CHAPTER 6

REMOVAL OF PHENOLS USING BIOACTIVE ACTIVATED CARBON SYSTEMS

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6.1 INTRODUCTION

Activated carbon units as a part of wastewater treatment are in use for many decades. It is established fact that the activated carbon is a very effective non-specific adsorbent (Vidic and Suidan 1993). It can adsorb a variety of matter from aqueous solutions. However, their use entails high capital and operating costs. Also, the activated carbon used is either to be discarded or to be regenerated after the carbon has reached saturation. Among several methods of regeneration, thermal regeneration is the most widely accepted method in industry. However, this method involves loss of carbon and high energy demand. This has motivated the researchers to think of methods of regenerating the carbon, which are less expensive and low on energy demand. One such method is biological regeneration. The results of the research work on this method have been found promising.

The present research work is concerned with the removal of phenol and its derivatives in a bioactive granular activated carbon system.

6.2 THEORY

6.2.1 General Description

As described in Chapter 1, adsorption of bacteria on activated carbon and formation of a biofilm on these activated carbon particles are essentially two steps involved in the bioactive activated carbon adsorbers. Adsorption and biodegradation are main removal mechanisms. The work on these two alternative methods of removal of phenols from aqueous solution have been presented and discussed in Chapters 4 and 5 respectively. Though biofilm covering the activated carbon employs the biodegradation as ultimate means of substrate removal, many factors related to the biofilm affect the process dynamics in one way or the other. Therefore, anything that affects the biofilm indirectly should have impact on the performance of bioactive activated carbon.

6.2.2 Biofilm Formation

Bacteria when come in contact with any surface submerged in an aqueous environment stick firmly to it. A matrix of polymers, mainly polysaccharide extends from the surface of the cell. The

bacteria attach to the surface by this matrix and form a mass of tangled fibers, which is called glycocalyx (Costerton et al 1978). As cells grow and reproduce at the surface, the biomass and associated material increase. Gradually, the entire surface is covered by bacteria. This coverage of bacteria on surface is termed as the biofilm.

Several physical, chemical and biological processes contribute in the development of a biofilm on a surface exposed to the fluid flow. These are as follows (Bryers 1987, Charaklis 1981):

i. Transport and adsorption of organic molecules to the surface.
ii. Transport of microbial cells to the surface.
iii. Microbial transformations at the surface (growth and extracellular polymeric substances), this results in formation of biofilm.
iv. Any detachment of the biofilm because of fluid shear stress.

First step occurs instantaneously, as the bacteria present in solution come in contact with the surface. This conditions the clean surface and it is a prerequisite for biological attachment. Fluid flow regime around the surface affects the transport of microbial cells from bulk to the surface. Molecular diffusion and turbulent eddy transport are dominant mechanisms in turbulent flow whereas in a static fluid by glycocalyx attachment. The commonly accepted view for attachment of microbes to the surface is that the production of a polysaccharide binding material is necessary (Lessard and Bihan 1996). Further, biofilm formation results from the reproduction of cellular mass and its binding by the polysaccharide. The rate of biofilm formation depends on the diffusion of nutrients into the film and their subsequent assimilation into attached biomass. When the biofilm has grown thick enough, the nutrients and/ or oxygen do not reach the inner most part of the biofilm, this becomes loose at those places. Biofilm thus is removed from those places and this is called sloughing of biofilm. Biofilm is also removed continuously because of shearing action of fluid flow around biofilm coated particles. Sloughing and shearing put together are called detachment of biofilm. The biofilm is the result of immobilization of cells on the surface.

6.2.3 Immobilization

To prevent the free movement of the cells by attaching them to some surface is called immobilization of cells. Formation of biofilm on activated carbon is also one of the excellent examples of whole cell immobilization. Several methods of immobilization include adsorption, covalent bonding, cell to cell cross linking, micro encapsulation and entrapment in polymeric networks. The adsorption method of immobilization is presented here.

6.2.3.1 Adsorption method of Immobilization

Adsorption of the whole cell on a solid support is a classical method of immobilization (Klein and Vorlop 1985). Vinegar production using woodchips as support for bacterial adsorption has been in existence since 1823. A suitable solid support is mixed with the cells in suspension and cells attach to the surface almost completely. As the solid support is inert and no additional chemical is used in this process, this technique of immobilization does not alter the physiological conditions of the cells. Therefore, this technique of immobilization is very much suitable for separation processes; however, presence of live cells is desirable (Annadurai et al. 2002).

6.2.4 Microbes on Activated Carbon

The granular activated carbon (GAC) has large surface area, porous structure and affinity for adsorption of organic nutrients. Therefore, these attributes make it ideal for bacteria attachment. Cario et al. (1979) conducted elaborate studies on the microbiology of granular activated carbon used in the treatment of potable water and found GAC to be an ideal habitat for bacterial growth. These bacteria in GAC have either a beneficial or detrimental effect. The beneficial effect includes the removal of organics through biodegradation. If the pathogenic bacteria develop in the bed, these may give health risk. Further, research work of Cario et al. (1979) suggests that the bacterial population follow a sigmoid growth. That is, the growth rate of bacteria increases exponentially after a short lag phase until their environment limits their growth. At this point, the bacterial population becomes stable with the given environmental conditions. And mixed culture of microbes establish in due course. However, in the present study, the pure culture of the *P. aeruginosa* (ATCC 9027) has been used to keep the ecology of the GAC simple.

6.3 EXPERIMENTAL

6.3.1 Materials

6.3.1.1 Activated Carbon

The activated carbon used for the immobilization of the *P. aeruginosa* was properly sterilized before adding to the batch reactor. This was achieved by wrapping the weighted activated carbon in aluminum foil and keeping it in oven at 110°C for 12 h.

6.3.1.2 Microorganism

The well-acclimatized culture of *Pseudomonas aeruginosa* (ATCC 9027) was used.

6.3.1.3 Stock Solutions

The stock solutions of phenol, 3-aminophenol and catechol were prepared by dissolving 5 g of each compound in sterilized distilled water and made up to one liter. Subsequently dilute solutions were prepared by adding appropriate volumes of the stock solutions to sterilized distilled water to get the working solution for each experimental run.

6.3.1.4 Growth Medium

These research studies were carried out in the same sterilized synthetic medium as used in biological studies and described in Chapter 4.

6.3.2 Methods

6.3.2.1 Experimental Method

The phenol, 3-aminophenol and catechol adsorption, immobilization of *P. aeruginosa* and removal of these phenols by bioactive activated carbon were studied in cotton plugged conical flask of 250 ml. Each flask contained 100 ml of the final solution. Solutions were prepared by pouring the calculated volumes of BSM solutions into the flask and subsequently, the required amount of the stock solutions of phenol, 3-aminophenol and catechol were added so as to get the solution of desired concentration.

