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REMOVAL OF 2,4-DICHLOROPHENOL BY SIMULTANEOUS ADSORPTION AND BIODEGRADATION (SAB) USING LOW COST ADSORBENT

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ABSTRACT

This study was aimed to investigate the use of Mustard stalk as a cheap, eco-friendly adsorbent with support matrix for the immobilization of microbial cell and for subsequent removal of 2,4-dichlrophenol(2,4-DCP) from waste water. A comparative batch study between adsorption as well as simultaneous adsorption and biodegradation (SAB) of 2,4-DCP by mustard stalk immobilized *Pseudomonas putida* MTCC1194 have been studied in conical flask having concentration ranges of 2,4-DCP from 100 to 1000 mg l^{-1} with adsorbent dose range 1 to 12 g l^{-1} at pH range 2 to 9 and temperature range 28°C to 35°C, placed in an orbital shaker. The results of the batch studies showed that simultaneous adsorption and biodegradation (SAB) shows the maximum percent (91%) removal of 2,4-DCP as compared to simple adsorption (86 %) at optimum temperature 32 °C of adsorbent dose 10 g l^{-1}, and pH 6 with MSAC having particle size 0.24 mm. The equilibrium data for 2,4-DCP degradation sorbent systems were well fitted with Langmuir isotherm.

Keywords: 2,4-dichlorophenol, SAB, AC, *Pseudomonas putida,* Adsorption, Mustard stalk.

1. Introduction

A variety of chemicals are commercially produced and newly synthesized every year. During their manufacture and use, these chemicals are often discharged into environment. Many of them degrade slowly and exert toxic effect on plants and animals, thus causing large scale environmental degradation (Annadurai *et al.,* 2007). Chlororinated phenolic compounds present in industrial waste water such as polymeric resins, petroleum refining, and pesticides are difficult to remove, highly toxic and difficult to degrade biologically. The chronic toxic effects of chlororinated phenolic compounds reported in humans including vomiting, difficulty in swallowing, anorexia, liver and kidney damage, headache, fainting and other mental disturbance. The Ministry of Environment and Forest (MOEF), Government of India and EPA, USA, have listed phenolic and phenolic compounds on priority-pollutants lists. The MOEF has set a maximum concentration level of 1.0 mg l^{-1} of phenolic compound in the industrial effluents for the safe discharge into the surface water, the WHO recommends the permissible phenolic concentration of 0.001 mg l^{-1} in potable water. Thus because of toxicity of chlororinated phenolic compounds many treatment processes have been applied for the removal of them from waste water. Some of these processes include: adsorption (Zhao *et al.,* 2010), photo-fenten degradation (Parida and Prahan, 2010), photocatalytic degradation (Devipriya and Yesodharan, 2010) and biodegradation (Agarry and Solomon,

2008). Combined methods like biochemical, electrochemical, physicochemical or simultaneous adsorption and biodegradation is nowadays gaining importance. Simultaneous adsorption and biodegradation increases the life of adsorbent, as biofilm on adsorbent degrade the adsorbate (Mondal and Balomajumder, 2007; Wang *et al.,* 2000).

Agricultural by-products have proved to be promising raw materials for the production of ACs They can be used for the production of AC with a high adsorption capacity, considerable mechanical strength, and low ash content and their availability at a low price. (Mahadevaswamy *et al.,*1997 and Chen *et al.,* 2011). Some of the agricultural waste products that has been developed as adsorbents include, orange and banana peels (Annadurai *et al.,* 2002), spent tea leaves (Hameed, 2009), tamarind fruit shell (Popuri *et al.,* 2007), soya bean hull (Ahmedna *et al.,* 2000), cotton seed hull and corn cobs (Reddad *et al.,* 2002), rubber fruit pericarp (Agarry and Owabor*,* 2005).

In this research paper, a detailed study on sorption potential of an abundantly available agricultural by-product Mustard stalk as non-conventional adsorbent. Mustard stalk activated carbon used as support matrix for *Pseudomonas putida* MTCC 1194 immobilization to remove 2,4-dichlorophenol from waste water. The effects of adsorbent dose, contact time, initial phenol concentration, pH and temperature on removal of 2,4-dichlorophenol by simultaneous adsorption-biodegradation(SAB) were studied.

