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*Full Length Research Paper*

# Decolorization of irgalite dye by immobilized *Pseuodomonas putida* on activated carbon, prepared from agriculture waste

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**A carbon sorbent derived from an agriculture waste, mustard straw was applied to study the removal of irgalite dye from aqueous solution. Comparative study on adsorption and simultaneous adsorption and biodegradation (+) of irgalite dye using *Pseuodomonas putida* (MTCC 1194) with activated carbon prepared from mustard straw showed removal of 83.3% higher SAB, with respect to adsorption (70%). For biodegradation of irgalite by *P. putida* at shake flask level Haldane's growth model fitted the best.**

**Key words:** activated carbon, *Pseudomonas putida,* adsorption.

## INTRODUCTION

Wastewater generated by the dye production industry and many other industries which use dyes and pigments is high in both colour and organic content. About 10,000 different commercial dyes and pigments exist, and over 7.105 tons are produced annually world-wide. It has been estimated that about 10 to 15% of these dyes are released in effluents during dyeing processes. Synthetic dyes are extensively used in many fields of up to-date technology, for example, in various branches of the textile industry (Sokolowska et al., 1996), of the leather tanning industry (Tu¨nay et al., 1999; Kabadasil et al., 1999) in paper production (Ivanov et al., 1996), in food technology (Slampova et al., 2001), in agricultural research a wide range of methods has been developed for the removal of synthetic dyes from waters and wastewaters to decrease their impact on the environment. Among various methods adsorption occupies a prominent place in dye removal. The growing demand for efficient and low cost treatment method and the importance of adsorption has given rise to low cost alternative adsorbent (LCAs). It was found that some LCAs, (Santhi et al., .2009; Parimaladevi and Venakateswaran, 2011; Carolyn et al., 2011) in addition to having wide availability, have first kinetic and appreciable adsorption capacities too. Advantages and disadvantages of adsorbent, favorable condition for particular adsorbate-adsorbent systems and adsorption capacities of various low cost adsorbents and commercial activated carbons are available in the literature are presented. (Gupta and Suhas, 2009; Kadirvelu et al., 2005) reported. Critical review of low cost adsorbents for waste and wastewater treatment has been represented by (Pollard et al., 1992; Mall et al., 1996; Bailey et al., 1999; Hao et al., 2000). The physical and chemical treatments available have limited use and are having high operational cost Synthetic dyes used are recalcitrant to remove by conventional wastewater treatments such as adsorption, photo-oxidation, flocculation, photodegradation and chemical degradation. Hence use of microorganism with addition of any physical methods shows better removal efficiency (Lamia et al., 2010; Chiing et al., 2007; Oranusi et al., 2005).

The purpose of this work is to investigate the possibility of mustard straw as activated carbon materials for removal of dyes from aqueous solution. This biomaterial is low cost agricultural waste residue and is easily available in large quantity in India. The dyes selected as sorbate is irgalite. The effects of various operating parameters on biosorption such as initial pH and dye Concentration, sorbent dosage, contact time was monitored and optimal experimental conditions are decide.

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## THEORY

In a batch study, for low concentration of dye, the dependence of specific growth rate, $\mu$, on substrate concentration, S, may be represented by Monod's Model.

$$\mu = \mu_{max} S/ (K_s+S) \quad (1)$$

This model is an over simplification and does not take care of inhibitory nature of substrate. For high concentration of dye, Haldane's inhibitory growth kinetic equation can be used (Kumar et al., 2005).

$$\mu = \mu_{max} S/(K_s+S+S^2/K_i) \quad (2)$$

where $K_s$ and $K_i$ are half saturation coefficient and inhibitor constant, respectively. For very high concentration of phenol that is, for $S>>K_s$, Linearized Haldane's equation may be used (Kumar et al., 2005).

$$\mu = \mu_{max} S/(S+S^2/K_i) \quad (3)$$

The above can be rearranged as,

$$(1/\mu) = (1/\mu_{max}) + ( S/K_i\mu_{max}) \quad (4)$$

For batch studies the specific growth rate may be expressed as:

$$\mu = (1/t) \ln(OD_t/OD_o) \quad (5)$$

where, $OD_o$ is the initial optical density and $OD_t$ is the optical density at time t.

## MATERIALS AND METHODS

Ciba® IRGALITE® Blue R-L can be used in the coloration of printing and writing paper and board grades and specialty grades such as decorative laminates and laundry tag papers. Activated carbon prepared from mustard straw with size range 2 to 4 mm, was used as adsorbent.