Broth obtained after complete biodegradation of 800 mg/l of phenol present in solution, was used for immobilization of bacteria on activated carbon. 50 ml of broth with 50 ml of fresh BSM solution was contacted with 0.25 g of oven dried activated carbon.

To initialize the immobilization process, 50 ml of fresh BSM solution was contacted with 0.25 g of oven dried granular activated carbon and turbidity of medium was observed at different intervals. After the turbidity of liquid medium stopped changing it was decanted. The activated carbon was washed again with the fresh BSM solution. Subsequently the BSM solution with desired concentration of phenol was poured into 250 ml flask containing washed activated carbon. In case of 3-aminophenol and catechol, broth of completely biodegraded solution having initial concentration of 500 and 600 mg/l respectively was taken. For each concentration, two or more flasks were used. All the experiments have been performed at 30°C in orbital incubator cum shaker of Scigenics Make, Saksham Technologies, Mumbai.

6.3.2.2 Scanning Electron Microscopy (SEM)

Biofilm coated granular activated carbon particles from each run were examined using a scanning electron microscope to observe the morphology and to determine the thickness of the biofilm on them. Biofilm coated GAC particles obtained at the end of a run carried out with initial phenol concentration of 800 mg/l, 3-aminophenol concentration of 500 mg/1 and initial catechol concentration of 600 mg/1 were taken for SEM after drying at 50°C for 15 min. SEM was also performed on the virgin activated carbon. SEM was performed at SICART, V. V. Nagar.

6.4 RESULTS AND DISCUSSION

6.4.1 Immobilization and Effect of Contact Time

Immobilization of *P. aeruginosa*(ATCC 9027) on granular activated carbon was studied in batch mode as described in experimental section. To monitor the immobilization process, visual observation has been made for turbidity of sample. The reduction in turbidity determines the quantity of bacteria attached to the activated carbon surface. Though the same bacteria *P. aeruginosa* was used for degradation of all the phenols, separate immobilization was carried out with each phenol. It may be noted that during acclimatization, surface properties of bacteria which may affect the adsorption of bacteria on activated carbon may change, therefore, may show different adsorbability. Even the same bacterial species acclimatized to one substrate may show different degradation potential for another substrate. Therefore it was thought appropriate to conduct separate immobilization step for all compounds. Most of the organisms are immobilized within 18 h of contact for all the systems. After about 18 h of contact, the adsorption was slow, since there was no change in the turbidity. All the systems showed the similar immobilization characteristics with respect to time.

6.4.2 Removal of phenols by Bioactive activated carbon

The removal of phenol, 3-aminophenol and catechol were studied in batch mode. In all the experiments, the dose of activated carbon added was 2.5 g/1. The initial concentration of phenols studied was 800, 1000 and 1200 mg/l. In case of catechol, the initial concentration of 600, 700 and 800 mg/l and concentration of 500, 600 and 700 mg/l for 3-aminophnenol has also been checked. Figs. 6.1, 6.2 and 6.3 show the concentration history of phenol, catechol and 3-aminophenol respectively.

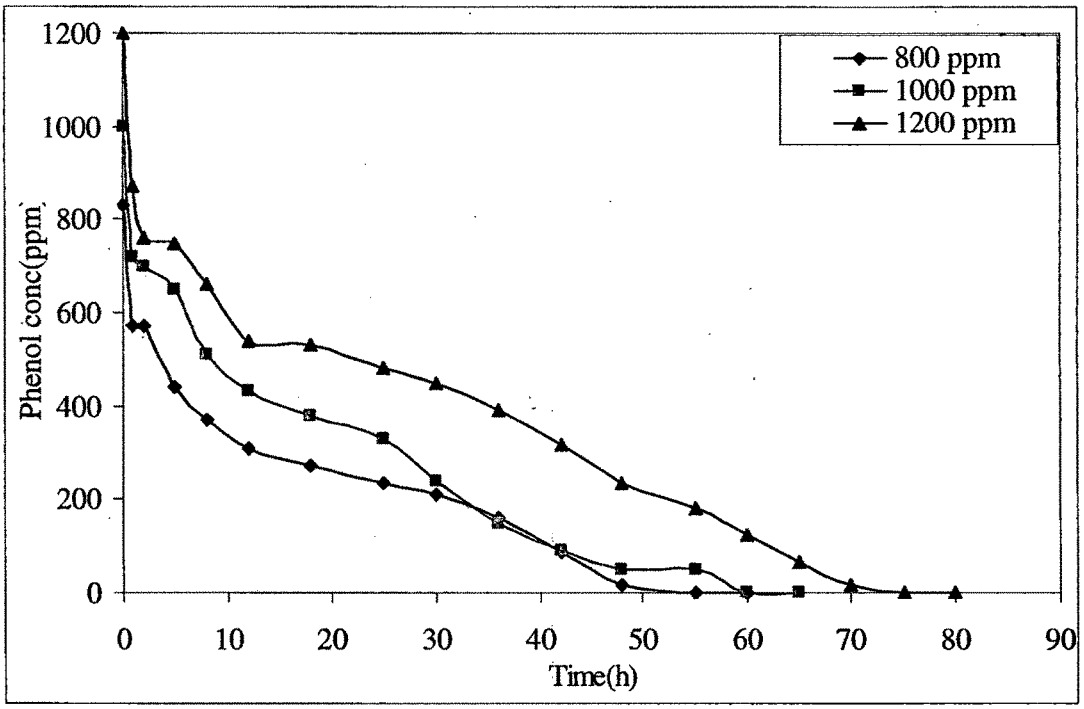

Figure 6.1. Phenol removal by a coupled system of *P. aeruginosa* and activated carbon in batch reactor [Temp.= 30 °C, pH=7]

