(NO_3)_2$, $CoCl_2$, $NaAsO_2$, $Ni(NO_3)_2$, $K_2Cr_2O_7$, $Pb(NO_3)_2$, polyvinyl alcohol (PVA), Phenol and bisphenol A were procured from Hi-media Labs. (Mumbai, India). All dyes were procured from CAB Chemicals, Ankleshwar (Gujarat, India). All mycological media and agar agar were also purchased from Hi-media Labs (Mumbai, India). White rot fungal strain, *Phanerochaete chrysosporium*, was obtained from gift cultures from the Institute of Frostbotanik, Gottingen, Germany. Culture was grown on malt extract agar at 28 °C for 8 days. For biomass production, spore suspension of *P. chrysosporium* (2.5×10^6 spores/ml) was inoculated in 100 ml of Sabouraud dextrose medium and incubated at 30 °C and 120 rpm for 10 days. Afterwards, obtained fungal pellets (free cells) were repeatedly washed three times with Na-acetate buffer (pH 5.0, 100 mM).

Simulated effluent preparation

Initially, stock solutions of individual metals and dyes (10 gL^{-1}) were prepared in autoclaved distilled water while stock solution of phenol and bisphenol A (10 gL^{-1}) was prepared in methanol (Table 1). For preparation of simulated effluent, 1 ml of each contaminant was mixed with autoclaved distilled water to obtain final concentration of each contaminant (100 mgl^{-1}) in simulated effluent. Prepared effluent was directly used for contaminant removal experiments.

P. chrysosporium coating using PVA

PVA solution was prepared by keeping the flask containing PVA flakes (10 gm) with 100 ml distilled water in water bath (~80 °C). Subsequently, PVA solution was allowed to cool for 10 min; fungal pellets were added individually to PVA solution in sterile condition and dropped into boric acid solution (7% w/v). Approx. 5 mm diameter of PVA coated *P. chrysosporium* pellets (PVA@PC) were selected for contaminants removal study. PVA@PC was stored in boric acid solution at 4 °C overnight to improve the strength. PVA@PC was activated

Table 1: List of Hazardous contaminants (dyes, phenolics and heavy metals) used in the present study

Azo dyes	Abbreviation	Metals	Abbreviation
Rubine Blue	R1	Al_2O_3	Al
Green MABL	R2	$NaAsO_2$	As
Acid Red B	R3	$Cd(NO_3)_2$	Cd
Reactive Violet 5B	R4	$CoCl_2$	Co
Reactobond Brown	R5	$K_2Cr_2O_7$	Cr
Reactive Black CNN	R6	$Ni(NO_3)_2$	Ni
Reactive Orange HE2R	R7	$Pb(NO_3)_2$	Pb
Phenolics			
Phenol		Bisphenol A	BPA

by keeping it for 24 h in autoclaved synthetic malt extract broth (malt extract, 10 gL^{-1}; dextrose, 20 gL^{-1}) at 4 °C.

Contaminants removal by free cells, PVA@PC and activated PVA@PC

A set of experiments of contaminant removal were conducted to infer overall contaminant (heavy metals, dyes and phenolics) efficacy by free cells of *P. chrysosporium*, PVA@PC and activated PVA@PC. In 250 ml Erlenmeyer flask, 10 g of free cells (fungal pellets), PVA@PC and activated PVA@PC were taken in autoclaved Na-acetate buffer (100 ml). Individual contaminants (100 mgl^{-1}) were added in buffer and incubated for 48 h at 140 rpm and 28 °C. Samples were periodically collected and % contaminant removal was calculated using following formula:

$$\% \text{ Contaminant removal} = \frac{(Ci - Cf)}{Ci} \times 100$$

Where, Ci = initial metal concentration, Cf = final metal concentration (Rudakiya and Pawar 2013a).

Batch Experiments

For individual contaminant removal experiments, 10 g of activated PVA@PC was taken in autoclaved Na-acetate buffer (100 ml, 50 mM) with 100 mgl^{-1} of individual contaminant and incubated for 48 h at 140 rpm and 28 °C. In simulated effluent experiments, 10 g of PVA@PC was taken in 100 ml of simulated effluent and incubated for 48 h at 140 rpm and 28 °C. All samples of individual and simulated effluent experiments were collected periodically and % contaminant removal was calculated.