### Microorganism and growth medium

*P. putida MTCC 1194* species was obtained from Institute of Microbial Technology, Chandigarh, India. Culture media was prepared as per the guidelines of Microbial type cell culture (MTCC). The culture media was acclimatized to dye environment with gradual exposure to increasing dye concentration. The composition of the Basal salt medium (BSM) used in this experiment as the growth medium is given in Table 2.

Shake flask experiments were carried out by using a gyratory incubator shaker. Five hundred ml erlenmayer flasks were charged with 190 ml of nutrient media. Different amounts of irgalite dye were added into each flask and dissolved by heating. The optimal growth conditions for the *P. putida* culture were used in the experimental studies. The initial pH was adjusted to 7 ± 0.2. Sterilized flasks were inoculated with 10 ml *P. putida* (DSM 6978) culture and were incubated in a shaker at 200 rpm and 30°C for 10 days (240 h). Shake flasks including the controls were prepared in duplicates. pH dropped slowly in experimental flasks as a result of HCl formation upon DCP degradation. pH was controlled at 7 ± 0.5 by addition of few drops of 0.1N NaOH everyday.

### Production of activated carbon

The mustard straw firstly cut into the small pieces, dried in sun light until the moisture was partially evaporated and was further dried in hot air oven at 60°C for 24 h. The dried straw were allowed for chemical activation by addition of conc. Sulphuric acid (1:1w/v) with constant stirring .The charred material was kept in hot air oven at 110+-5°C for 12 h. The resulting material was washed with distilled water, Soaked in 2% sodium bicarbonate solution and allowed over night to remove the residual acid from the pores of carbon. Then material was washed with distilled water, dried material was ground and particles retained of the desire size were collected and used for the study. (Madhava et al., 2006).

### Acclimatization

The acclimatization of *P. putida* (MTCC1194) in dye environment was performed as follows: The revived culture was first grown in basal salts medium (BSM) with glucose in a 250 ml conical flask. After 48 h, significant bacterial growth was observed in the flask and the synthetic medium in the flask turned milky. Appropriate quantity of stock solution of irgalite was added into the flask containing BSM to get a concentration of 10 mg/L of dye. It was kept aside, initially growth of *P. putida* was inhibited and degradation of irgalite started after 10 h. Thereafter, the dye was periodically added in increments of 10 mg/l in a series of 250 ml flasks till the dye concentration in the growth media reached 100 mg/L. The content of glucose was decreased and irgalite was increased over a period of one month. For inoculum, a further sub culturing was done and all the inoculums transfers were done in exponential phase. The temperature was maintained at 30°C.

### Analytical measurement

The determination of concentration of dye was done by finding out the absorbance characteristics wave length of these using UV-spectrophotometer ((Model - Perkin Elmer). A standard solution of dye was taken and the transmittance was determined at different wavelength to optain a plot of absorbance versus wavelength. The wavelength corresponding to maximum transmitance ($\lambda_{max}$) was determined from this plot; the $\lambda_{max}$ 588 for irgalite .Calibration curve was ploted between absorbance and solution concentration.

### Experimental procedure

To study the effect of important parameters like the pH, contact time, initial dye concentration and temperature on the removal of irgalite, batch experiments were conducted. For adsorption study experimental run, 50 ml of dye solution of known concentration range 50 to 200 mg/l, known pH varied from 2 to 9 and a known amount of the adsorbent varied from 1 to 10 g/l were taken in a 200 mL stoppered conical flask. This mixture was agitated in a temperature controlled shaking water bath at a constant speed of 150 rpm. Samples were withdrawn at different time intervals (0 to 120 min), filtrated and analyzed for remaining dye concentration.