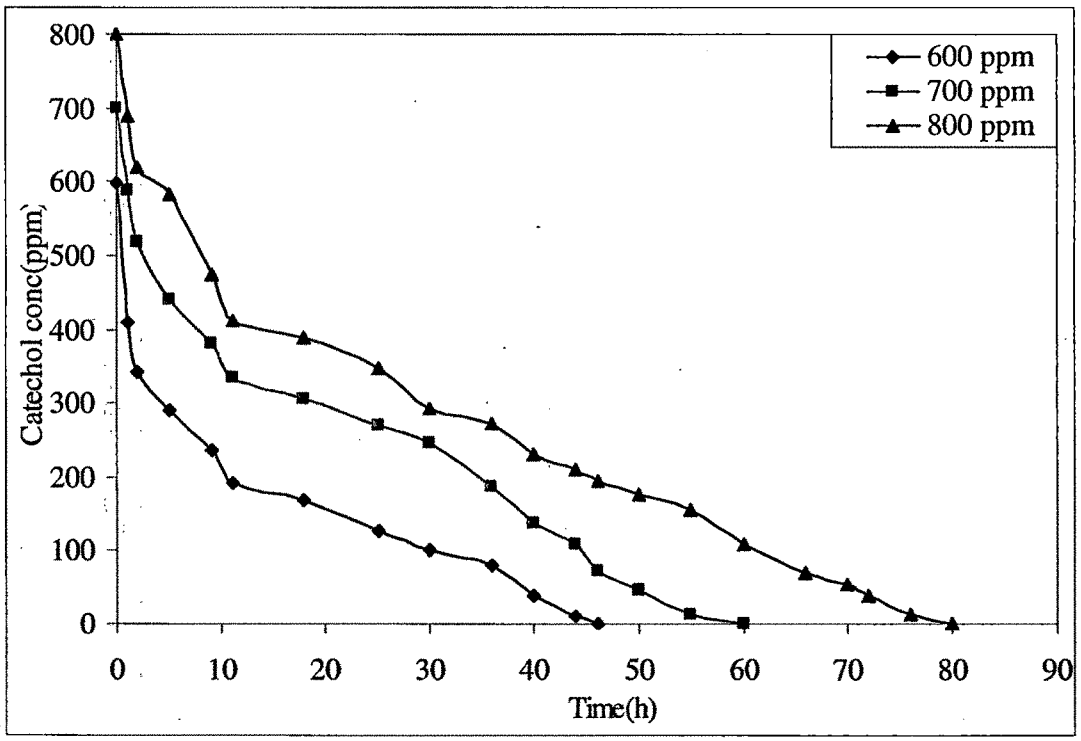

Figure 6.2. Catechol removal by a coupled system of *P. aeruginosa* and activated carbon in batch reactor [Temp. = 30 °C, pH=7]

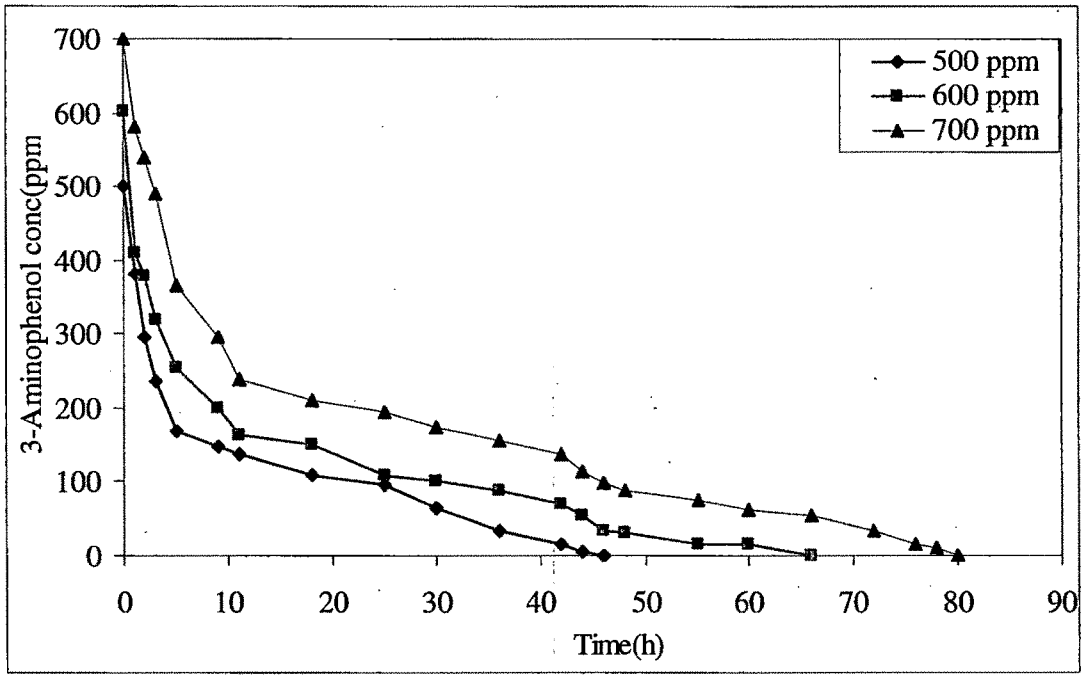

Figure. 6.3. 3- aminophenol removal by a coupled system of *P. aeruginosa* and activated carbon in batch reactor [Temp. = 30 °C, pH=7]

All the plots show the similar trend in that the removal is initially faster and then a slow phase follows. The initial faster removal may be due to the adsorption and then the slow removal is accomplished through degradation. This may be substantiated by the fact that there was no or little biomass growth during initial periods of removal and after that a phase of consistent biological growth was observed. The phenomenon of initial faster removal by adsorption followed by biological reduction may be regarded as characteristic of such systems, was observed, both in batch and continuous reactors also by Ehrhardt and Rehm (1985) and Lin and Weber (1992).

When phenol was removed by biodegradation alone, the well- acclimatized bacterial strain of *P. aeruginosa* (ATCC 9027) could metabolize the initially present phenol concentration of 800 mg/1 in 136 h with a lag phase of 3 days and could not metabolize 1000 mg/1 even up to 23 days. Similarly 3-aminophenol and catechol could metabolize at an initial concentration of 500 mg /1 in 160 h with lag phase of 2.5 days and at 600 mg/1 in 85 h with 2 days lag phase respectively. They could not be metabolized at all even with a well acclimatized culture of *P. aeruginosa* (ATCC 9027) with initial concentration of 600 and 700 mg/l of 3-aminophenol and catechol respectively. Therefore, the removal behavior of phenol at initial concentration of 1000 and 1200 mg/ 1, of 3-aminophenol at 600 and 700 mg/l and of catechol at 700 and 800 mg/l was studied on bioactive activated carbon. These concentrations of compounds were 110% to 150% of those concentrations to which cultures were acclimatized. At all these initial concentrations and for all compounds,

removal was complete in bioactive activated carbon system. Also, there was no lag phase observed. This suggests that reactor using bioactive activated carbon can be operational with no start up time. Also, the functioning of such reactors would not be disturbed in case of shock loads (at least up to additional 50% of design load). It may be concluded that the degradative capacity of bacteria increases by immobilization on activated carbon.

Initially adsorption of phenols might be taking place and thereafter due to lowering of concentration of phenol / 3-aminophenol/catechol in liquid phase, a negative concentration gradient is established and phenol / 3-aminophenol / catechol desorb back into the liquid phase. Now, the phenol may either be degraded by the actively growing biofilm on activated carbon or may be degraded by the bacteria suspended in liquid phase. Therefore, it may be concluded that some sort of active sites creation takes place as a result of desorption of adsorbed phenol, 3-aminophenol or catechol. This phenomenon has also been reported by Ehrardt and Rehm (1985). And if this happens than active site generation through bacteria may be the cause of regeneration. This type of regeneration is called as bio-regeneration.