Reusability of free cells, PVA@PC and activated PVA@PC

Reusability of fungal biomass can reduce the cost of remediation so overall efficiency of free cells, PVA@PC and activated PVA@PC were checked for 10 subsequent cycles. After each cycle, respective pellets were washed twice with distilled water and incubated in synthetic malt medium for 12 h at 4 °C to improve the efficacy of cells. All contaminants were extracted after each cycle, analyzed using specific techniques and overall % contaminant removal efficiency were calculated.

Hazardous contaminant analysis

After completion of biosorption experiments, aqueous solution was extracted and centrifuged at 4 °C (8000 rpm, 15 min). For heavy metal analysis, clear supernatant of individual sample was digested using microwave digestion system (Titan MPS 8, Perkin Elmer) and analyzed using inductively coupled plasma spectrophotometer (Optima-3300 RL, Perkin Elmer). For dye analysis, the aliquots of decolorized and control dye samples were analyzed with respective wavelength using UV-Visible spectrophotometer (Shimadzu UV 1800) to observe the changes in absorbance spectrum during decolorization. UV-Visible spectrophotometry was used to detect phenol and bisphenol A content in individual and simulated effluent at 260 nm and 278 nm, respectively. Baseline of mixture of the hazardous compounds was corrected using individual compounds.

Light microscopy analysis

P. chrysosporium cells were tested for light microscopy analysis to detect the hazardous contaminants (metals, dyes, etc.) were absorbed and/or metabolized by fungal hyphae. Fungal spores (2.5×10^6 spores/ml) were inoculated in Sabouraud dextrose medium and incubated at 30 °C and 120 rpm for 10 days. Fungal pellets were washed three times with distilled water, incubated with mix metals (set 1; 100 mgL^{-1} of each metal) and mix dyes (set 2; 100 mgL^{-1} of each dye) for 48 h at 140 rpm and 28 °C and further proceed for microscopy analysis. A set of fungal pellets without any hazardous contaminants also proceed for microscopy analysis which was served as control. For light microscopy, thin section of fungal pellets of *P. chrysosporium* were cut and observed under optical microscope with a magnification of 400×.

Toxicity analysis

The microbial toxicity of control contaminant and fungal treated samples using important microorganisms such as *Bacillus subtilis, Bacillus cereus, Bacillus megaterium, Proteus vulgaris, Salmonella typhi, Escherichia coli* and important agricultural microorganisms, *viz. Azotobacter sp., Rhizobium radiobacter.* In the well diffusion method, control contaminants were compared with degraded

contaminants. Furthermore, inhibition zone was measured after 24 h of incubation.

RESULTS AND DISCUSSION

Contaminants removal by free cells, PVA@PC and activated PVA@PC

It was observed that the overall contaminant removal efficiency of activated PVA@PC and PVA@PC was higher than the free cells. PVA coating provides mechanical strength to the free cells, which increases the physical and chemical resistance of the microbial cells. Activated PVA@PC showed higher contaminant removal efficiency than PVA@PC, depicting the nutrients provide during each cycle enhance the efficacy of the free cells. Overall, metal removal efficiency free cells, PVA@PC and activated PVA@PC were 24.45%, 39.56% and 75.30%, respectively (Fig. 1).

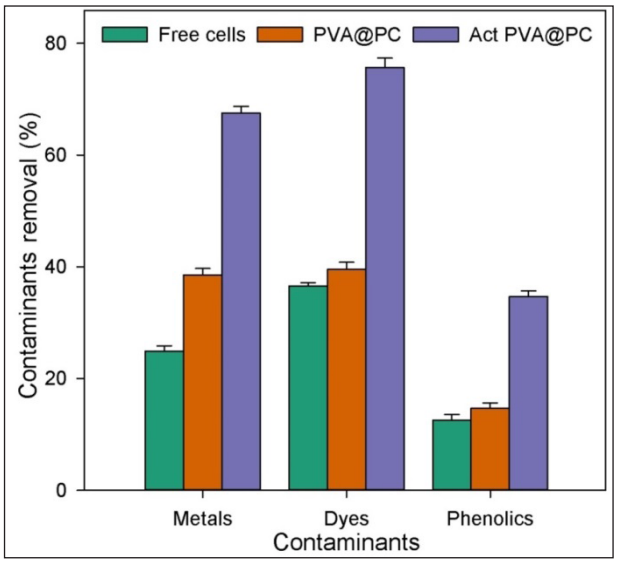

Fig. 1: Overall metals, dyes and phenolics removal by free cells of *P. chrysosporium*, PVA@PC and activated PVA@PC within 48 h of incubation

Results suggested that activated PVA@PC showed higher contaminant removal efficacy by three times. In case of phenolics, free cells and PVA@PC showed 18-20% of removal efficiency while activated PVA@PC showed 37.5% of removal efficiency.

