International Journal of Advanced Biotechnology and Research
ISSN 0976-2612, Online ISSN 2278–599X,
Vol 4, Issue 1, 2013, pp 20-28
http://www.bipublication.com

UTILIZATION OF FLY ASH AS BIOLOGICALLY ACTIVATED CARBON ON MICROBIAL CONSORTIUM FOR THE REMOVAL OF POLLUTANTS FROM WASTE WATER

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[Received-07/12/2012, Accepted-09/01/2013]

ABSTRACT

Fly ash based low cost absorbent was prepared, characterized and used for the removal of dyes such as Methylene blue & Congo red from wastewater. This research work also reports the potential of mixed bacterial population (bacterial consortium, co-culture having *Bacillus Sp*. & *Kurthia Sp*.) were used for the decolonization of textile dye waste water for the biodegradation of simulated textile dye waste water. The percentage of colour removed increase with increasing adsorbent dosage & increase with increasing contact time. Optimum contact time for equilibrium to be achieved is found to be 2 hours (120 min). It is basically due to saturation of the active site which does not allow further adsorption to take place. Optimum adsorbent dose for the dye was found to be 30g/l. The linear form of Freundlich isotherm model fitted the adsorption data. And the adsorption kinetics also follows pseudo first order kinetics. The studies revealed that fly ash based activated carbon can be fruitfully employed as an adsorbent for the dye removal from textile waste water. Dye decolourization by the biologically activated carbon (BAC) also prepared by mixing fly ash based activated carbon & co-culture of *Bacillus* & *Kurthia Sp*., and was very fast as reflected from the studies. Using such a system, the decolorization of dye solutions or textile waste water containing these dyes is quite cheaper as compared to the independent process either by using ACs or by using bacterial consortium alone and the other conventional treatment process.

Key words: Fly ash, microbial consortium, adsorption equilibrium, adsorption kinetics, biologically activated carbon (BAC)

[I] INTRODUCTION

The textile industries produce effluents which are often contaminated with harmful or poisonous substances and these industries also plays an important role in the world economy as well as in our daily life. At the same time, it consumes large quantities of water (upto 150 liter of water to dye 1 kg of cotton) and generates huge amounts of wastewaters (1, 2). The major chemical pollutants present in textile wastewater are dyes containing

carcinogenic amines, toxic heavy metals, pentachlorophenol, chlorine bleaching, halogen carriers, free formaldehyde, biocides, fire retardants, and softeners (3, 4).

Globally, over 600 million tonnes of fly ash are generated every year from coal combustion by thermal power-plants and its disposal as landfill poses major challenges and serious economic and environmental problems (5). Thus, the amount of coal waste (fly ash), released by factories and thermal power plants has been increasing throughout the world, and the disposal of the large amount of fly ash has become a serious environmental problem. Therefore, it is desirable to take proper and efficient control measures to overcome this problem. Now a day many of researchers are using fly ash as an adsorbent for the removal of various pollutants. It is revealed from the studies that the treatment of domestic wastewater can be done by fly ash generated from thermal power plant to reduce the organic load. It is physically viable and economically viable approach. Appreciating the overall concern for environmental and management issues pertaining to fly ash, which otherwise is a very useful by-product of thermal power plants, the Technology Information, Forecasting & Assessment Council (TIFAC), Department of Science & Technology (DST), Government of India identified "Safe Disposal and Gainful Utilization of Fly Ash" as a thrust area and commissioned a Techno-Market Survey "Technologies for Disposal of Thermal Power Station Fly Ash".

Previous studies have shown that biodegradation of organic chemicals by using pure cultures usually can produce toxic intermediates. This problem may be overcome by the use of mixed cultures that have a wider spectrum of metabolic properties (6-10). Multi species microbial granules or microbial consortia are emerging as most promising agents for state-of-the art wastewater treatment and bioremediation technologies. High satiability, high stability in variable organic load, effective mineralization and amenability for bioaugmentation are some of the superior parameters which make them one of the best choices for bioremediation and wastewater treatment & its decolonization (11).