2. Materials and methods

All the reagents and 2,4-dichlorophenol were procured from by Hi-Media Company make. An accurately weighed quantity of the 2,4-dichlorophenol was dissolved in double-distilled water to prepare a stock solution (1000 mg l^{-1}). The desired concentration range 100–1000 mg l^{-1} were obtained by successive dilutions with double-distilled water. *Pseudomonas putida* MTCC 1194 was obtained from Institute of Microbial Technology, Chandigarh, India. The composition of the basal salt medium (BSM) used in this experiment as the growth medium contained 1.5 g l^{-1} K_2HPO_4, 0.5 g l^{-1} KH_2PO_4, 0.5 g l^{-1} $(NH_4)_3PO_4$, 0.5 g l^{-1} NaCl, 3 g l^{-1} Na_2SO_4, 2 g l^{-1} Yeast extract, 0.5 g l^{-1} Glucose, 0.002 g l^{-1} $FeSO_4$ and 0.002 g l^{-1} $CaCl_2$. Activated carbon prepared from chemically treated mustard stalk, the procedure mention by Madhava *et al.* 2006, having surface area 129 $m^2 g^{-1}$ was used as adsorbent and solid support matrix for microbial cell immobilization.

2.2 Acclimatization

The acclimatization of *Pseudomonas putida* (MTCC1194) in phenolic environment was performed as follows: The revived culture were first grown in basal salt medium with glucose as sole carbon source. The culture were acclimatized to phenol by exposing the cultures in a series of shake flasks (250 ml), After 48 hr, significant bacterial growth was observed in the flask and the turned milky. Appropriate quantity of stock solution of 2,4-DCP was added into the flask containing BSM to get a concentration of 10 mg l^{-1} of 2,4-DCP It was kept aside, initially growth of *Pseudomonas putida* was inhibited and degradation of 2,4-DCP started after 5 hr. Thereafter, the 2,4-DCP was periodically added in increments of 10 mg l^{-1} in a series of 250 ml flasks till the 2,4-DCP concentration in the growth media reached 1000 mg l^{-1}. For inoculums, a further sub culturing was done and all the inoculums transfers were done in exponential phase.

3. Experimental procedure

For Adsorption studies concentration ranges of 2,4-DCP from 100 to 1000 mg l^{-1} were kept in the conical flasks along with adsorbent dose range 1 to 12 g l^{-1} at pH range 2 to 9 having temperature range 25 °C to 38 °C and kept in the orbital shaker at 180 rpm. The initial pH of the solution was adjusted by addition of dilute aqueous solutions of HCl or NaOH (0.1 M). The samples were collected at definite intervals, filtered and analyzed spectophometerically for remaining concentration. For SAB studies 2,4-DCP was performed in 500 ml cotton-plugged flasks containing 150 ml of BSM with 20 ml inoculum of acclimatized *Pseudomonas putida* with 2,4-DCP, having biomass concentration 32.50 mg l^{-1}. Aliquots of

40 ml from stock solutions of 2,4-DCP and adsorbent dose (1–12 g l^{-1}) was added to the reaction mixture and agitated in orbital shaker at constant speed of 180 rpm. Initial concentration of 2,4-DCP was varied between 100 to 1000 mg l^{-1}. The pH and temperature ranges were from 2 to 9 and 25 °C to 38 °C, respectively. Samples were collected at definite intervals of time. All the collected samples were centrifuged at 10,000 rpm for 15 min. The supernatant was separated and analyzed spectrophotometrically at suitable wavelength. The percentage removal of adsorbate was calculated using the following relationship eq.(1),

$$\text{Removal (\%)} = \frac{C_o - C_t}{C_o} \times 100 \quad (1)$$

where C_o (mg l^{-1}) is the initial adsorbate concentration and C_t is a concentration at time t. The isotherms were determined by analyzing adsorptive uptake of the 2,4-DCP from aqueous solution at distinct time intervals.

4. Results and discussion

4.1 Adsorbent dose

The percentage removal of 2,4 -DCP with adsorbent dose range 1 to 12 g l^{-1} for adsorption and SAB is shown in fig 1. The removal of 2,4-DCP was 86 and 91% for adsorption and SAB respectively as mentioned in Table 1.

