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Remediation of silver nitrate activated spent adsorbent prepared from cassava peel by *Trichoderma reesei*

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ABSTRACT

Remediation is a feasible method for degrading dyehouse effluents. Carbon prepared from cassava (*Maniot esculenta* Crantz) peel, activated with heat, acid, alkali and silver nitrate, was utilized for removal of dyes. Silver nitrate activated adsorbent was proved to be the potential candidate in removing dyes. Hence, the present study aims at degradation of silver nitrate activated spent adsorbent (Adsorbent left after removal of dyes) with 11 fungal species such as *Mucor racemosus* MTCC 7382, *Rhizopus stolonifer var stolonifer* MTCC 7370, *Fusarium moniliforme* MTCC 2015, *Fusarium oxysporum* MTCC 284, *Trichoderma viride* MTCC 2417, *Aspergillus terreus var terreus* MTCC 3006, *Aspergillus ochraceus* MTCC 1810, *Penicillium citrinum* MTCC 8009, *Saccharomyces cerevisiae* MTCC 2376, *Kluyveromyces maxianus* MTCC 4059 and *Trichoderma reesei* MTCC 3193. Out of 11 organisms selected for screening, *Trichoderma reesei* MTCC 3193 showed significant decourization. Further parameter screening by Plackett-Burman design (PBD) and process optimization by Box-Behnken design (BBD) showed maximum spent adsorbent degradation of 96.78% at initial concentration of 0.2 mg/mL, contact time of 2 days and inoculum size of 5.38% (w/v).

Keywords: Degradation, Dyes, Effluent, Fungal

INTRODUCTION

Many dyes are visible in water at concentrations as low as 1 ppm. Dyehouse effluents typically with dye content in the range 10-200 ppm are therefore usually highly coloured and discharge in open waters presents an aesthetic problem. As dyes are designed to be chemically and photolytically stable, they are highly persistent in natural environments. The release of dyes may therefore present an ecotoxic hazard and introduces the potential danger of bioaccumulation that may eventually affect man by transport through the food chain.

Adsorption is one of the best methods for treatment of dyehouse effluents. Carbon prepared from cassava (*Maniot esculenta Crantz.*) peel was activated with heat, acid, alkali and silver nitrate for effective removal of dyes from effluents. Silver nitrate activated adsorbent was proved to be the potential candidate in removing dyes (Parvathi et al., 2013; Parvathi et al., 2011). Hence, the present study aims at mycoremediation of silver nitrate activated spent adsorbent, adsorbent left after removal of dyes.

Fungi are microscopic, eukaryotic organisms exhibiting growth on various substrates and are capable of continuing their function almost indefinitely. These organisms, including the molds, yeasts, and filamentous fungi are a diverse group of organisms which are ubiquitous in nature, having their own specific adaptations to survive in their particular ecosystem. Their contribution ranges from natural to industrial use. They contribute to maintaining the ecosystem through their complex mycelial network which aids in exchanging nutrients.

Singh et al (2013) was attempted to examine the potential of aerobic fungal culture for decolourization of brown GR textile dye. Tripathi and Srivastava (2011) studied the potential of different bacterial strains for decolorization of acid orange 10 (azo dye) in batch reactors. They have characterized and identified dye degrading efficiency of *Pseudomonas putida* MTCC 102. Shah et al. (2013) studied a decolorizing bacterial strain, *Bacillus* spp. ETL 1982, was isolated from activated sludge. *Bacillus* spp. ETL 1982 showed decolorizing activity through a degradation mechanism rather than adsorption, and it could tolerate high concentrations (up to 1000 ppm) of reactive red.

MATERIALS AND METHODS

Culture collection: Eleven fungal species, *Mucor racemosus* MTCC 7382, *Rhizopus stolonifer var stolonifer* MTCC 7370, *Fusarium moniliforme* MTCC 2015, *Fusarium oxysporum* MTCC 284, *Trichoderma viride* MTCC 2417, *Aspergillus terreus var terreus* MTCC 3006, *Aspergillus ochraceus* MTCC 1810, *Penicillium citrinum* MTCC 8009, *Saccharomyces cerevisiae* MTCC 2376, *Kluyveromyces maxianus* MTCC 4059 and *Trichoderma reesei* MTCC 3193. Out of 11 organisms selected for screening, *Trichoderma reesei* MTCC 3193 were procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. These cultures were preserved under specified growth media as prescribed by IMTECH.