[II] MATERIALS AND METHODS

2.1 Fly Ash (Adsorbent) Collection

In modern thermal power stations, pulverized coal is used and fly ash is obtained as a waste product in large quantities. A representative sample of the raw material (fly ash) was collected from National Thermal Power Corporation Plant, Dadri, UP, India.

2.2 Dyes (Adsorbate)

The methylene blue dye used was discovered by Caro in 1878. It is a basic cationic dye, heterocyclic aromatic chemical compound with molecular formula: $C_{16}H_{18}N_3SCl$, Molecular Weight=319.85. λmax = 663 nm.

The congo red is a red color dye. Its color changes from blue to red at pH 3.0-5.2, so can be used as a pH indicator. It is the sodium salt of benzidinediazo-bis- 1-naphthylamine-4-sulfonic acid (formula: $C_{32}H_{22}N_6Na_2O_6S_2$; molecular weight: 696.66 g/mol). It is a secondary diazo dye. λmax = 497 nm

2.3 Biological Material

Bacterial Strain: *Bacillus sp.* (MTCC No. 2443) & *Kurthia Sp.* (MTCC No. 2442) strain was used in the present study that was procured from Microbial Type Culture Collection and Gene Bank, Institute of Microbiology (IMTECH), Chandigarh, India, in lyophilized form.

2.4 Chemicals

Chemicals used during the study were procured mainly from Hi-Media Laboratories and Sisco Research Laboratories (SRL) Pvt. Ltd., India. All the chemicals used were of analytical grade.

2.5 Adsorbent Development

The fly ash collected from NTPC, Dadri was in the form of small, spherical grayish-black particles. The collected fly ash was sieved to the desired particles sized ranges, including -16 to +16, -16 to +30 and -30 to +72 meshes. Each of

these particle size lots was washed a no. of times with distilled water to remove the adhering organic material and then dried at 100 ± 5 ^{0}C for 24 hr and kept in a separate plastic bag in a vacuum dedicator until required in the adsorption studies.

2.6 Preparation of Activated Carbon with KOH activation

The UC in bottom ash was ground in this work, and that with a particle size ranging from 0.83 to 1.65 mm was screened and activated. The activation was done by dissolving KOH in water in a stainless steel beaker, to which the UC was added. The weight ratio of water, KOH, and FA was 3:3:1. They were uniformly mixed, dried at 130 ^{0}C, and then placed in a high temperature oven. Nitrogen gas was introduced into the oven, where was heated to 780 ^{0}C and kept there for 1 h. The product was neutralized by HCl (600 cm^3) having an equal equivalent to KOH solution until most of CO_2 bubbles were disappeared, to which a large amount of 10% HCl solution was then added. It was placed in water bathed at 80 ^{0}C for 1 h, after which washing was continued with distilled water until the water became neutral (12, 13, 14).

2.7 Adsorption Studies

2.7.1 Effect of Contact Time

150 ml of dye solution with dye concentration (50 mg/l) is to be prepared in a conical flask with adsorbent concentration (30 g/l) and kept inside the shaker. Dye concentration to be estimated spectrophotometrically at the wavelength corresponding to maximum absorbance, λ_{max}, using a spectrophotometer. The samples to be withdrawn from the incubator shaker at predetermined time intervals and the dye solution should be separated from the adsorbent by the help of a micropipette. The absorbance of solution is then measured. The dye concentration is to be measured after 20, 40, 60, 80, 100, 120, 140, 160 & 180 min until equilibrium reaches. A graph is to be plotted with q_e vs time. The q_e is expressed as

$$q_e = (C_0 - Ce) / X \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where, q_e = Amount of dye adsorbed per unit mass of adsorbent (mg/g).