Table 1. Effect of adsorbent dose on 2,4-DCP removal onto MSAC

	Adsorption		SAB	
Adsorbent dose (g)	**%Removal**	**Amount adsorbed/g of adsorbent (mg g^{-1})**	**%Removal**	**Amount adsorbed/g of adsorbent (mg g^{-1})**
1	52	208	65	260
5	65	52	75	60
7	72	42.2	88	50.8
10	86	34.4	91	34
12	86.2	32.4	91.5	30.7

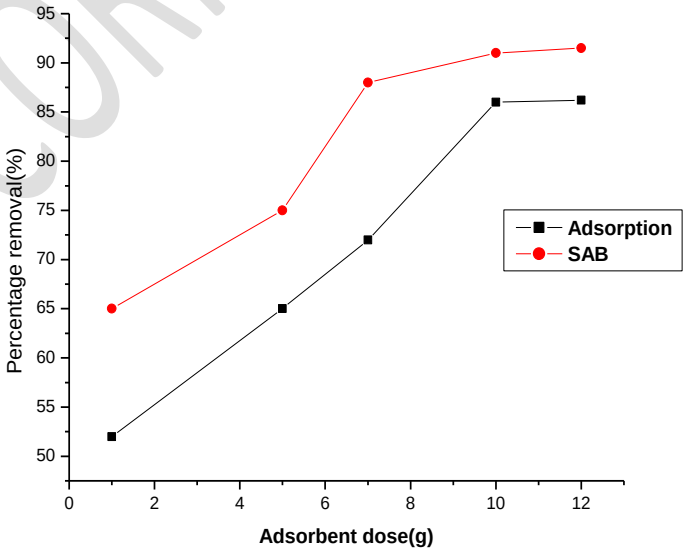

Figure1. Effect of adsorbent dose on removal of 2,4-DCP onto MSAC (At initial conc. 400 mg l^{-1} and pH 7)

From fig.1 it is evident that the percent removal of 2,4-dicholrophenol reaches its constant value at the dose of around 10 g l^{-1}. In case of SAB the difference between the percent removal of SAB and adsorption is lower at the starting of the experiment (up to around one hour) but gradually increases and then remains constant. From the above observation it seems that for SAB, the adsorption dominates at the initial stage and bio-sorption dominates after reaching adsorption equilibrium. This agrees the mechanism of bio-filtration (Chaudhury *et al.,* 2003) where bio-layer is formed followed by degradation of compounds.

4.2 Effect of pH

In this experiment the percent removal for SAB was maximum at pH 6. Figures 2 and 3 show percentage removal of 2,4-DCP at different pH by adsorption and SAB, respectively.

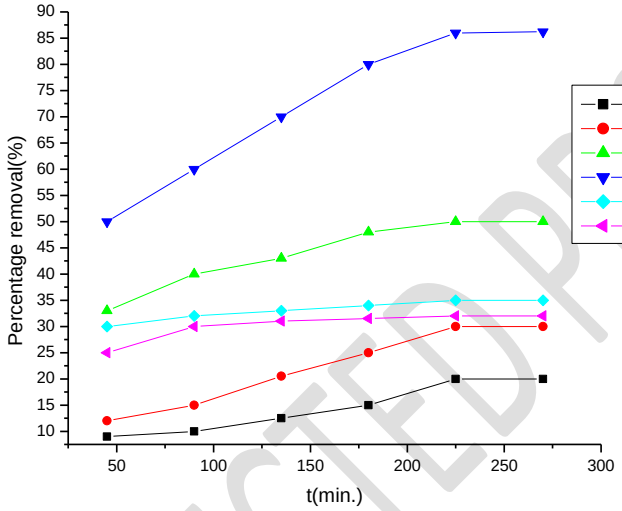

Figure 2. Effect of pH on removal of 2,4-DCP onto MSAC (Adsorption study)
(At initial conc. 400 mg l^{-1} and adsorbent dose 10 g l^{-1})