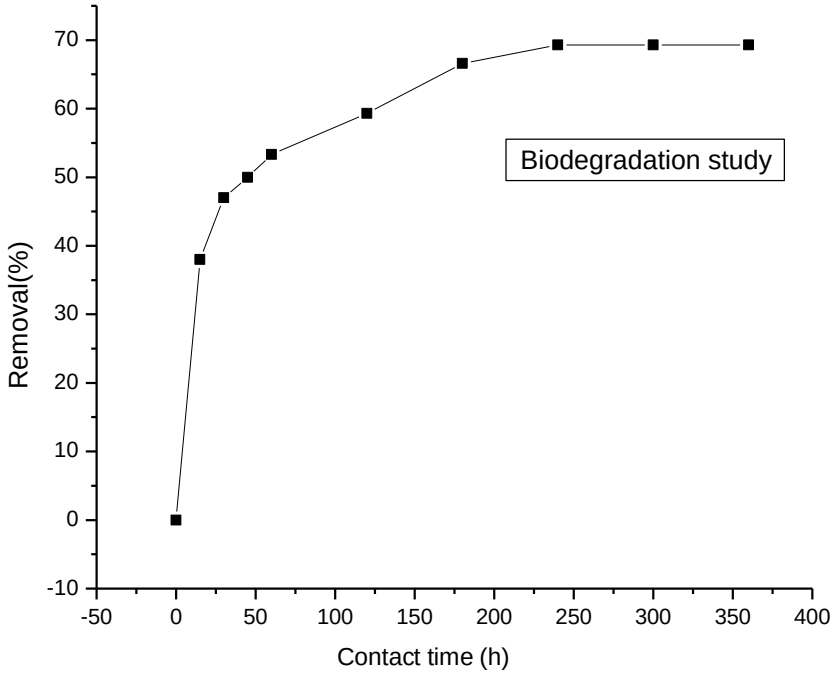



**Figure 1.** Biomass growth kinetics during irgalite degradation by free *P.putida.*

The initial pH of the solution was adjusted by addition of dilute aqueous solutions of HCl or NaOH (0.1M). For SAB study experiments were conducted in BSM with 20 ml inoculum of irgalite acclimatized *P. putida* at 0.9125 gm cell (dry wt)/l in 500 ml flasks at 30ºC and 150 rpm with various initial concentrations of irgalite to determine the parameters for different kinetic models for biodegradation. Unless specified otherwise, all the reaction mixtures in the batch experiments consisted of 100 ml BSM at pH 7. To vary the initial concentrations of irgalite aliquots of 40 ml were taken from different stock solutions of irgalite and added to the reaction mixture. Effects of various parameters like initial concentration of dye, pH, adsorbent dose, etc. on percentage removal of irgalite were also investigated. In all the experiments partical size of adsorbent was 2 to 4 mm. To find the optimum dose of adsorbent different quantities of AC were taken in 500 ml conical flasks containing BSM for removal of dye by simple adsorption. The percentage removal of irgalite was calculated using the following relationship % color removal:

$$= \frac{Co\text{-}Ct}{Co} \times 100$$

where *Co* and *Ct* ($mgL^{-1}$) are the initial dye concentration and concentration at time *t*, respectively. Kinetics of adsorption was determined by analyzing adsorptive uptake of the dye from aqueous solution at different time intervals.

## RESULTS AND DISCUSSION

### Kinetic studies of irgalite biodegradation

Figure 1 shows that for initial concentration of 150 mg irgalite/L, 69.3% irgalite was removed in 300 h. A lag phase of 45 h followed by a steep log phase growth was evidenced till 180 h. The long lag phase of 45 h shows inhibitory effect of dye as substrate, which can possibly be decreased by application of larger amount of well acclimatized inoculums. Growth kinetics of *P.putida* on irgalite removal was studied taking initial concentration as, 50, 100,150 and 200 mg irgalite/L at pH 7. The specific growth rate was calculated using Equation (5) and the values were used to check the validity of various kinetic models like Monod's model, Haldane's model and linearized Haldane's model. Specific growth rate data from low concentration region (50 to 200 mg irgalite/L) were fitted to Monod's model (Figure 2) and those from higher concentration region (50 to 200 mg irgalite/L) fitted to linearize Haldane's model (Figure 3). Haldane's model (Figure 4) shows that specific growth rate initially increases with the increase in the initial irgalitel concentration and then starts decreasing with the increase in irgalite concentration which indicates inhibitory nature of irgalite. In general, Haldane's growth kinetic model is used to represent growth kinetics data of an inhibitory substrate. Therefore, it is evident that the entire range of present experimental data fits best to Haldane's growth kinetic model. Values of various kinetic constants for the above models have been recorded in Table 1.

### Effect of mass of adsorbent

The effect of adsorbent dose on uptake of irgalite on activated carbon was studied (Figures 5 and 6). These