C_0= Initial dye concentration (mg/l)

C_e= Final dye concentration (mg/l)

X = Dose of adsorbent (g/l)

2.7.2 Effect of Adsorbent Dose

150ml of dye solution was prepared in different conical flasks with dye conc. (50mg/l) and adsorbent concentration 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 & 4.0 g /100 ml. The final dye concentration readings were taken after putting the 4 flasks inside the shaker for 2 hours. A plot of q_e vs adsorbent dose is taken. The q_e is expressed as equation 1.

2.8 Revival of the strains

The lyophilized strain was revived on the Nutrient Broth Agar Medium. The culture plates were inoculated in laminar flow cabinet with all sterilized instruments and incubated at 30˚C for 16 hours. The strain grew well and then these plates were stored in the refrigerator at 4˚C.

2.9 Growth Medium

The media used for the growth of *Bacillus sp. & Kurthia sp.* was Nutrient Broth Agar Medium. Composition of this media is given in Table 1. The salts were added and mixed one by one in distilled water to avoid precipitation. pH of the medium was maintained at 7.0. The medium was autoclaved and stored at room temperature under aseptic conditions.

Table 1: Composition of Nutrient Broth Agar Medium

S. No.	Component	Amount (g/l)
1.	Beaf Extract	01
2.	Yeast Extract	02
3.	Peptone	05
4.	NaCl	05
5.	Agar	15

2.10 Acclimatization of culture

The revived cultures were first grown in MSM with glucose as sole carbon source. The cultures were acclimatized to dyes by exposing the cultures in a series of shake flasks (250 ml)

wherein the content of glucose, initially 2%, was decreased and that of dyes increased gradually. All the batch experiments were performed at 30˚C. Once the acclimatization was over and certain enzymes were induced in the bacteria to participate in the metabolism in the presence of dyes, experiments were set for the degradation of dyes by *Bacterial Strains* at different initial concentrations of dyes.

2.11 Inoculums' Preparation

To develop the seed culture 200 ml of NB media was inoculated with a loop full of the culture freshly grown from the revived culture plates and incubated on an incubator shaker at 30˚C overnight. The obtained cells were then isolated by following three steps below:

a) The culture broth was transferred to outchlery tubes and centrifuged at 6000 rpm for 10 minutes at 4˚C.
b) The supernatant was discarded and the pellets were resuspended in 1 ml MSM and again centrifuged at 6000 rpm for 5 minutes at 4˚C.
c) Supernatant was again discarded and the pellets were resuspended in 1 ml MSM to be used as inoculum.

The OD value of inoculum was recorded with MSM as control in the spectrophotometer.

2.12 Analytical Methods

2.12.1 Assay of decolonization activity

The bacterial strains were grown on nutrient agar plates and were streaked on plates containing dyes in MM media. Decolourization of the dye was visually observed for the extent of zone clearing on the plates. The extent of dye decolorization by the microbial co-culture *(Bacillus+Kurthia)* in broth (nutrient broth and MM broth) was determined by spectrophotometer at the maximum absorbance of the respective dyes in the cell free extracts. The percentage of dye decolorization by the cells was done using the modified method of Yatome et al (1991). Co-cultures of bacterial strains were grown in 50 ml of nutrient broth for overnight at 37^0C and 80 rpm to an OD of 1.00 at 600 nm. The co-culture was centrifuged at 10,000 rpm for 10 min. and washed twice with sterile saline (0.85%) and resuspended in 10 ml of saline solution. 0.1ml of the inoculum was added to the broth containing dye and incubated at 37^0C, 85 – 110 rpm for 24 hr. The supernatant was collected after centrifugation for absorbance measurement at respective wavelengths. The percentage decolorization was calculated as follows:

% age Decolourization = (Initial O.D - Final O.D) / Initial OD

2.12.2 COD Removal

COD of the samples can be determined by standard dichromate reflux method (15).