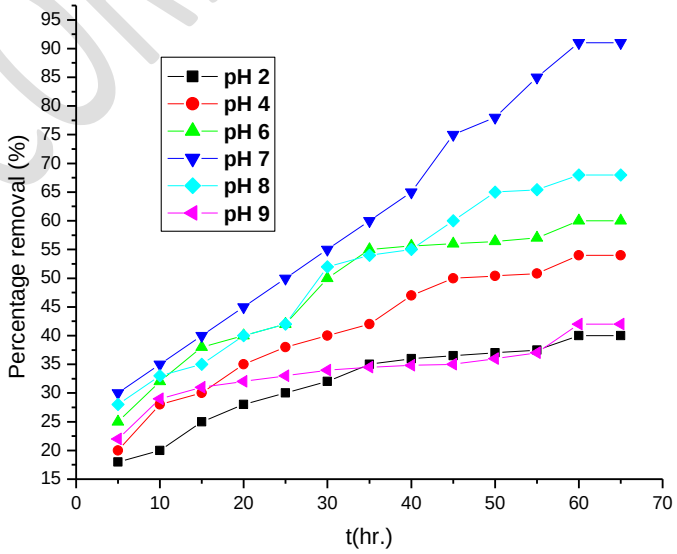

Figure 3. Effect of pH on removal of 2,4-DCP onto MSAC (SAB study)
(At initial conc. 400 mg l^{-1} and adsorbent dose 10 g l^{-1})

The amount adsorbed of 2,4-DCP by MSAC varies pH 2 to 9 as shown in Table 2. This observation agrees the fact that at near to neutral pH the specified microbes achieve maximum efficiency. The pH range 2 to 9 was chosen to study the effect of pH on the percent removal for SAB as well as adsorption studies. As the pH increased, overall surface charge on the cells became negative and sorption decreased.

Table2. Effect of pH on 2,4-DCP removal onto MSAC

	Adsorption		SAB	
pH	**%Removal**	**Amount adsorbed/ g of adsorbent(mg g^{-1})**	**%Removal**	**Amount adsorbed/ g of adsorbent (mg g^{-1})**
2	20	8	40	16
4	30	12	54	21.6
6	50	20	60	24
7	86	34.4	91	34
8	35	14	68	27.2
9	32	12.8	42	16.8

Whereas the reduction in adsorption capacity are noticed at pH 9. The amount of phenol adsorption are high at pH 6 on the other hand, the steep reduction in adsorption uptake capacity are found beyond pH 6, due to ionization of adsorbate molecules and the electrostatic repulsion between the surface charge of MSAC and phenolic anions (Hameed, 2009).

4.3 Effect of contact time

For study of effect of contact time, 2,4-DCP concentration range 100 -1000 mg l^{-1} at optimum pH 6 and dose 10 g l^{-1} were taken for 65 hr for SAB study where as for adsorption study 270 min. The plot of 2,4-DCP concentration vs. time for adsorbent were shown in fig. 4 and 5. The fig.4 shows that removal rate of 2,4-DCP is found to be very rapid during the initial period of 30 min. and then slows down. No significant change in the elimination of 2,4-DCP is observed after equilibrium time (Table 3). It can be seen from fig. 5 that the percent removal of SAB is not as much in the initial stage of experiment but it gets a rise after certain hrs and reached its optimum value at a particular point of time. These findings correlate with the study of Chaudhury *et al.*, (2003) where a bio-layer has been formed due to the degradation of compounds and termed as the bio-filtration mechanism.

Table 3. Effect of contact time on removal of 2,4-DCP onto MSAC

Adsorption			SAB		
Contact time(min.)	**%Removal**	**Amount adsorbed/ g of adsorbent (mg g^{-1})**	**Contact time(hr.)**	**%Removal**	**Amount adsorbed/ g of adsorbent (mg g^{-1})**
45	50	20	5	30	12
90	55	23.2	10	35	14
135	60	24	15	40	16
180	75	30	20	42	16.8
225	86	34.4	25	45	18
270	86	34.4	30	55	22
			35	58	23.2
			40	60	24
			45	70	28
			50	73	29.2
			55	75	30.4
			60	91	35
			65	91	35