It may be concluded that activated carbon and *P. aeruginosa* function together in degrading/removing the phenolic compound and are rather complementary to each other. Ehrardt and Rehm (1985), while investigating the phenol degradation behavior by species of *Candida* and *Pseudomonas* immobilized on activated carbon reported that the activated carbon operated like a buffer and it protected the immobilized microorganism by adsorbing toxic phenol concentrations. The result of present study corroborates this view.

6.4.3 Morphology of Biofilm

This is evident from the discussion under biofilm formation in theoretical background section, that a biofilm may be developed on any solid surface exposed to the liquid growth medium containing bacteria. In the present study, the bioactive activated carbon particles obtained after the complete consumption of 800 mg/l phenol, 500 mg/l of 3-aminophenol and 600 mg/l of catechol in batch reactors were examined for the formation, nature and morphology of biofilm formed on these particles. Figs. 6.4, 6.8, 6.12 and 6.16 show the photomicrograph of virgin carbon at 350X, 650X, 800X and 200X magnification. Figs. 6.5 to 6.7, 6.9 to 6.11 and 6.13 to 6.15 show the bacterial mass growing on phenol, 3-aminophenol and catechol respectively along with particles of activated carbon. Comparison of Figs. 6.4 to 6.7 shows the partial film on activated carbon whereas Figs. 6.8 to 6.19 clearly show clusters of biomass with reference to virgin carbon. The individual bacteria *P. aeruginosa* is also seen there. The one striking feature is that the growth of bacteria has different but uniform morphology for different phenols. This reinforces the contention

that the integrity and purity of the bacterial culture of *P. aeruginosa* was maintained till the end of the experiments.

Smear on 650X magnification has appeared in virgin carbon also, hence it can be considered as inherent property of activated carbon rather than of any impurity. Different morphology for the degradation of different phenols can be explained on the basis that specific enzymes are excreted for adaptation.

Figs. 6.9 to 6.11 also reveal the growth of bacteria in the inside of the macro pores of the activated carbon particles. This is possible as the activated carbon used in the present study is relatively macro-porous with 41 % of total pores having diameters greater than 5 μm. For the accessibility of the inner surface of the pores of the porous material by bacteria and yeast, a ratio of pore to cell diameter of 4 to 5 has been reported (Messing et al 1979). The diameter of macro pores of the activated carbon particles used in this study is approximately ten times the size of the rod shaped *P. aeruginosa* species (approximately 0.5 μm).

On examination of the plates on SEM, it was observed that most of the particles were not fully covered with the biofilm. Rather, the smooth surface shown is that biomass is present in the pits and crevices of the particle. This is quite possible as the activated carbon particles were scanned only after 3 to 7 days of initiation of immobilization process. Craik et al (1992) also found that the granular activated carbon particles could not be fully covered even after 276 days of operation of the biological reactor. They consider that this non-establishment of biofilm even after so many days of operation is due to the shear field around the particles. The present studies were conducted in batch reactor under good mixing conditions for aerobic bacteria, therefore, the full establishment of biofilm might have been restricted by the turbulent fluid flow around the granular activated carbon particles.

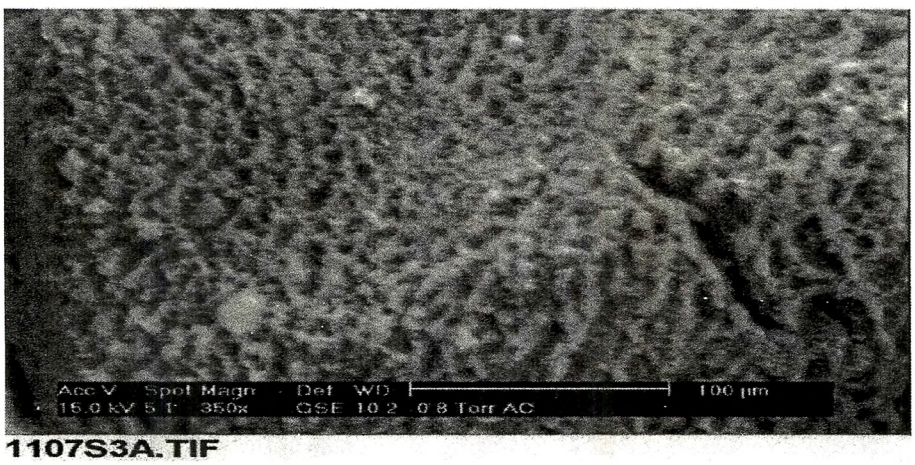

Figure 6.4. Scanning Electron Micrograph of Virgin Carbon at low magnification

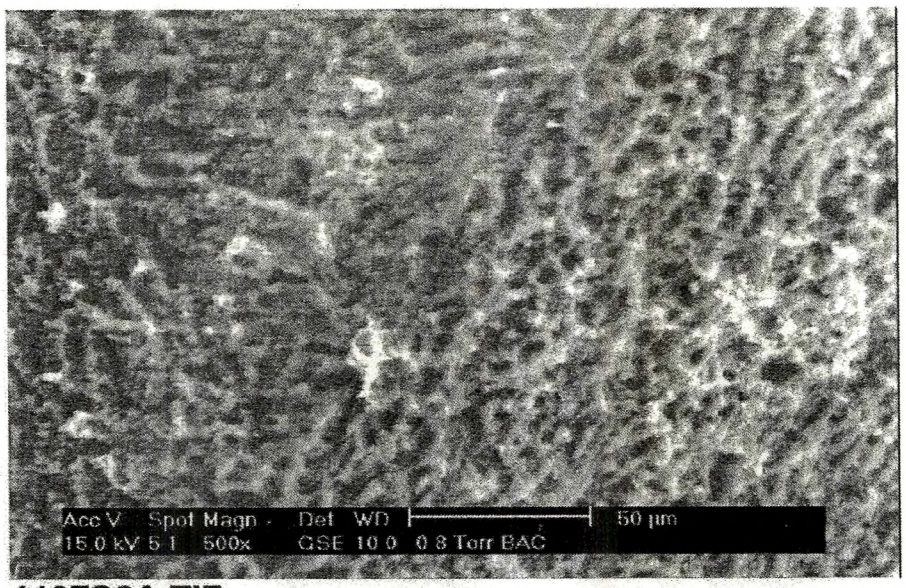

Figure 6.5. Scanning Electron Micrograph showing partial biofilm on activated carbon [*P. aeruginosa*/ Activated carbon / Phenol system]