2.12.3 Studies on decolonization of dyes by BACs

Using biologically activated carbon (fly ash along with bacterial cells), batch decolorization studies of dyes was carried out. To 40 ml of the dye solution 10 g each of fly ash was added separately. The dye decolorization was then studied by taking the samples at regular intervals, centrifuged and the supernatant was assayed for the extent of decolorization.

[III] RESULTS AND DISCUSSIONS

3.1 Adsorption Studies

3.1.1 Effect of contact time

The effect of contact time can be seen from Fig 1 & Fig 2 for the dyes Methylene Blue (MB) & Congo Red (CR) respectively. It is clear that the extent of adsorption is rapid in the initial stages and becomes slow in later stages till saturation is allowed. The final dye concentration did not vary significantly after 2 hours from the start of adsorption process. This shows that equilibrium can be assumed to be achieved after 2 hours (120 min). It is basically due to saturation of the active site which does not allow further adsorption to take place. It was observed that the interactions between adsorbent & dyes could be better explained on the basis of pseudo first order

kinetics. It was depicted from Fig 3 & Fig 4 that results obey Pseudo First Order Kinetics. Using fly ash based activated carbon as an adsorbent, the advantage is twofold; it not only acts as an effective & economic tool as compared to other existing commercial activated carbons for solving the problem of color pollution but also help in an effective & useful disposal of fly ash released from thermal power plant as waste residue.

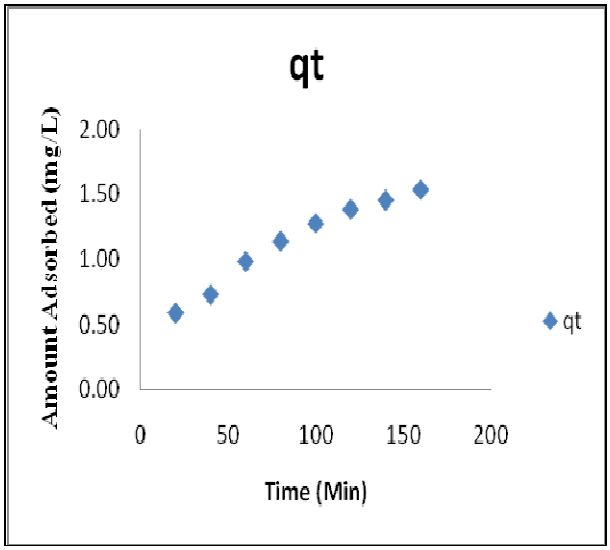

Fig 1: Effect of contact time on adsorption of dye solution (MB) on fly ash based activated carbon at Co- 50 mg/l & adsorbent dosage- 30mg/l

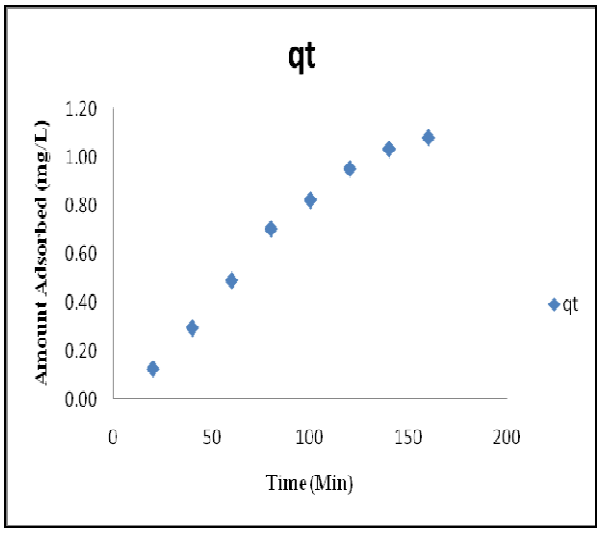

Fig 2: Effect of contact time on adsorption of dye solution (CR) on fly ash based activated carbon at Co- 50 mg/l & adsorbent dosage- 30mg/l