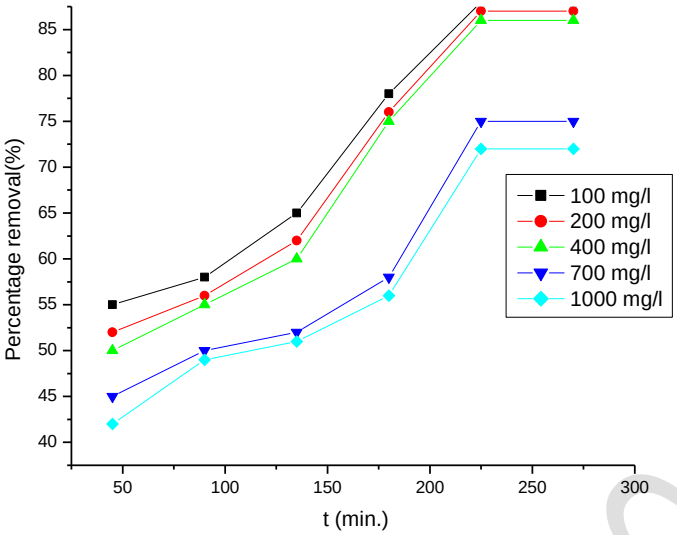

Figure 4. Effect of contact time on removal of 2,4-DCP onto MSAC (Adsorption study) (At adsorbent dose 10 g l^{-1} and pH 7)

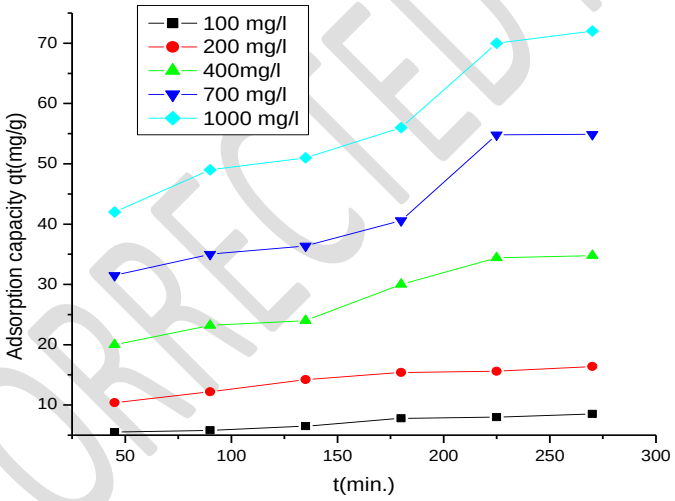

Figure 5. Effect of contact time on removal of 2,4-DCP onto MSAC (SAB study) (At adsorbent dose 10 g l^{-1} and pH 7)

4.4 Effect of initial 2,4-DCP concentration

From fig. 6 and 7 it could be seen that the amount of 2,4-DCP adsorbed per unit mass of adsorbent increased with increase in initial concentration until equilibrium was reached at about definite time.

Table 4 show that percent removal of 2,4-DCP decreased with the increase in initial concentration.

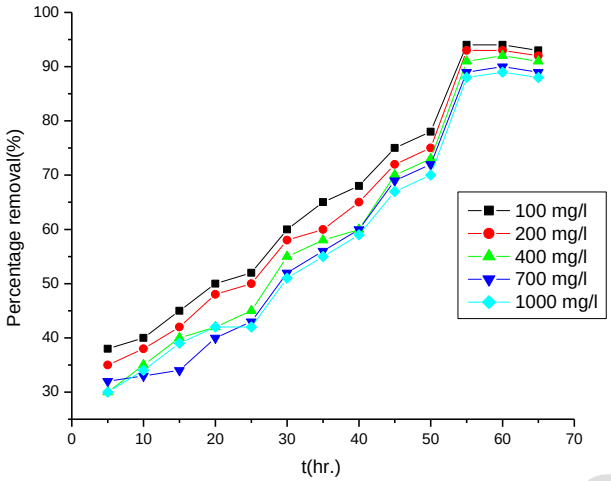

Figure 6. Effect of initial concentration on removal of 2,4-DCP onto MSAC (Adsorption study) (At adsorbent dose 10 g l^{-1} and pH 7)