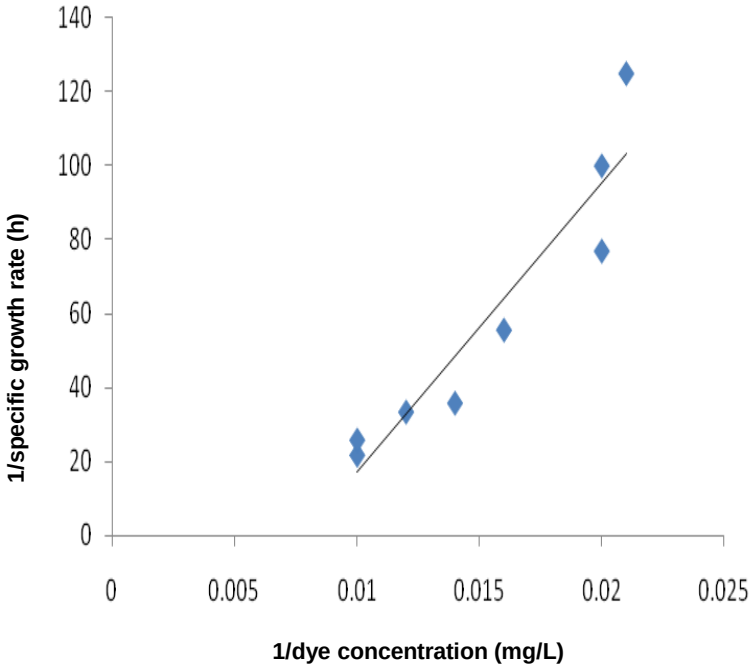



**Figure 2.** Biodegradation kinetics (Monod's model) for free *P. putida* at 50,100,150 and 200 mg irgalite /l initial concentration in the reaction mixture.

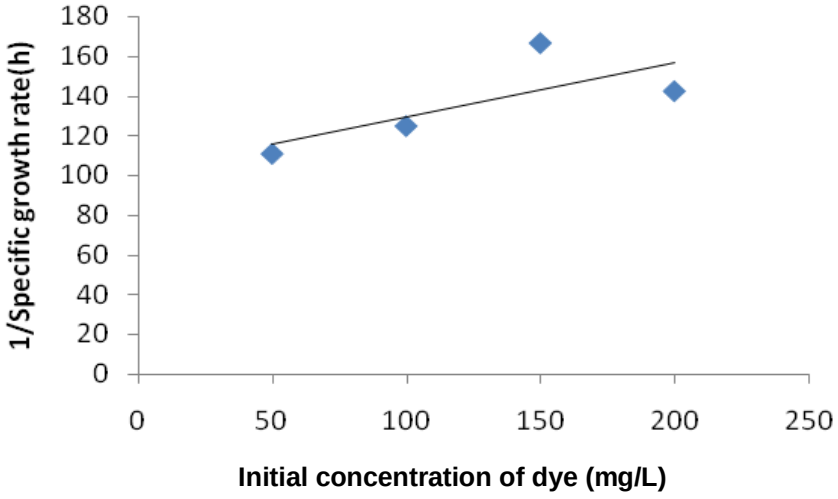



**Figure 3.** Biodegradation kinetics (Linearized Haldane's model) for free *P. putida* at 50.100,150,200 mg dye/l initial concentration in the reaction mixture.

figures reveals that removal of irgalite increase 55.3 to70% in case of adsorption, 63.3 to 83.3% in simultaneous adsorption and biodegradation with increase in adsorbent doses that is 2 g to 8 g. The removal of irgalite at adsorbent dosages is larger than 8 g/l remain almost unchanged. An increase in the adsorption with the adsorbent dosages can be attributed to greater surface area and availability of more adsorption sites. At adsorbent dose less than 6 g/l, the adsorbent surface become saturated with dye and residual concentration in the solution is large. The dye removal increases due to increased dye uptake by increased amount of adsorbent. At adsorbent dose more than 6 g/L, the incremental dye removal becomes very low. As the surface dye concentration and the solution dye concentration come to equilibirium with each other, about 8 g/L the removal efficiency becomes almost constant.