Individual contaminant removal experiments

Individual hazardous contaminant removal efficiency was studied by activated PVA@PC wherein higher removal efficiency was observed in metals and azo dyes. In dyes and phenolics, higher removal efficiency was in R5, R6, R1, R4 and R7 dyes, which were higher than 80% (Fig. 2).

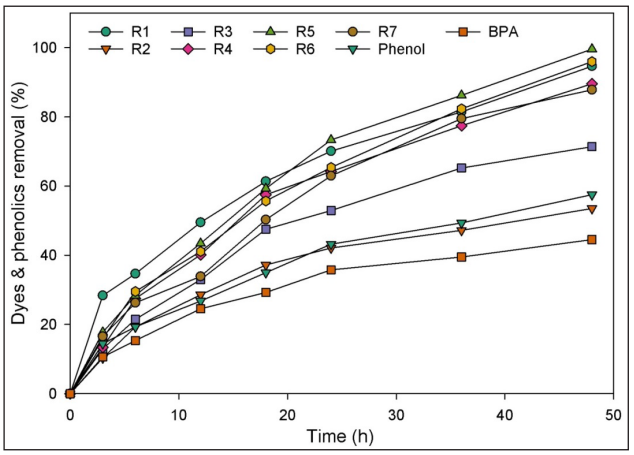

Fig. 2: Removal of individual dyes and phenolics by activated PVA@PC within 48 h

Activated PVA@PC showed the lower degradation potential towards R3 and R2 dyes that showed the dyes structure also influenced on the degradation efficiency. Rudakiya and Pawar (2014) showed that structure, complexity and molecular weight of dye effect on the degradation. In case of metal removal efficiency, the order of efficiency is as follows: Pb>Al>As>Cd>Cr>Ni>Co (Fig. 3). Activated PVA@PC showed highest binding and removal efficiency towards lead and the least affinity towards cobalt.

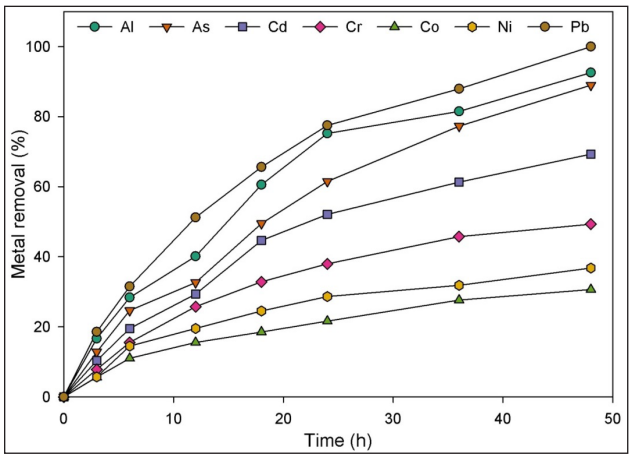

Fig. 3: Removal of individual heavy metals by activated PVA@PC within 48 h.

Simulated effluent removal experiments

In case of simulated effluent, all hazardous contaminant removal efficiency was studied by activated PVA@PC wherein higher removal efficiency was obtained in metals and azo dyes. As

shown in Fig. 4, R5, R6, R4 and R1 dyes showed higher removal efficiency among all reactive dyes, which were higher than 80%. Activated PVA@PC depicted the lower degradation potential towards R2 and R3 dyes that showed the dyes structure also influenced on the degradation efficiency (Rudakiya and Pawar 2014).