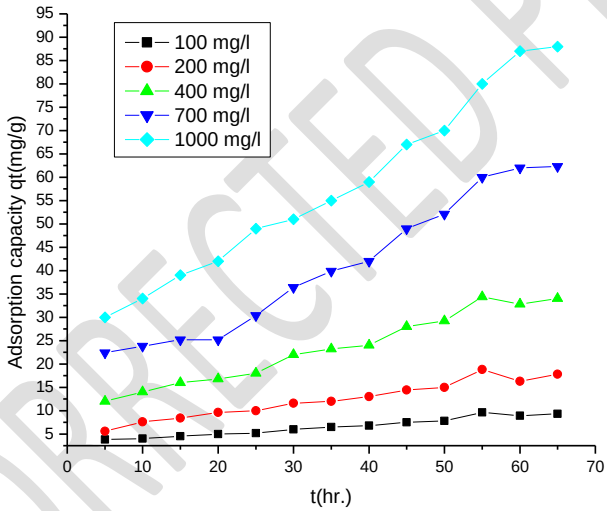

Figure 7. Effect of initial concentration on removal of 2,4-DCP onto MSAC(SAB study) (At adsorbent dose 10 g l^{-1} and pH 7)

Table 4. Effect of initial concentration on removal of 2,4-DCP onto MSAC

Adsorption			SAB	
Initial concentration of 2,4-DCP (mg l^{-1})	**%Removal**	**Amount adsorbed/ g of adsorbent (mg g^{-1})**	**%Removal**	**Amount adsorbed/ g of adsorbent (mg g^{-1})**
100	85	8.5	93	9.3
200	82	16.4	92	17.8
400	86	34.4	91	34
700	75	54.9	89	62.3
1000	72	72	88	88

4.5 Effect of temperature

The fig. 8 and 9 show that with the increase in temperature removal of 2,4-DCP was increased. Table 5 shows maximum removal of 2,4-DCP at 32 °C temperature, after that only a slight difference are seen for percentage removal. This is basically due to the fact that the diffusion process is an endothermic

process (Weber, 1972). With an increase in temperature, the mobility of the phenolate ions increases and retarding forces acting on the diffusing ions decrease, thereby increasing the sorptive capacity of adsorbent. The increase in 2,4-DCP sorption capacity of the carbonaceous adsorbent with the increase in temperature has also been reported by other investigators (Banat *et al.,* 2004, Vijayalakshmi *et al.*, 1998).

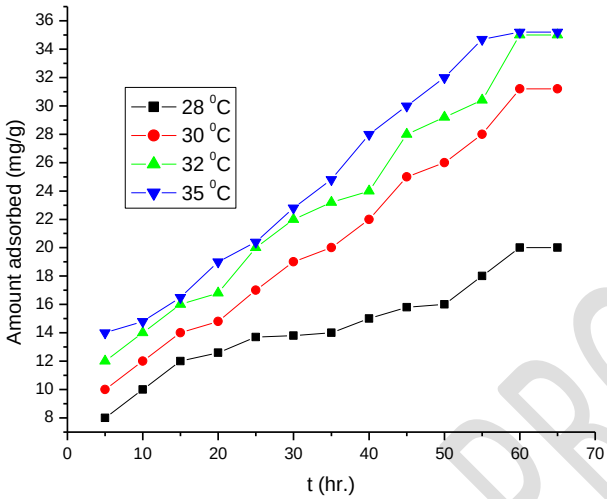

Figure 8. Effect of temperature on removal of 2,4-DCP onto MSAC (SAB study) (At pH 7, adsorbent dose 10 g l^{-1}, initial conc. 400 mg l^{-1})

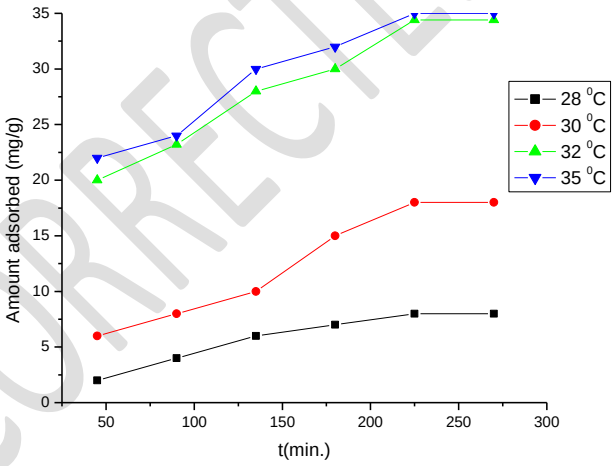

Figure 9. Effect of temperature on removal of 2,4-DCP onto MSAC (Adsorption study)(At pH 7, adsorbent dose 10 g l^{-1}, initial conc. 400 mg l^{-1})