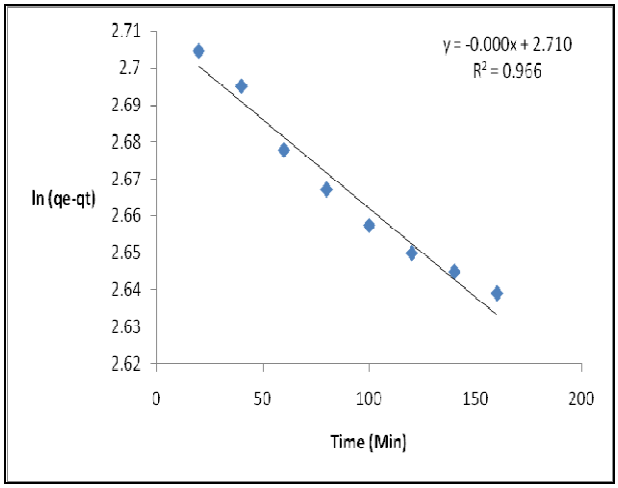

Fig 3: Plot for MB obeying Pseudo First Order Kinetics

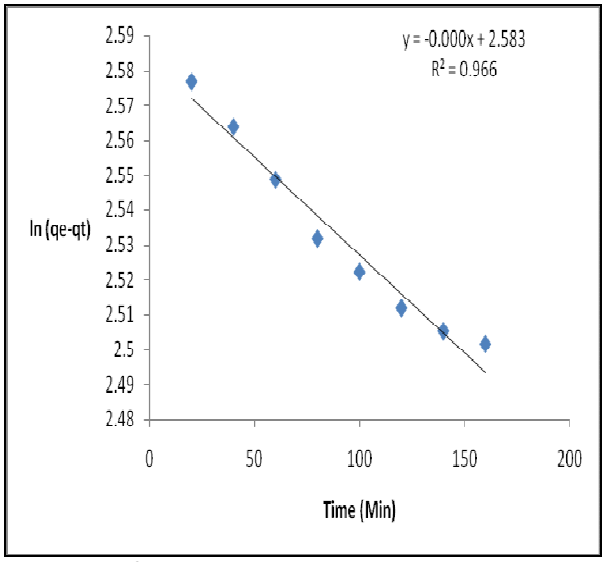

Fig 4: Plot for CR obeying Pseudo First Order Kinetics

3.1.2 Effect of adsorbent dose

From Fig 5 & Fig 6, we see that the optimum dose for the dye is 3g /100ml. Though at 3.5g /100ml, there is slight increase in qe value but if we get nearly the same result as we get at adsorbent dosage of 03g /100ml then going for 3.5g /100ml will be expensive and loss of adsorbent. It is obvious as with increasing amount the active sites for adsorption of mixture of three dyes increases which results in an increase in removal efficiency. The decrease in adsorption capacity with an increase in the adsorbent concentration could be ascribed to the

fact that some of the adsorption sites remained unsaturated during the process. Fly ash concentration increases the adsorption rates of the mixture due to increases in the surface area available for the adsorption. The adsorption by fly ash & coal in different concentrations showed that a good adsorption capacity towards metal containing dyes.

The adsorption of methylene blue & congo red dyes on fly ash based activated carbon is well explained by Freundlich isotherm models in all the cases which indicate the formation of monolayer coverage of the adsorbate on the outer surface of the adsorbent. Similar results were also reported by Kahn et al (16).