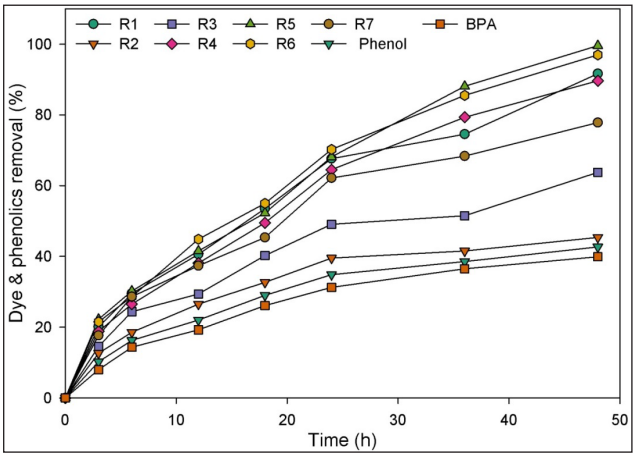

Fig. 4: Removal of hazardous contaminants (azo dyes and phenolics) from simulated effluent by activated PVA@PC within 48 h

In case of metal removal efficiency, Cr, Ni and Co showed least removal efficiency, suggesting the activated PVA@PC showed different metal binding behavior in case of simulated effluent. Al, Pb and As metals showed highest removal efficiency that were nearly 100% within 48 h of incubation.

Reusability of free cells, PVA@PC and activated PVA@PC

In the present study, free cells, PVA@PC and activated PVA@PC were examined for 10 times in batch-type process for the removal of simulated effluent. Fig. 4 shows that activated PVA@PC were highly efficient to removal all hazardous contaminants presented in simulated effluent till 6th cycle. Activated PVA@PC could remove all hazardous contaminants above 10% till 9th cycle while PVA@PC could remove the contaminants till 6th cycle. Free cells of *P. chrysosporium* showed very less efficiency in terms of reusability wherein contaminant removal efficiency above 10% was till 4th cycle (Fig. 6). Similar results were obtained by Rudakiya and Pawar (2014) wherein activated Ca-alginate immobilized *C. acidovorans* were showed higher efficiency compared to non-activated and free cells.

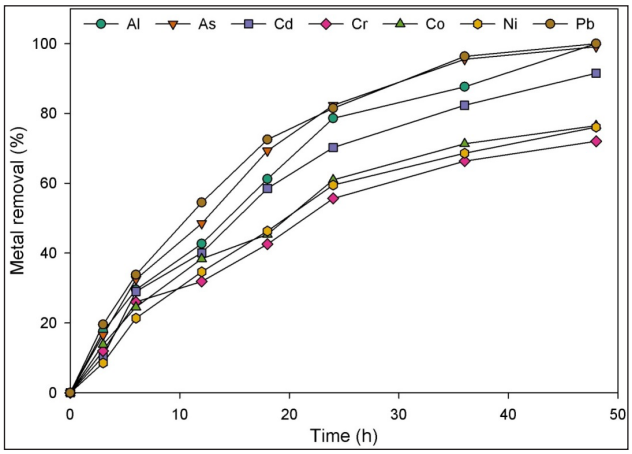

Fig. 5: Removal of heavy metals from simulated effluent by activated PVA@PC within 48 h

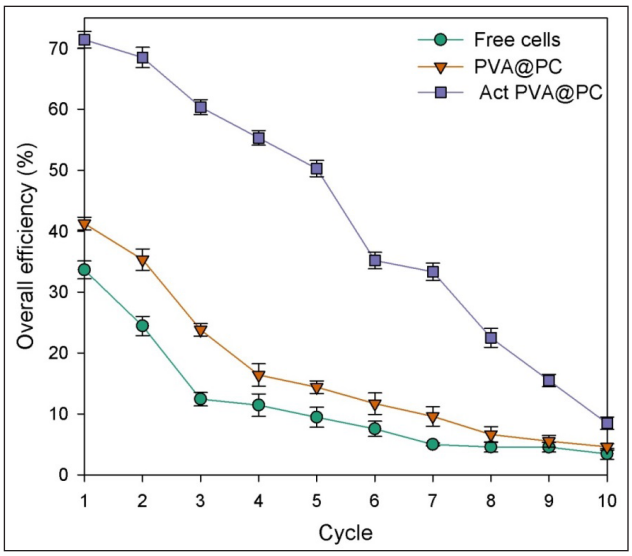

Fig. 6: Reusability efficiency of free cells of *P. chrysosporium*, PVA@PC and activated PVA@PC for the removal of heavy metals, dyes and phenolics

Light microscopy analysis

In control samples (fungal without hazardous contaminants), long, broad (~ 5-8 mm) and smooth-walled *P. chrysosporium* hyphae were clearly observed in Fig. 7a. Similarly, rough-surfaced hyphae (~ 4-9 mm) with precipitated metals were observed in mix metal set, suggesting the fungi had ability to secrete certain organic acids (specially, oxalic acid, malic acid) which could precipitate the metals extracellularly (Fomina *et al.* 2005; Rudakiya and Gupte 2017). Some precipitates were clearly observed inside the hyphae, suggesting the fungi had the ability to metabolize the metals in certain amounts (Fig. 7b). In mix dye set, it was clearly

seen that all dyes were observed in the center of the fungal hyphae, showing the fungal hyphae had the ability to degrade these contaminants. Similarly, Gu *et al*. (2017) observed in light microscopy that *P. chrysosporium* mycelia were smooth when it was in the soil with phenanthrene.