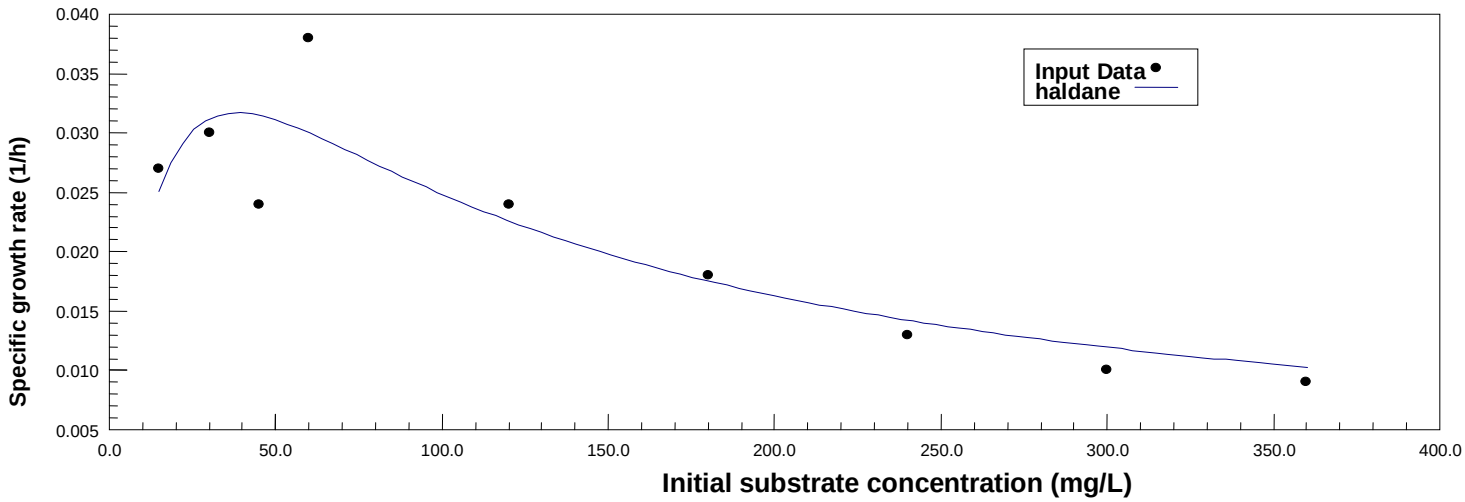



**Figure 4.** Biodegradation kinetics (Haldane's model) for free *P. putida* for initial concentration ranging from 10 to 350 mg irgalite /l in the reaction mixture.

**Table 1.** Values of constants for different kinetic models.

| Monod's model | | Linearized | Haldane's model | Haldane's model | | |
|---|---|---|---|---|---|---|
| $\mu_{max}(h^{-1})$ | $K_s$(mg/) | $\mu_{max}(h^{-1})$ | $K_i$(mg/l) | $\mu_{max}(h^{-1})$ | $K_s$(mg/l) | $K_i$ (mg/l) |
| 1.75 | -8.09 | 3.1 | 4.52 | 0.11 | 53.8 | 141.7 |

**Table 2.** Composition of Basalt Salt media.

| | |
|---|---|
| $K_2HPO_4$ | 1.5 gm/l |
| $KH_2PO_4$ | 0.5 gm/l |
| $(NH_4)_2PO_4$ | 0.5 gm/l |
| NaCl | 0.5 gm/l |
| $Na_2SO_4$ | 3 gm/l |
| Yeast extract | 2 gm/l |
| Glucose | 0.5 gm/l |
| Ferrous Sulphate | 0.002 gm/l |
| Calcium chloride | 0.002 gm/l |

## Effect of pH on biodegradation of irgalite in batch mode

Figure 7 gives a comparative picture of effect of various pH values on corresponding irgalite removal percentages, measured at regular time intervals. The Study shows that at pH 2 percentage removal of irgalite is 50 and 52.6 where as at pH 9 percentage removal is 48 and 62 for adsorption and SAB. Hence this study reveals that pH values around 7 are favorable for higher removal percentage of irgalite that is, 70 and 83.3 for adsorption and SAB.Thus irgalite biodegradation occurs best near neutral pH due to neutrophilic behavior of *P.putida,* and becomes less effective in highly acidic or alkaline conditions.

## Effect of initial concentration of irgalite dye

Figure 8 shows that the rate of removal in the reaction mixture was highest in case of 150 mg irgalite/L solution (that is, 70 and 83.3%) for adsorption and SAB followed by 50 mg/L (that is, 65 and 70%) and 100 mg/L (that is, 68 and 80%). The higher biodegradation found in case of 150 mg/l indicates that higher amount of available carbon source, possibly has increased the cell numbers, hence the dye consumption is faster. Slower substrate degradation in case of 200 mg/L (that is, 75 and 80%) may be attributed to number of possible reasons. Growth inhibition may be caused by higher concentration of irgalite as substrate or may be due to accumulation of toxic end products of irgalite degradation that might have caused blockage of pores. The unfavorable pH values arising during the course of degradation may also discourage the growth and biodegradation activities of *P. putida* hence diminish the removal rate of dye.

## Comparative study of adsorption and SAB

Figure 9 shows the comparative patterns of irgalite dye removal by adsorption and SAB. It is evident from Figure 9 that initially up to 1 h, there is no variation in percentage removal of irgalite in both the processes, while after 1 h the variation starts, where higher percentage removal of dye has been observed in case of