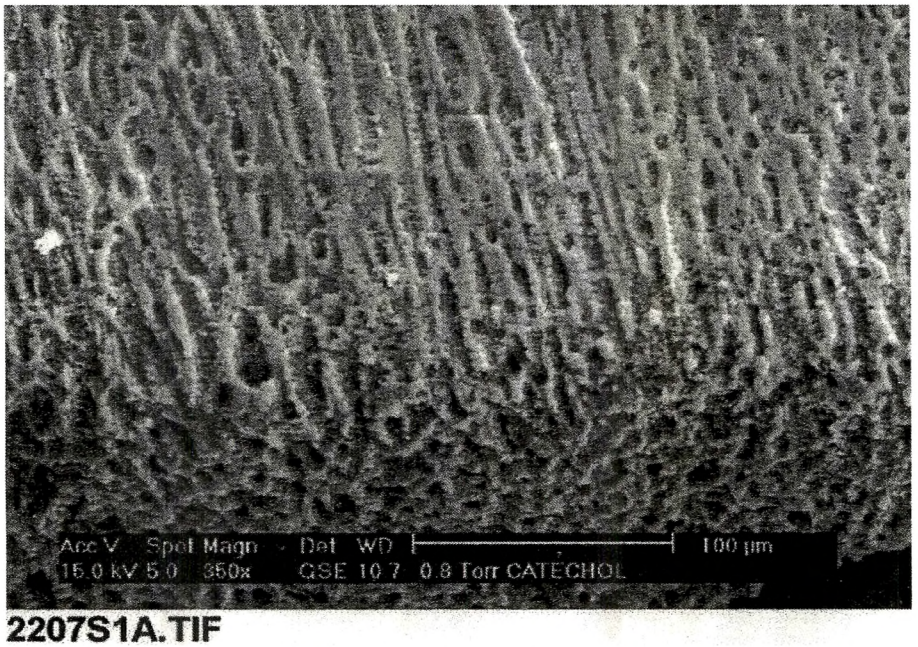

Figure 6.6. Scanning Electron Micrograph showing partial biofilm on activated carbon [*P. aeruginosa*/ Activated carbon / Catechol system]

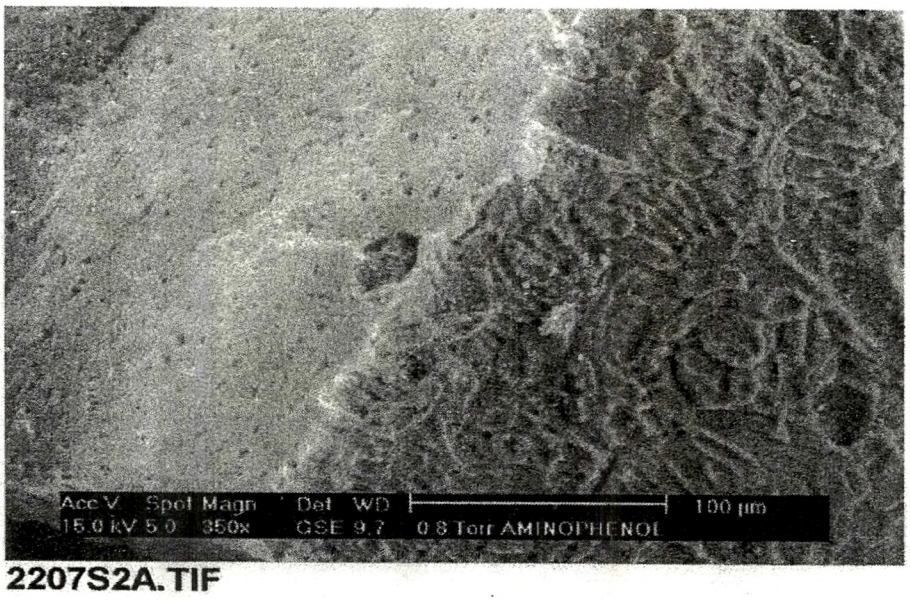

Figure 6.7. Scanning Electron Micrograph showing partial biofilm on activated carbon [*P. aeruginosa*/ Activated carbon / 3-Aminophenol system]

Figure 6.8. Scanning Electron Micrograph of Virgin Carbon at medium magnification

Figure 6.9. Scanning Electron Micrograph showing biofilm in pits and crevices of activated carbon [*P. aeruginosa*/ Activated carbon / Phenol system]

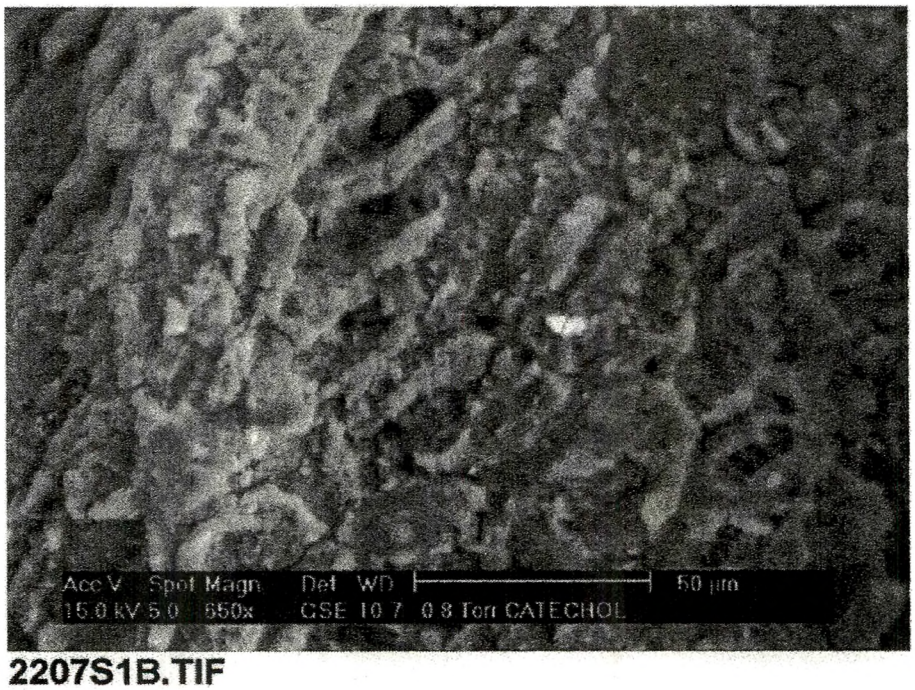

Figure 6.10. Scanning Electron Micrograph showing biofilm in pits and crevices of activated carbon [*P. aeruginosa*/ Activated carbon / Catechol system]