Table 5. Effect of temperature on removal of 2,4-DCP onto MSAC

	Adsorption		SAB	
Temperature (°C)	%Removal	Amount adsorbed/ g of adsorbent (mg g^{-1})	%Removal	Amount adsorbed/ g of adsorbent (mg g^{-1})
28	20	8	50	20
30	45	18	78	31.2
32	86	34.4	91	35
35	86.2	34.6	91.5	35.2

5. Adsorption study

The adsorption isotherms are important to describe the sorbate-adsorbent interaction. The isotherm data were analyzed by fitting them into Langmuir and Freundlich isotherm to find out the suitable model. The Langmuir eq.(2) is represented in the linear form as follows:

$$\frac{C_e}{q_e} = \frac{1}{K_L Q_m} + \frac{C_e}{Q_m} \quad (2)$$

where K_L is Langmuir adsorption constant (l mg^{-1}), Q_m is the theoretical maximum adsorption capacity (mg g^{-1}), Ce is equilibrium liquid phase concentration (mg l^{-1}), q_e is sorption capacities at equilibrium (mg g^{-1}).

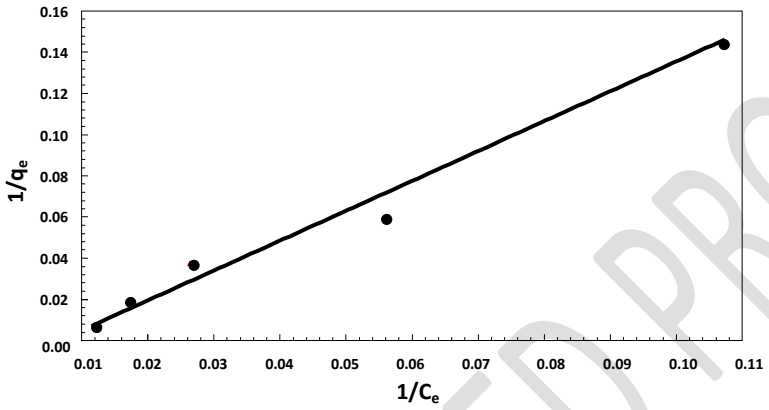

Figure 10. Langmuir isotherm for removal of 2,4-DCP onto MSAC (SAB study)
(At pH 7 and adsorbent dose 10 g l^{-1})

Figure 10 shows the Langmuir ($1/C_e$ vs.$1/q_e$) plot of 2,4-DCP for SAB onto MSAC, which are found to be linear over the whole concentration range. The Q_m is the theoretical maximum adsorption capacity 39.2 and 28.5 mg g^{-1} and the correlation coefficient (R^2) are extremely high 0.998 and 0.985(SAB and adsorption, respectively) as shown in Table 6. The separation factor (R_L) which is a measure of adsorption favorability, R_L values are in between 0 and 1, thus validating a favorable adsorption process (Table 6).

Table 6. Constant values of adsorption isotherms for 2,4-DCP onto MSAC

Isotherm model	SAB	Adsorption
Langmuir		
Q_m (mg g^{-1})	39.2	28.5
K_L (l mg^{-1})	99.1	20.7
R_L	0.022	0.032
R^2	0.998	0.985
SEE	1.015	1.017
Freundlich		
K_f ((mg g^{-1}) (mg l^{-1}) $^{-1/n}$)	1.407	1.543
1/n	0.215	0.016
R^2	0.992	0.962
SEE	1.487	2.371

The fitness of the Langmuir isotherm indicated the formation of monolayer coverage of the sorbate on the outer surface of the adsorbent. The linear Freundlich isotherm is expressed as eq. (3):

$$\log q_e = \log K_f + \frac{1}{n}\log C_e \quad (3)$$

where K_f is Freundlich constant and 1/ n is Heterogeneity factor. Figures 11 shows that linear plot (log q_e vs. log C_e) of 2,4-DCP for SAB onto MSAC also follows freundlich isotherm.