Preservation of pure cultures: Growth medium for the fungal species was prepared and sterilized at 121°C for 15 minutes. 2 mL of sterile media was taken to serve as blank. 2% w/v (on wet basis) of mycelia was inoculated into 100

mL of the medium and the flask was swirled for even suspension of mycelia in the medium. An initial absorbance of this broth (0^{th} h) was measured at 600 nm using visible spectrophotometer (Systronics 106, India). The culture was incubated at room temperature and 100 rpm. Every 3 hours, 2 mL of this culture was aseptically transferred to a cuvette and absorbance was noted. The growth was spectrophotometrically recorded and the growth curve was plotted till the fungal growth reached its stationary phase.

Batch fungal remediation studies: The growth medium was inoculated with a loop full of fungal culture under sterile conditions. The inoculated medium was incubated at optimal temperature with continuous shaking for 3-5 days, depends on fungal culture. Spent adsorbent solution was prepared by adding known quantity of spent adsorbent in water and the pH was adjusted. The spent adsorbent solution was inoculated with 5 to 10% (w/v) of mid log phase culture under sterile conditions and incubated at suitable growth conditions of temperature and agitation speed. The sample was collected at regular intervals of time for the determination of biomass growth. The collected sample was centrifuged at 10,000 rpm for 5 min. The cell free supernatant was characterized for dye concentration in spent adsorbent solution using water as blank at suitable absorption maxima of the dye spectrophotometrically.

Optimization studies on mycoremediation of synthetic dyes by design of experiments: Optimization of process parameters by classical method of changing one variable while fixing the others at constant level is laborious and time consuming, especially when the number of variables is more than 6. An alternative and more efficient approach to this method is design of experiments. There are two types of experimental design: screening and optimal designs. Screening designs are used to investigate significant factors. Optimal designs are used to determine the optimal settings of the significant factors.

Plackett-Burman Design: For mycoremediation of dyes, the factors influenced were dye concentration in spent adsorbent solution, contact time, incubation temperature, pH of solution, inoculum size and agitation speed of shaker. When more than five independent variables are to be investigated, the Plackett-Burman design may be used to find the most significant variables in a system which is then optimized in further studies. This method allows for the evaluation of X-1 variables by X experiments where X must be a multiple of 4 starting from 8. PBD was selected to screen the six variables for dye degradation. But PBD requires a minimum of 8 experiments and 7 variables. So, 1 dummy factor was added to the design.

Table.1.Actual levels of variables tested with Plackett-Burman design

	VARIABLES	L	H
A	Dye concentration (ppm)	20	120
B	Time(days)	2	4
C	Temperature(°C)	27	37
D	pH	4	9
E	Inoculum size (%)	5	15
F	Agitation speed (rpm)	0	100
G	Commercial activated carbon (mg)	0	0

Eight run PBD with 7 factors were tested for their effects on dye degradation. Low and high levels were assigned for each variable (Table 1). The experimental design for myco-remediation of dyes is shown in Table 2. The percentage (%) dye removal was used as the response in this design. The significance of variables was determined by calculating their effects on dye removal. The dye removal percentage for each experiment was calculated using the formula,

$$\text{Percentage dye removal} = \frac{(C_i - C_o)\, x\, 100}{C_i} \quad (1)$$

where C_i and C_o are the initial and final dye concentrations.

Table 2. PBD matrix for 7 factors and 8 experiments

Trial	Variables						
	A	B	C	D	E	F	G
1	H	H	L	H	L	L	H
2	H	H	H	L	H	L	L
3	L	H	H	H	L	H	L
4	L	L	H	H	H	L	H
5	H	L	L	H	H	H	L
6	L	H	L	L	H	H	H
7	H	L	H	L	L	H	H
8	L	L	L	L	L	L	L

The effect of each variable on the dye removal percentage were calculated using the formula,

$$\text{Effect of each variable} = (\sum H - \sum L)/(N/2) \quad (2)$$

where N is the number of experiments. The variables were ranked based on the effect values i.e., variable with highest effect secure first rank and so on. The most significant variables influencing the experiment were identified by the rank.

Box-Behnken Design: The significant variables screened by PBD were optimized by Box-Behnken Design (BBD). The number of experiments for k factors in BBD based on 3 level is given by 2k(k-1)+c where c is number of centre points. According to BBD table, 15 experiments were performed for 3 factors with 3 central points. A coefficient of the quadratic model was calculated using the following equation,

$$Y = b_0 + \sum_{i=1}^{k} b_i X_i + \sum_{i=1}^{k} b_{ij} {X_i}^2 + \sum_{i_{i<j}}^{k} \sum_{j}^{k} b_{ij} X_i X_j \quad (3)$$

where Y is the predicted response and i,j are linear, quadratic coefficients respectively, b and k are regression coefficients and the number of factors studied in the experiment respectively.

The significance of each coefficient was determined and the results were analysed by trial version of design expert. Three dimensional surface plots were obtained to study the interaction effect between variables. The optimum values were obtained based on the humps in 3-D plots.