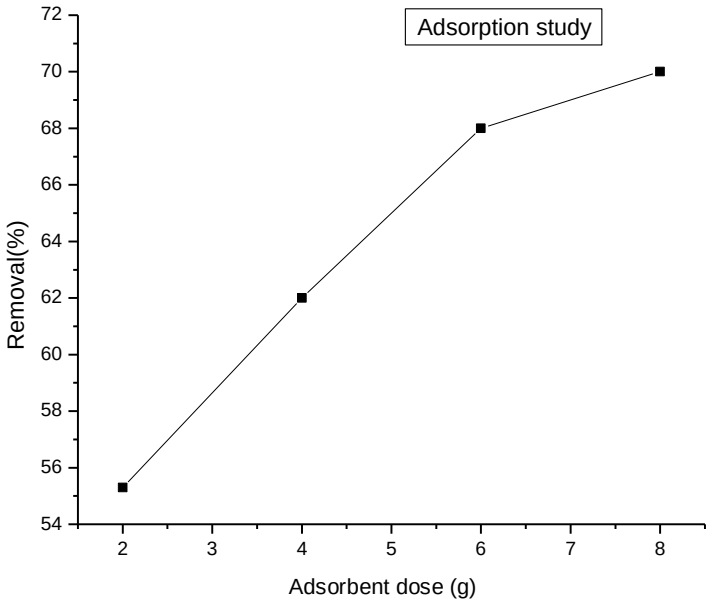



**Figure 5.** Effect of adsorbent dose on irgalite removal. Initial concentration of irgalite was 50 mg /l in the solution. Irgalite concentrations were measured after 360 min.

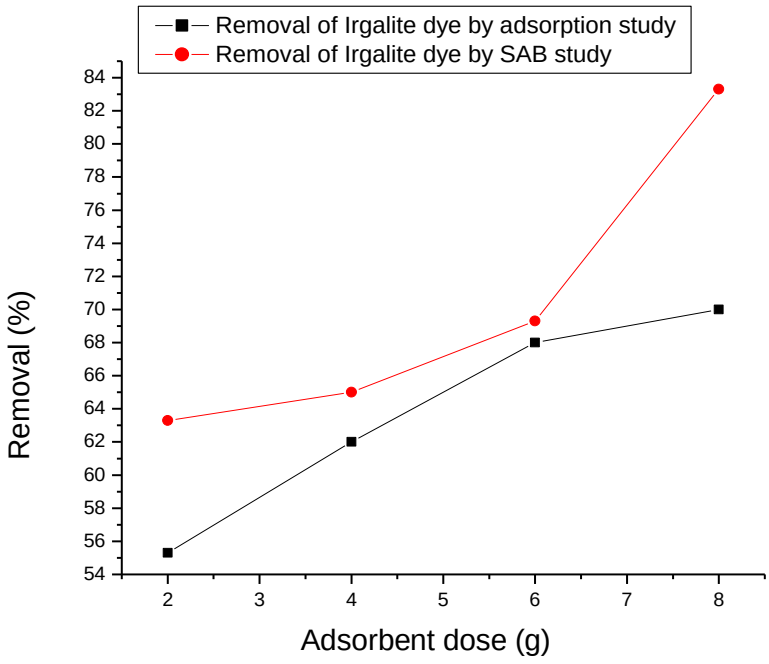



**Figure 6.** Comparison Effect of adsorbent dose on %removal of irgalite dye between adsorption and SAB study at conc. 150 mg/l, ph 7.

SAB (83.3%) with respect to adsorption (70%). Such an observation is evidenced in case of SAB due to occurrence of additional dye removal by biodegradation of *P. putida* cultures with progress of time, resulting in higher dye removal. This increase of removal rate in SAB is due to the dominating role of bio degradation of irgalite by the microbes attached to the bio-layer formed on the surface of the adsorbent. Formation of bio-layer during SAB of irgalite is evident by comparing Figure 10A and B. SEM images shows the adsorption and biodegradation