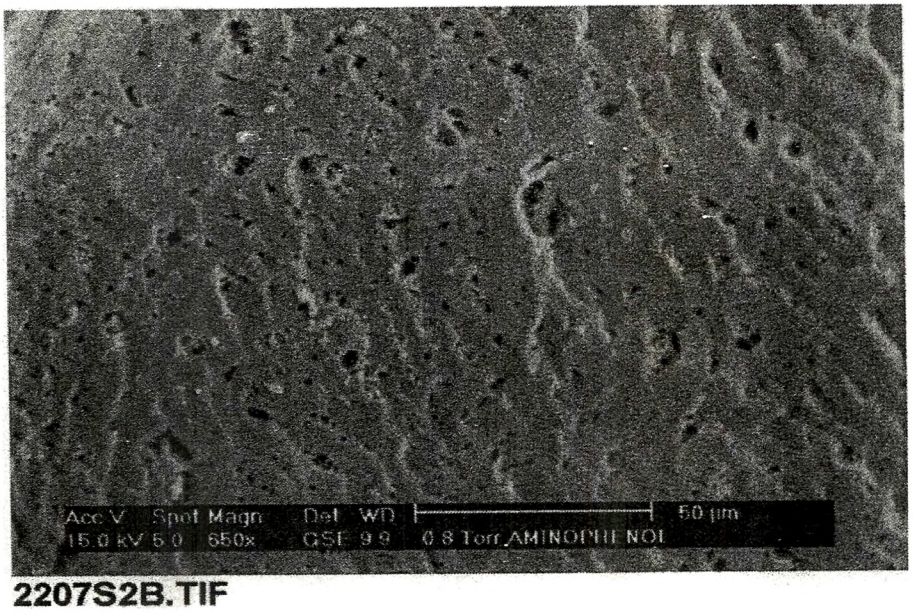

Figure 6.11. Scanning Electron Micrograph showing biofilm in pits and crevices of activated carbon [*P. aeruginosa*/ Activated carbon / 3-Aminophenol system]

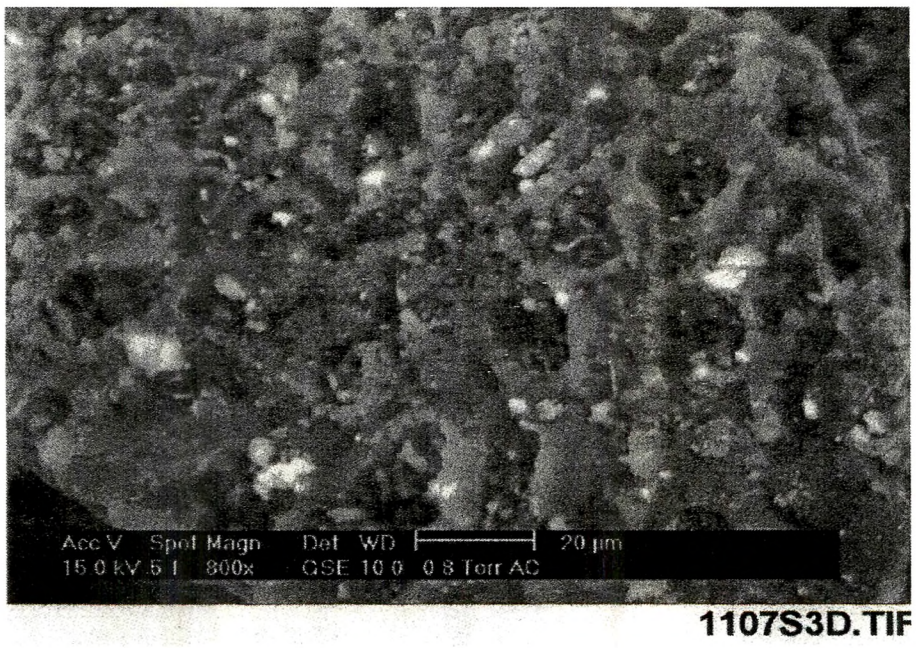

Figure 6.12. Scanning Electron Micrograph showing smear on virgin carbon at high magnification

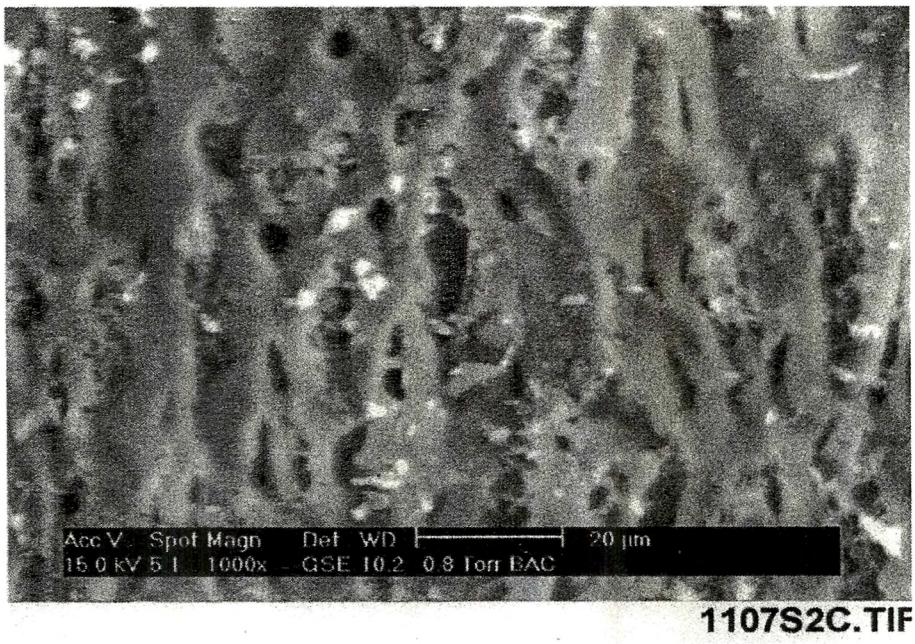

Figure 6.13. Scanning Electron Micrograph showing smear in biofilm on activated carbon [*P. aeruginosa*/ Activated carbon / Phenol system]

Figure 6.14. Scanning Electron Micrograph showing smear in biofilm on activated carbon [*P. aeruginosa*/ Activated carbon / Catechol system]

Figure 6.15. Scanning Electron Micrograph showing smear in biofilm on activated carbon [*P. aeruginosa*/ Activated carbon / 3-Aminophenol system]