RESULTS

Plackett-Burman Design: Based on literature, 11 fungal species were selected and their effect on spent adsorbent solution degradation was screened (Table 3). For mycoremediation of dyes, the factors influenced were dye concentration in spent adsorbent solution, contact time, incubation temperature, pH of solution, inoculum size and agitation speed.

Table.3.Screening experimental results for dye degradation

FUNGI	$AgNO_3$ activated spent adorbent
Penicillium citrinum	-
Aspergillus terreus	-
Aspergillus ochraceus	-
Fusarium oxysporum	-
Saccharomyces cerevisciae	-
Trichoderma reesei	+
Trichoderma viride	-
Kluyveromyces marxianus	-
Rhizopus stolonifer	-
Mucor racemosus	-
Fusarium moniliforme	-

[+: Significant decolourisation and -: No significant decolourisation]

Eight experiments were conducted and the percentage degradation for each trial was calculated (Table 4). Using the percentage removal the effect of each variable on dye decolourisation was calculated. Concentration of dye in spent adsorbent solution, time and inoculum size showed statistical significance after ANOVA for PBD is performed. These significant variables were selected for further optimization using BBD.

Table.4.Plackett-Burman experimental Design

Trial	Variables							Dye removal (%)
	A	B	C	D	E	F	G	
1	H	H	L	H	L	L	H	94.32
2	H	H	H	L	H	L	L	55.28
3	L	H	H	H	L	H	L	19.53
4	L	L	H	H	H	L	H	28.23
5	H	L	L	H	H	H	L	69.84
6	L	H	L	L	H	H	H	29.76
7	H	L	H	L	L	H	H	38.15
8	L	L	L	L	L	L	L	50.19

Box-Behnken Design: To optimize the dye degradation experiments, Box-Behnken design was chosen for response surface optimization with a 3-level-3-factor. Tables 5 and 6 list the experimental factor settings and results on the basis of the experimental design. All the 15 designed experiments were conducted and the results were analysed by regression. This showed 3 linear coefficients (A, B, C) and 3 quadratic coefficients (A^2, B^2, C^2) and 3 cross product coefficients(AB, BC, AC) were significant (Table 7).

Table.5.Process variables and level used for Box-Behnken Design

Variable	Symbol	Unit	Levels		
			-1	0	1
Dye concentration	A	Ppm	20	70	120
Time	B	d	2	3	4
Inoculum Size	C	% (w/v)	5	10	15

The coefficients of this model is given in Equation (4) were also evaluated. A *p-value* showed that all of the linear coefficients were more significant than their quadratic and cross product terms. However to minimize the error, all of the coefficients were considered in the design. According to ANOVA analysis, non-significant lack of fit was observed. This indicates that the model does indeed represent the actual relationships between parameters, which are well within the selected ranges (Table 7).

Table .6. Box-Behnken experimental design

Trial	Dye concentration (ppm)	Time (d)	Inoculum size (% w/v)	Dye removal (%)
1	120	2	10	76.66
2	20	2	10	84.15
3	120	4	10	61.66
4	20	4	10	76.66
5	120	3	5	43.55
6	20	3	5	51.49
7	120	3	15	3.25
8	20	3	15	25.47
9	70	2	5	96.25
10	70	4	5	80.19
11	70	2	15	75.625
12	70	4	15	51.25
13	70	3	10	6.25
14	70	3	10	5.08
15	70	3	10	15.62

The final estimative response model equation (based on the actual value) is

$$y=7.62+4.94*A-7.970*B-14.84*C+5.46*A*B+4.33*A*C-2.03*B*C+10.52*A^2+56.47*B^2 +11.69*C^2 \quad (4)$$

where y is the dye removal, A,B,C are the actual values of dye concentration (mg/mL), time(d) and inoculum size (% w/v) respectively. The model coefficients and probability values are shown in Table 7. The model proved suitable for adequate representation of the real relationship among the selected factors (Daneshvar et al., 2007)

Fig. 1(a) represents the effect of dye concentration and time at a constant inoculum size on dye degradation. At any designed dye concentration from 20 to 120 ppm, the dye removal percentage slightly decreased and then increased rapidly. Similarly the dye removal decreased and then increased when time varied from 2 to 4 days. This show that time is more significant than dye concentration at constant inoculum size (Yogita et al., 2011).

Fig. 1(b) represents the effect of dye concentration and inoculum size at a constant time. It is evident here that dye removal decreases initially and then increases with the increase in inoculum size from 5 to 15 % (w/v). When dye concentration increases from 20 to 120 ppm the dye removal also increases slightly (Nawahwi et al., 2013). This shows that inoculum size is more significant than dye concentration at constant time.