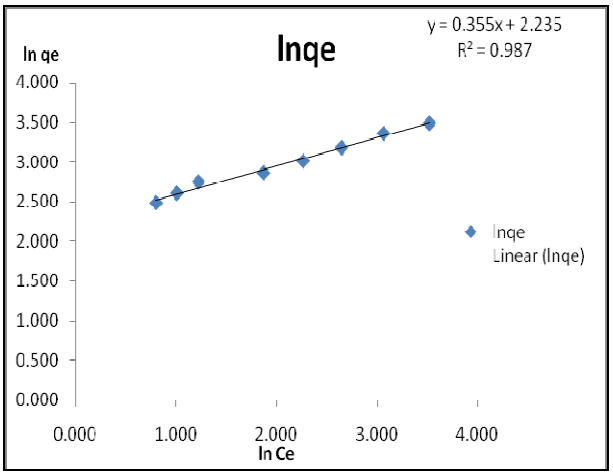

Fig 5: Freundlich Isotherm Plot for Methylene Blue

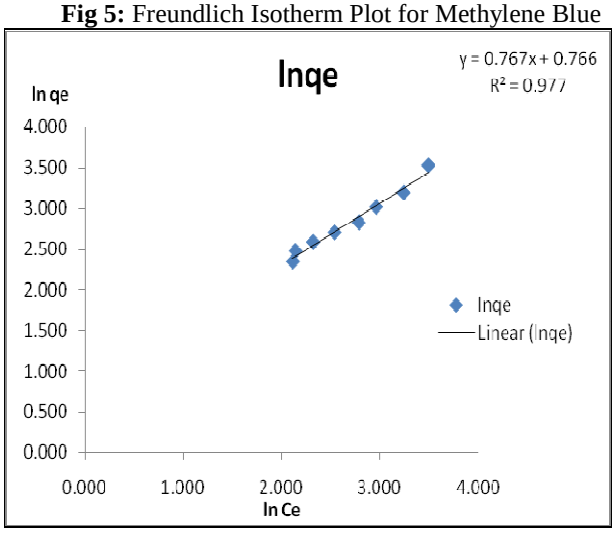

Fig 6: Freundlich Isotherm Plot for Congo Red

3.2 Decolorization of Dyes using Bacterial Consortium (Co-culture)

Aerobic bacterial isolates were found capable of growing in media containing dyes. They were checked for the extent of dye decolorization on solid nutrient agar media & minimal media plates. Visual decolorization indicated that decolorization was higher in case of nutrient broth as compared to minimal media. Bacterial consortium was studied further for dye decolorization. Decolorization of MB (3gm/100ml) was about 62 % (Fig 7) and for CR it was 61.45 % (Fig 8) on day 6. The rate of decolorization of MB by bacterial consortium was found to be higher than CR.