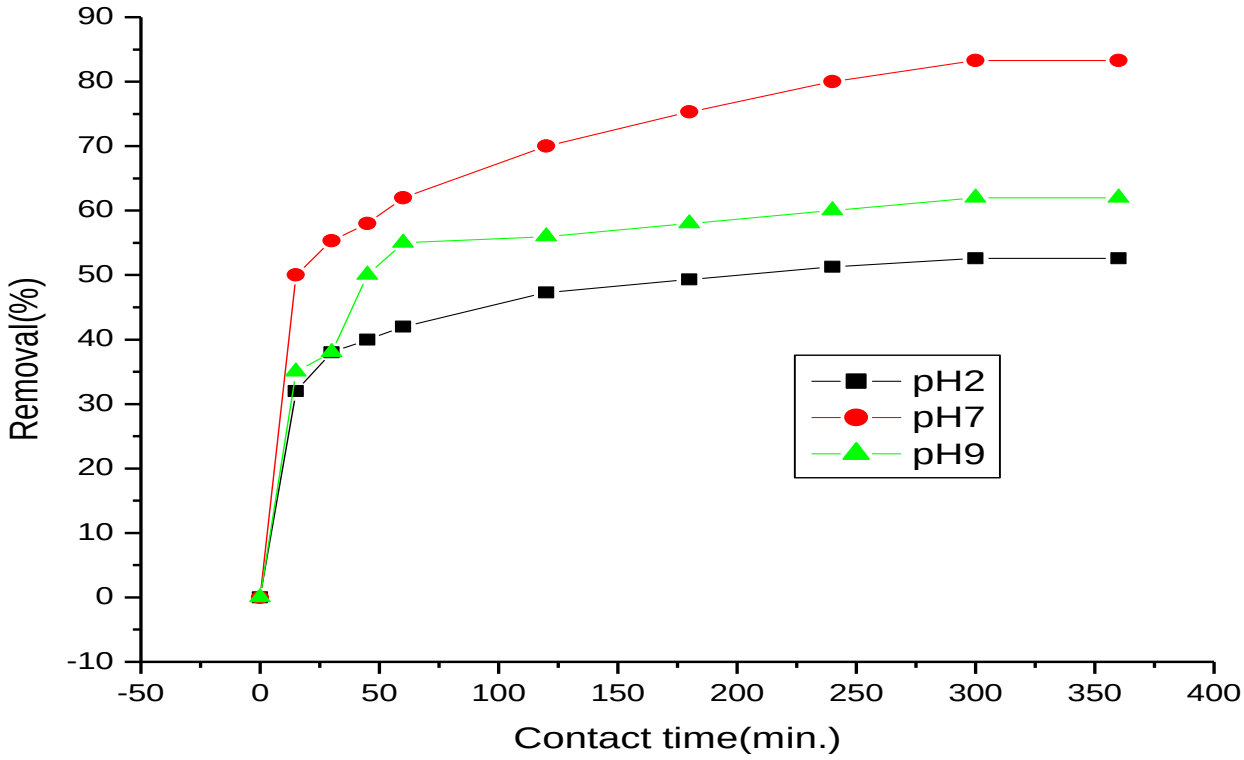



**Figure 7.** Removal of Irgalite by free *P.putida* at different pH values taking initial concentration of irgalite as 150 mg /l in the reaction mixture. AC dose was 8 gm/l. pH **2**; pH 7; pH9.

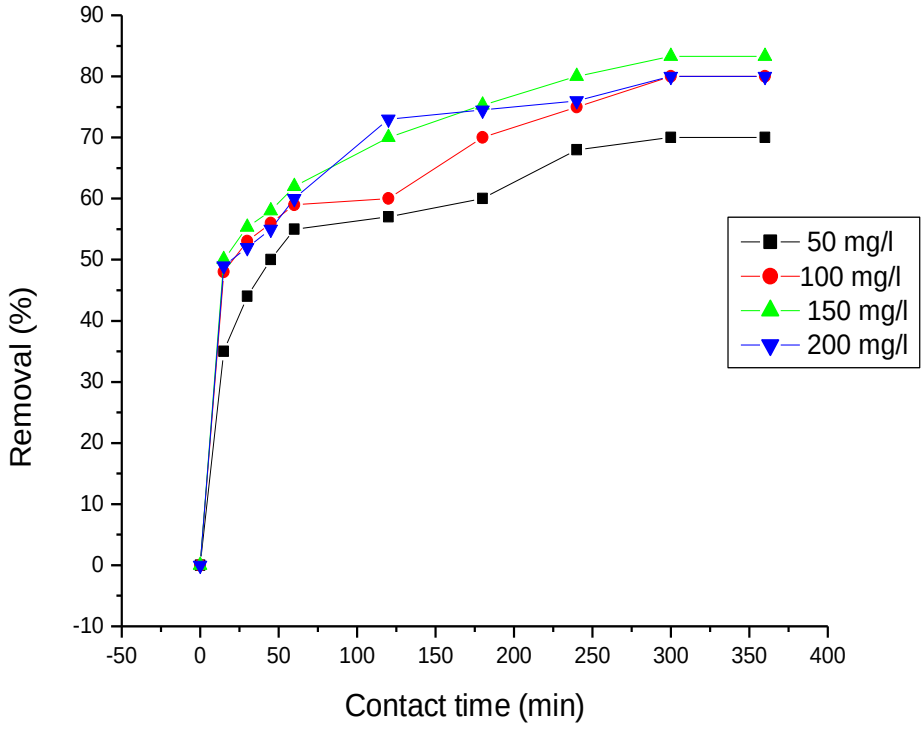



**Figure 8.** Removal of irgalite by *P. putida* at various initial concentrations in the reaction mixtures. AC dose was 8gm/l. Initial concentration= 50, 100, 150 and 200 mg/L, pH 7.