Fig. 1(c) represents the effect of time and inoculum size at a constant dye concentration. As inoculum size increased from 5 to 15% (w/v), dye removal reduced and then increased rapidly. Similarly an increase in time from 2 to 4 days has the same effect on dye removal. Comparatively inoculum size is more significant than time at constant dye concentration (Selvam, 2012; Singh et al., 2013; Tekere et al., 2011). From these figures, the optimal conditions for dye degradation were found. The optimal values were obtained by solving the regression equation using Design Expert 7 trial software.

Table.7.Model coefficients estimated by multiple linear regression and analysis of variance

Source	Sum of squares	df	Mean square	F-value	p-value
Model	14769.77	9	1641.085	26.273	0.0011
A	195.228	1	195.228	3.125	0.137
B	508.406	1	508.406	8.139	0.035
C	1761.36	1	1761.359	28.198	0.003
AB	119.137	1	119.137	1.907	0.225
AC	75.082	1	75.082	1.202	0.322
BC	16.503	1	16.503	0.264	0.629
A^2	408.774	1	408.774	6.544	0.050
B^2	11774.52	1	11774.516	188.505	< 0.0001
C^2	504.198	1	504.198	8.072	0.036
Residual	312.312	5	62.462		
Lack of Fit	171.281	3	57.093	0.809	0.593
Pure Error	141.031	2	70.515		

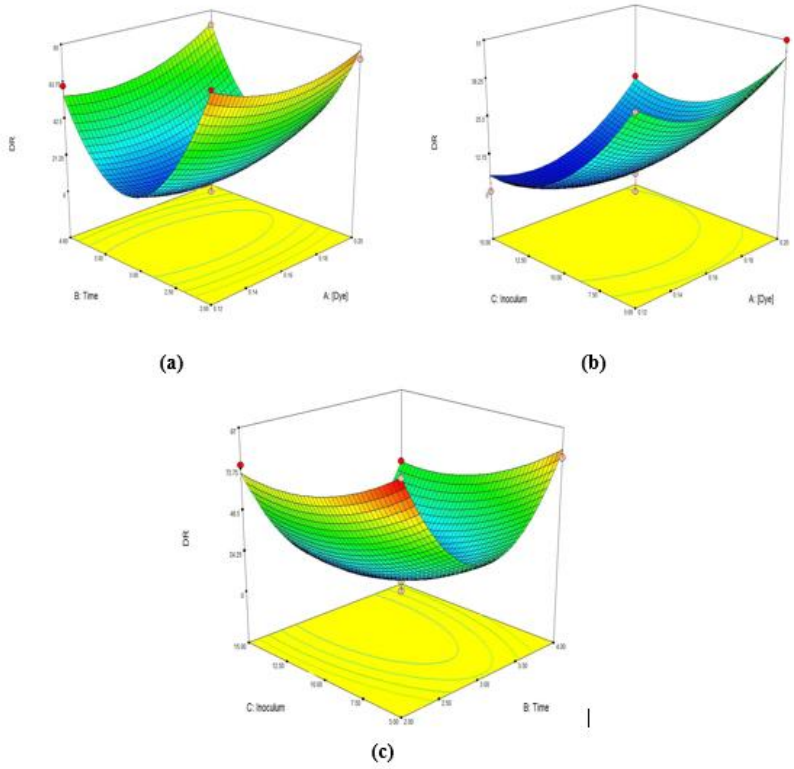

Fig.1. Surface plots showing the effect of dye concentration in spent adsorbent solution, inoculums size and time on dye removal

CONCLUSION

Carbon prepared from cassava (*Maniot esculenta Crantz.*) peel, activated with heat, acid, alkali and silver nitrate, was utilized for removal of dyes. Silver nitrate activated adsorbent was proved to be the potential candidate in removing dyes. Hence, the present study aims at degradation of silver nitrate activated spent adsorbent (Adsorbent left after removal of dyes) with 11 fungal species such as *Mucor racemosus* MTCC 7382, *Rhizopus stolonifer var stolonifer* MTCC 7370, *Fusarium moniliforme* MTCC 2015, *Fusarium oxysporum* MTCC 284, *Trichoderma viride* MTCC 2417, *Aspergillus terreus var terreus* MTCC 3006, *Aspergillus ochraceus* MTCC 1810, *Penicillium citrinum* MTCC 8009, *Saccharomyces cerevisiae* MTCC 2376, *Kluyveromyces maxianus* MTCC 4059 and *Trichoderma reesei* MTCC 3193. Out of 11 organisms selected for screening, *Trichoderma reesei* MTCC 3193 showed significant decourization. Further parameter screening by Plackett-Burman design (PBD) and process optimization by Box-Behnken design (BBD)

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