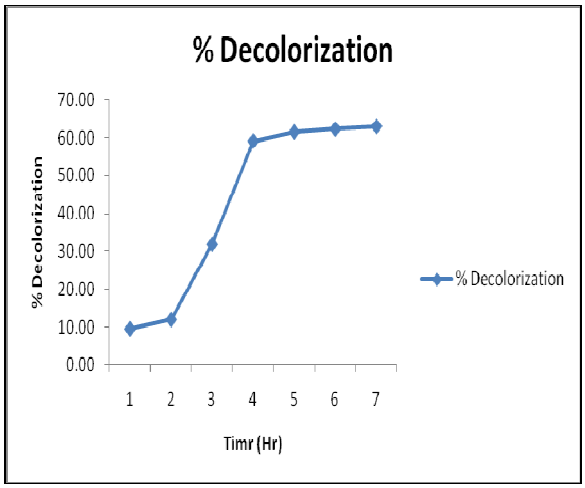

Fig 7: Decolorization of MB using BC

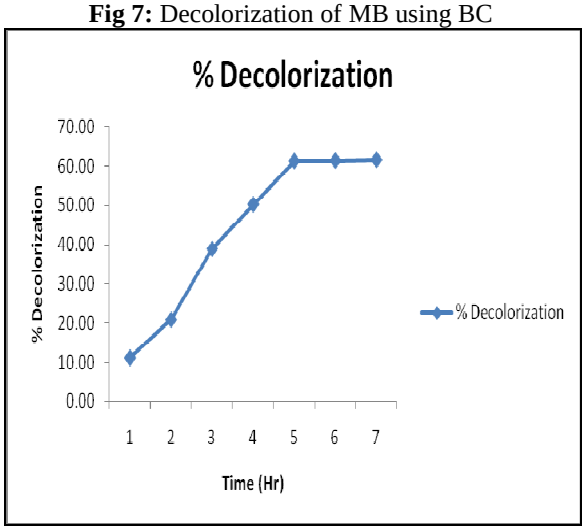

Fig 8: Decolorization of CR using BC

3.3 COD Removal by the Bacterial Consortium

During the experimental set up COD removal was investigated daily. Fig 9 & Fig 10 illustrate the COD during the experimental conditions. The COD of the sample was determined by standard dichromate reflux method. The initial COD concentration of the dye solutions was very high. COD reduction was found to be near about 82% in case of MB & about 80% in case of CR after the incubation period of 7 days under shaking conditions.

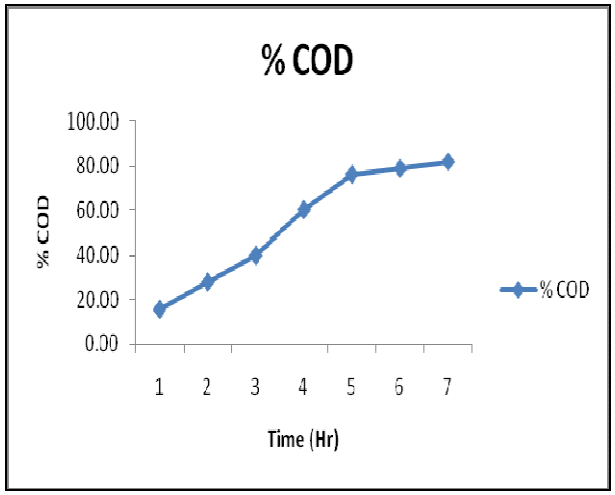

Fig 9: COD Removal by the BC from MB containing dye solution

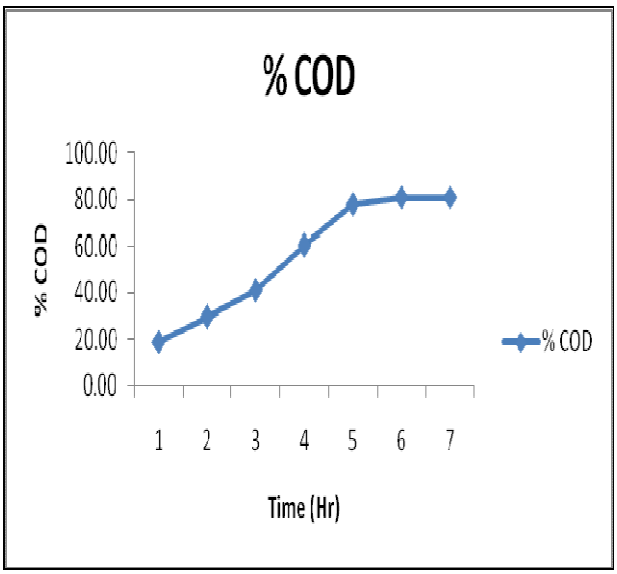

Fig 10: COD Removal by the BC from CR containing dye solution

3.4 Decolorization of Dyes using Fly Ash based Biologically Activated Carbon (BAC)

The immobilization of the bacterial consortium as Biologically Activated Carbon (BACs) was carried out using a mixture of fly ash based activated carbon and bacterial consortium in equal proportion. It was seen that 62.54 % MB (Fig 11) and 61.94% CR (Fig 12) was decolorized by biologically activated carbon just after incubation of 96 hr. & 108 hr. duration respectively. Although an increase was further reported in the decolorization of dyes but that was of no significance keeping in mind the cost & other factors.