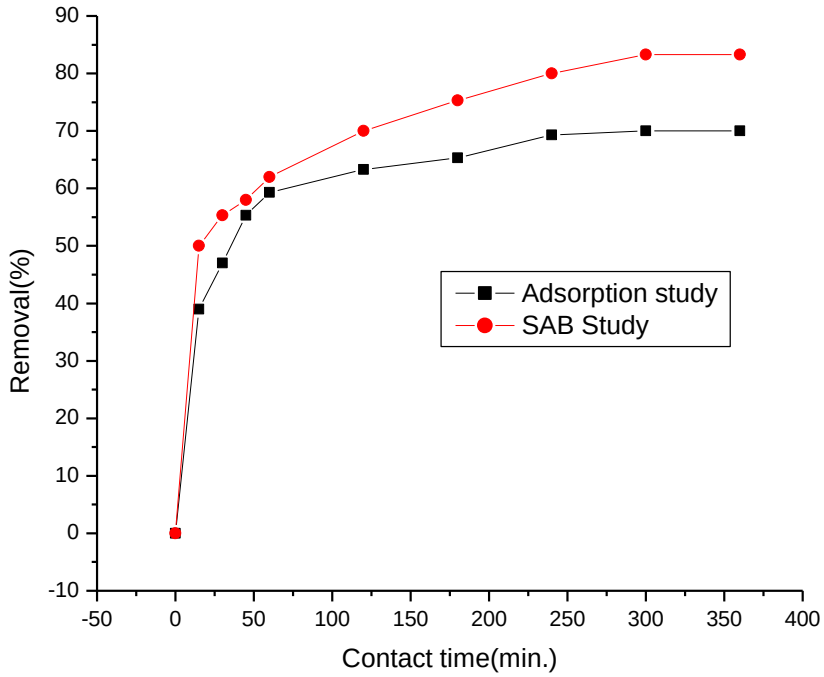
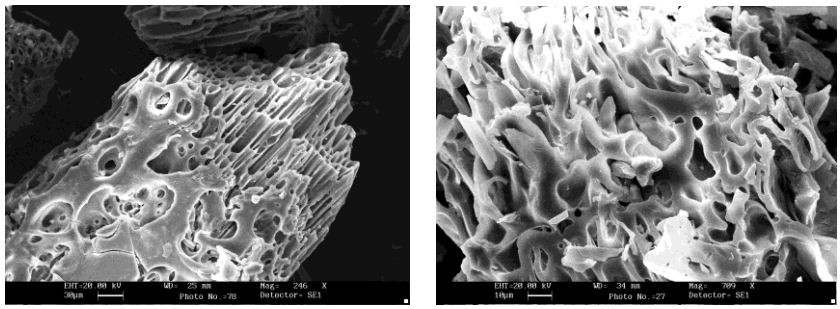



**Figure 9.** Comparison of dye removal by simple adsorption and SAB. For both the experiments initial concentration of irgalite dye was 50 mg /l in the reaction mixture. AC dose was 8 mg/l. For SAB 20 ml *P. putida* inoculums was used. SAB and adsorption.



**(a)** **(b)**

**Figure 10.** (A) Adsorption study .Scanning electron micrograph of 2 to 4 mm carbon particles at 1000x magnification after adsorption of irgalite; (B), SAB study. Scanning electron micrograph of 2 to 4 mm, carbon particles at 1000x magnification after, SAB of irgalite

capacity.

## Conclusion

1) It can be concluded that activated carbon prepared from agriculture waste material is effective for the removal of irgalite dye; higher percentage removal of irgalite has been observed in case of SAB (83.3%) with respect to adsorption (70%).

2) Haldane's growth kinetic model also verifies the practically optimized parameters for the removal of the irgalite dye.

## Nomenclature

Kh =Inhibition coefficient of metabolic intermediates (mg/l); Ki =Inhibition constant for cell growth (mg/L); Ki9 =Inhibition constant for substrate consumption (mg/L); Kp =Proportionality constant (mg/L)

KS =Saturation constant for cell growth (mg/L); KS9 =Saturation constant for substrate consumption(mg/L); Ks= Saturation constant for substrate consumption(mg/L); Qs= Specific utilization rate of substrate (mg/(mg 3 h)); Rm= Maximum specific consumption rate of substrate(mg/(mg 3 h)); Rm9 Maximum specific consumption rate of substrate(mg/(mg 3 h)); S =Substrate concentration (mg/l); S0= Initial substrate concentration (mg/L)t Time (h); X= Biomass; concentration (mg/L); X0= Initial biomass concentration (mg/l); Y= Observed cell mass yield (g/g); YC =Theoretical cell mass yield on dye(g/g); YE =Yield of cell mass on dye for energy (g/g); Kd= decay coefficient (h−1); Ks= half saturation coefficient (mg/l); T= time (h); Greek symbol

µg specific growth rate (h−1); µmax maximum specific growth rate (h−1); µnet _g −kd, net specific growth rate (h−1).

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