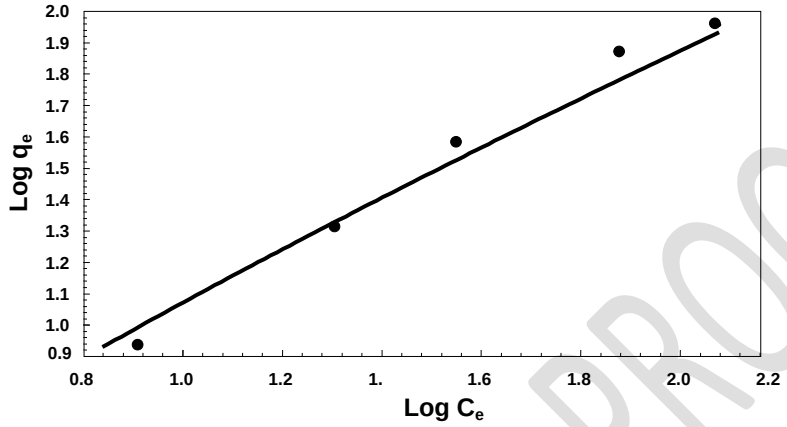

Figure 11. Freundlich isotherm for removal of 2,4-DCP onto MSAC (SAB study)
(At pH 7 and adsorbent dose 10 g l^{-1})

The freundlich constant value, 1/n (0.21 and 0.16) and correlation coefficient (R^2) 0.992 and 0.962 (SAB and adsorption, respectively) were reported in Table 6. The less value of K_f in case of SAB indicates adsorption capacity of the adsorbent and higher values of 1/n (shift towards 1.0) indicates more adsorption intensity. The values of Langmuir and Freundlich isotherm constant and correlation coefficient (R^2) for adsorption and SAB of 2,4-DCP are given in Table 6. The correlation coefficient for Langmuir isotherm are greater than those of Freundlich isotherm which confirms that Langmuir isotherm is better fit for the description of adsorption process.

6. SEM micrographs

The morphologies of MSAC after adsorption and SAB of 2,4-DCP removal were examined under scanning electron microscope. The SEM micrographs of MSAC are shown in fig. 12 and 13. The fig. 12 have linear type of fibers with holes which filled with 2,4-DCP after their adsorption. Figure 13 shows the development of micro-organisms inside the voids of activated carbon and cover the surface of MSAC by simultaneous adsorption and biodegradation of 2,4-DCP.

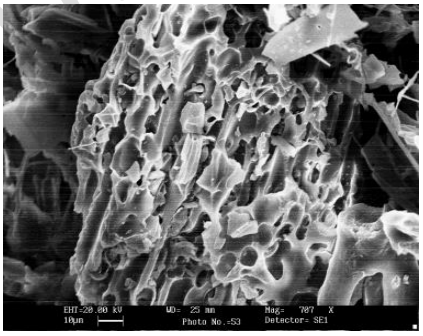

Figure 12. SEM Micrograph of MSAC after Adsorption of 2,4-DCP

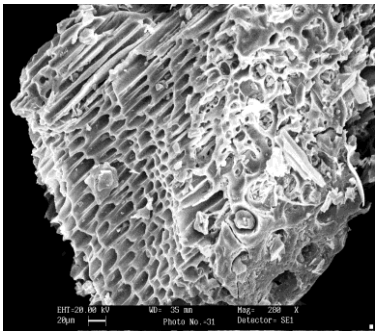

Figure 13. SEM Micrograph of MSAC after SAB of 2,4-DCP

7. Conclusions

1. Under the experimental study the optimum condition i.e. adsorbent dose 10 g l^{-1}, pH 6 and 32 °C temperature the 2,4-DCP removal efficiency of SAB (91%) is more than that of adsorption(86%).
2. The theoretical models verify the practically optimized parameters for the removal of the 2,4-DCP.
3. Langmuir isotherm is better fit than the Freundlich isotherm to explain the adsorption. The fitness of Langmuir's model indicated the formation of monolayer coverage of the sorbate on the outer surface of the adsorbent.
4. The results indicated that the MSAC with a support matrix of *pseudomonas putida* MTCC1194 as adsorbent is capable for the removal of 2,4-DCP with high affinity and capacity and use as a low cost adsorbent.
5. MSAC is an inexpensive, indigenous and easily available in large quantity and its use as sorbent would significantly lower the cost of wastewater treatment and in near future also tried for other toxic compounds removal.

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