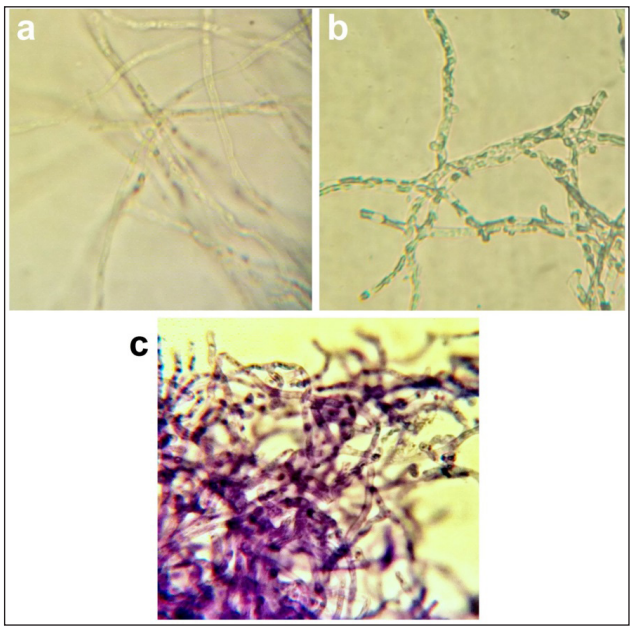

Fig. 7: Light microscopy analysis of hyphae of control cells (without hazardous contaminants) (a), mixed metal absorbed hyphae of *P. chrysosporium* (b) and simulated effluent absorbed hyphae of *P. chrysosporium* (c)

Toxicity analysis

Bacteria are fundamental components of ecological balance so it is frequently used for toxicological studies (Baek *et al.* 2017; Rudakiya and Pawar 2017). Antibacterial activity of simulated effluent and degraded samples suggested that higher toxicity was observed in simulated effluent in Gram –ve, Gram +ve and agricultural important bacteria (Table 2). Fungal treated samples showed comparative less toxicity compared to control samples while PVA@PC samples showed less toxicity compared to free cells (Table 2). The zone of inhibition of *E. coli* was higher in case of simulated effluent (24.6 mm), moderate in case of free cells sample (19.2 mm), PVA@PC (18.9 mm) and very less inactivated PVA@PC (12.5 mm). Similarly, Ozturk and Abdullah (2006) reported azo and indole dye toxicity studies against pathogenic and non-pathogenic strains and suggested that most of the azo dyes and indole dyes were toxic on majority of micro-organisms (Öztürk and Abdullah 2006).

Table 2: Antibacterial activities of hazardous contaminant and degraded products against soil and important agricultural microorganisms

Bacteria	Zone of inhibition			
	Simulated effluent	Degraded products		
		Free cells	PVA@PC	Activated PVA@PC
Gram negative bacteria				
E. coli	24.6	19.2	18.9	12.5
P. vulgaris	15.4	13.1	12.8	10.4
S. typhi	19.8	16.4	15.6	12.1
Gram positive bacteria				
B. subtilis	22.4	18.9	16.8	10.5
B. cereus	20.5	15.5	14.0	13.3
B. megaterium	21.6	19.2	15.3	11.0
Agricultural important bacteria				
Azotobacter sp.	19.5	16.9	15.4	15.1
R. radiobacter	19.0	17.8	17.7	14.6

CONCLUSION

Based on the experimental results, activated PVA@PC has shown great potential for the removal of toxic dyes, heavy metals and phenolic compounds. Activated PVA@PC depicted higher efficacy in individual batch as well as in simulated effluent experiments. Among dyes, activated PVA@PC has shown higher degradation potential for reactive dyes than acidic dyes. Activated PVA@PC was efficient to remove metals like Pb, Al, As and Cd than other metals. Repeated use of activated PVA@PC was also efficient for 6th cycle so it can be used for continuous cycle experiments.

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