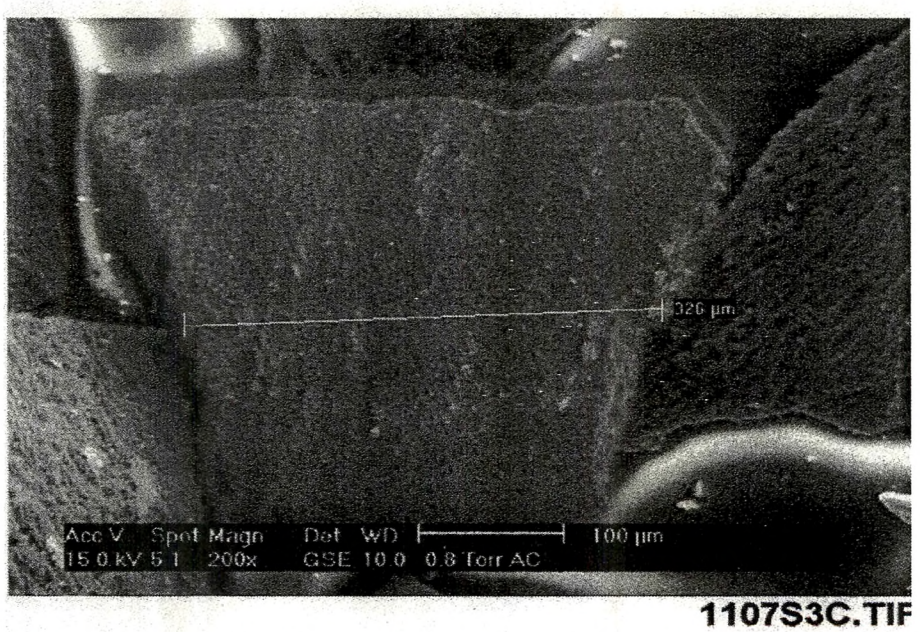

Figure 6.16. Scanning Electron Micrograph of virgin carbon for biofilm thickness determination

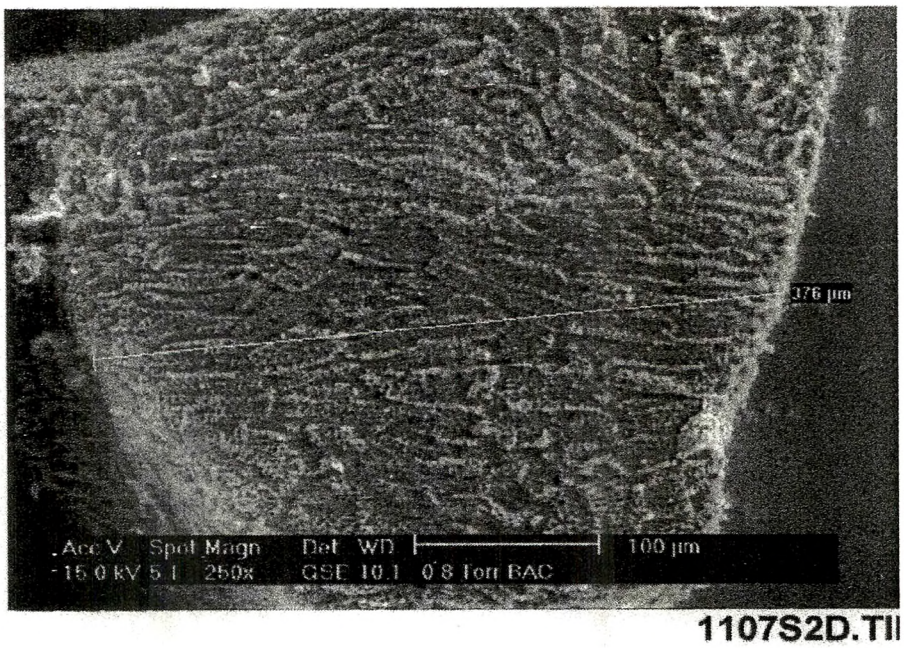

Figure 6.17. Scanning Electron Micrograph for biofilm thickness determination [*P. aeruginosa*/ Activated carbon / Phenol system]

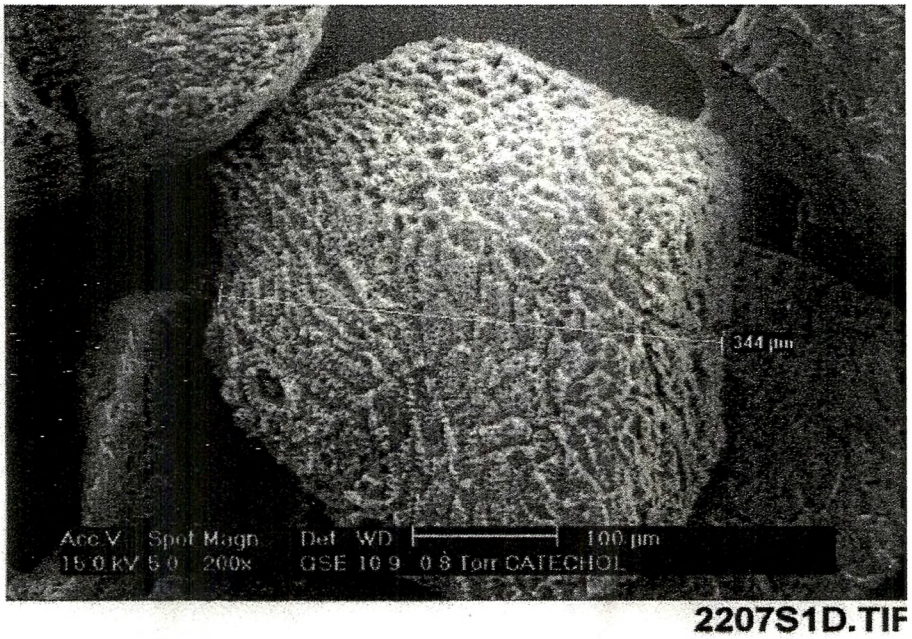

Figure 6.18. Scanning Electron Micrograph for biofilm thickness determination [*P. aeruginosa*/ Activated carbon / Catechol system]

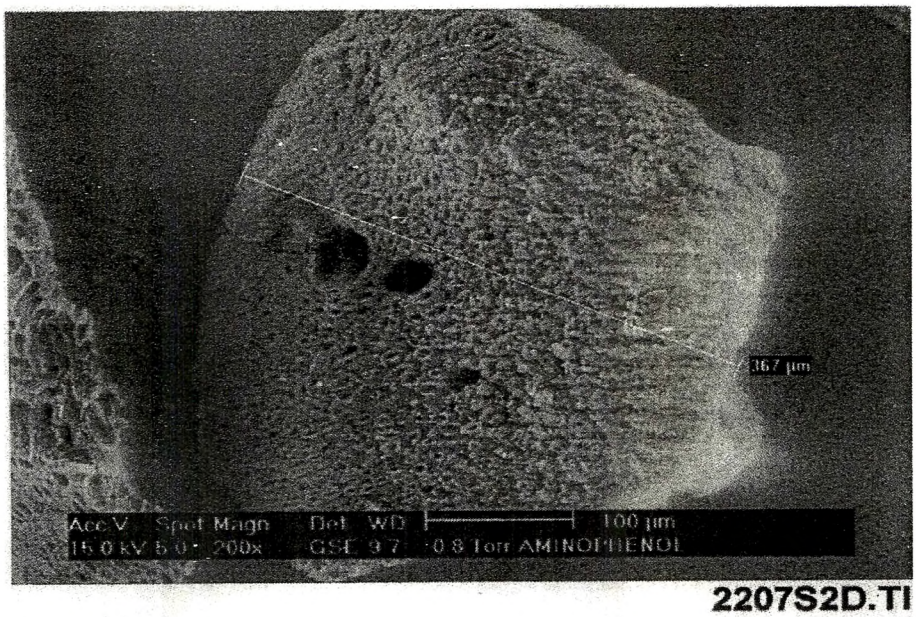

Figure 6.19. Scanning Electron Micrograph for biofilm thickness determination [*P. aeruginosa*/ Activated carbon / 3-Aminophenol system]