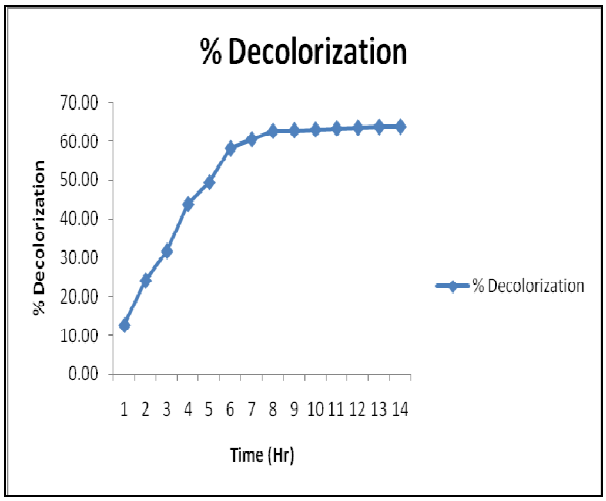

Fig 11: Decolorization of MB using BAC

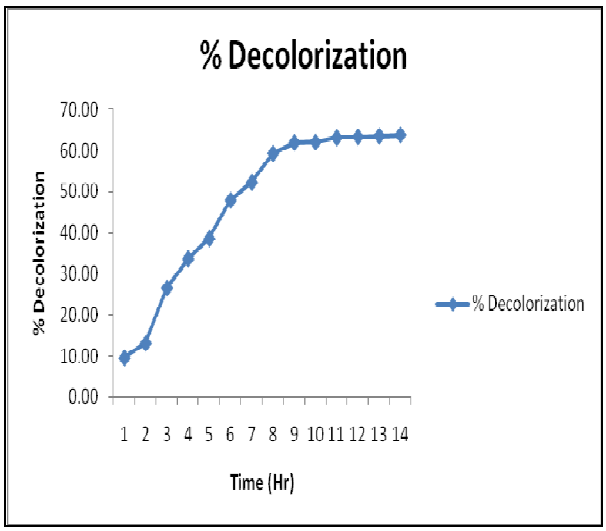

Fig 12: Decolorization of CR using BAC

[IV] CONCLUSIONS

Removal of dyes, mixture of methylene blue (MB) and congo red (CR) from aqueous solutions by adsorption with fly ash based activated carbon has been experimentally determined and the following observations are made:

- The percentage of colour removed increase with increasing adsorbent dosage & increase with increasing contact time.
- The adsorption rates increases with increasing temperatures.
- Optimum contact time for equilibrium to be achieved is found to be 2 hours (120 min). It is basically due to saturation of the active site which does not allow further adsorption to take place.
- Optimum adsorbent dose for the dye is found to be 3g /100ml. It is obvious as with increasing amount the active sites for adsorption of mixture of three dyes increases which results in an increase in removal efficiency. The decrease in adsorption capacity with an increase in the adsorbent concentration could be ascribed to the fact that some of the adsorption sites remained unsaturated during the process and agglomeration of activated carbons as a result all the surface area is not available for adsorption process.
- The adsorptions of days also follow the pseudo first order kinetics.
- Bacterial consortium (co-culture of *Bacillus* Sp. & *Kurthia* Sp.) has enormous potential to degrade the dye solutions or textile waste and resolve the problems of unnecessary dyes & high COD present in the effluents of textile industries.
- Methylene blue was the dye most remarkably decolorized than the Congo red.
- Dye decolorization by the biologically activated carbon was very high as reflected from the results. Using such a system, the decolorization of dye solutions or textile waste water containing these dyes is quite cheaper as compared to the independent process either by using ACs or by using bacterial consortium alone and the other conventional treatment process.
- Results concluded that BAC prepared by mixing fly ash based activated carbon & co-culture of *Bacillus* & *Kurthia* Sps. May be a promising biologically activated carbon to depollute the textile waste water effluent containing MB & CR.

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