6.4.4 Biofilm Thickness

As a result of consumption of all the substrate from the liquid medium and the solid adsorbent, the biomass on the solid adsorbent keeps on building up. Also, this is removed by the action of shearing and sloughing. Thus, the biomass on the particle is the net result of above factor. For spherical particles of known diameter, the net biomass is characterized in terms of biofilm thickness. In the present study, the biofilm thickness was determined on the biofilm coated particles obtained at the end of the complete removal of initially present 800 mg/1 of phenol, 500 mg/l of 3-aminophenol and 600 mg/1 of catechol by bioactive activated carbon in batch reactors. Biofilm thickness can be either measured microscopically or calculated gravimetrically. Both the methods have been reported in the literature (Livingston and Chase 1989, Rittman 1980).

In present research program, diameter of a number of particles of virgin carbon and biofilm coated bioactive activated carbon particles from the experiments with phenol, 3-aminophenol and catechol have been measured. By comparing diameters of virgin carbon particle size shown in Fig. 6.16 with biofilm coated carbon particle shown in Figs. 6.17 to 6.19, the value of biofilm thickness for bioactive activated carbon obtained at the end of complete removal of initially present 800 mg/l of phenol, 500 mg/l of 3-aminophenol and 600 mg/l of catechol were found to be 25, 21 and 9 µm respectively. Whereas, the average thickness for 10 particles was found to be 28, 22 and 14 µm

respectively for phenol, catechol and 3-aminophenol. This method would be applicable for particles that are fully covered with the biofilm.

Another method based on gravimetry, was also employed for calculating the biofilm thickness. This approach is based on the premise that the biofilms are composed of 99% of water and 1% biomass. Therefore, the biofilm thickness, z can be calculated by the expression (Kim and Pirbazari 1989):

$$Z = \frac{W_e}{N\left(4\ \pi r_p^{\ 2}\right)\rho_l\,(0.99)} \qquad (6.1)$$

where, W_e is weight of evaporated water, ρ_l is density of water, r_p is radius of particle and N is number of particles in the sample.

5 to 10 activated carbon particles covered with biofilm were taken on a slide to determine their diameter by simple microscope. Average value of the radius of different particles was taken for calculating biofilm thickness. These values of average biomass thickness obtained for the three phenols are reported in Table 6.1. The values of biofilm thickness obtained are in the range 14 to 34 μm whereas reported in the literature are 25 to 50 μm (Annadurai 2002).

6.4.5 Bio-regeneration

The biological growth on the bioactive activated carbon increases the service life of activated carbon by degradation of phenolic compound in the liquid medium as well as phenolic compound present on the activated carbon. Thus, active sites are regenerated at the activated carbon surface. This effect was investigated by conducting the experiments with BAC using initial concentrations of 800, 500 and 600 mg/l for aqueous solution of phenol, 3-aminophenol and catechol respectively. The BAC obtained at the end of these experiments was washed with distilled water and again contacted with fresh liquid medium containing phenol, 3-aminophenol and catechol of 800, 500 and 600 mg/l concentration respectively for 48 h. From material balance, the quantity adsorbed was calculated. Also, the liquid medium at same concentration was contacted with virgin activated carbon for 48 h, and the quantity of the phenolic compound adsorbed was calculated. The difference between the two quantities gives the bio-regeneration (i.e. increase in capacity of the activated carbon). Table 6.2 presents the percentage bio-regeneration of the carbon saturated with phenol, 3-aminophenol and catechol. These experiments have been performed for higher

concentrations also viz. for phenol 1000 and 1200 mg/l, for catechol 700 and 800 mg/l and for 3-aminophenol 600 and 700 mg/l to check bio regeneration at these concentrations.

The percentage regeneration was found in the range of 33-66% in 48 h contact time. However, the regeneration was not complete. It may be due to insufficient contact time for regeneration. In case of phenolic compounds, the phenomenon or irreversible adsorption as a result of activated carbon catalyzed polymerization reactions has earlier been reported in aerobic systems (Vidic and Suidan 1993).

Table 6.1. Thickness of Biofilm on Bioactive activated carbon particles

	Activated carbon particles exposed to initial phenol concentration			**Activated carbon particles exposed to initial catechol concentration**			**Activated carbon particles exposed to initial 3-aminophenol concentration**		
	800 ppm	1000 ppm	1200 ppm	600 ppm	700 ppm	800 ppm	500 ppm	600 ppm	700 ppm
Biofilm thickness, µm	30	34	31	20	22	23	14	16	22

Table 6.2. Percentage Bio-regeneration of Activated carbon

	Activated carbon particles exposed to initial phenol concentration			**Activated carbon particles exposed to initial catechol concentration**			**Activated carbon particles exposed to initial 3-aminophenol concentration**		
	800 ppm	1000 ppm	1200 ppm	600 ppm	700 ppm	800 ppm	500 ppm	600 ppm	700 ppm
% Regeneration	58.1	48.8	40.8	62.9	47.5	40.6	66.4	37.8	33.1

6.5 CONCLUDING REMARKS

In this chapter the phenol, 3-aminophenol and catechol / bioactive activated carbon systems have been studied. The immobilization of P aeruginosa on activated carbon was done. The removal of phenol at initial concentration of 800, 1000 and 1200 mg/1, of 3-aminophenol at 500, 600 and 700 mg/l and of catechol at 600, 700 and 800 mg/l have been studied. Highest concentration selected was 50% greater than the highest concentrations at which *P. aeruginosa* (ATCC 9027) had been acclimatized to these phenolic compounds.

It was observed that most of the bacteria was adsorbed on activated carbon within approximately 18 h. The effect of initial concentration of the phenolic compound was to completely remove the phenols at all the concentrations studied without any lag phase. The biological activity in the liquid medium might have started after the concentration in the liquid medium has reached at a certain lower level.

After the complete consumption of the phenolic compounds, the particles were examined using SEM for the biofilm morphology. The complete biofilm was not observed, rather, clusters of biomass in the pits and crevices of the carbon particles and inside the macro pores were seen. Further, the biofilm thicknesses was found in the range 14 to 34 μm. The experiments were also conducted to determine the extent of bio-regeneration. The bio-regeneration was not complete and found in the range 33-66%.

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