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Comparative Studies on Simultaneous Adsorption and Biodegradation, Adsorption and Biodegradation for Treatment of Wastewater containing Cyanide and Phenol

Bhumica Agarwal[1*], Ravi Kiran Vedula[2] and Chandrajit Balomajumder[3]

1,3Department of Chemical Engineering, IIT-Roorkee, Uttrakhand, INDIA.
2Rashtriya Chemical and Fertilizers Ltd., Thal Alibag, Maharashtra, INDIA.

*Corresponding author: bhumica.agarwal@gmail.com

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Abstract

This paper presents a comparative study of the efficiency of biodegradation, adsorption and simultaneous adsorption and biodegradation (SAB) process for the remediation of industrial wastewaters containing both cyanide and phenol. Adsorption was carried out using granular activated carbon (GAC), while biodegradation was achieved by co-fermentation with *Pseudomonas putida* and *Azotobacter chroococcum.* During co-metabolism *P. putida* utilized phenol as carbon source while *A. chroococcum* utilized cyanide as nitrogen source for growth. The biodegradation efficiency decreased with increasing concentrations of phenol and cyanide and was observed as 99.99, 92.45, 86.12, 75.21 and 60.34% for cyanide and 99.61, 85.62, 79.15, 64.21 and 56.63% for phenol respectively after 60 h of agitation when initial concentration was increased from 50-350 mg L^{-1}. With adsorption on GAC, the removal efficiencies were found to be 85.8, 77.67, 75.51, 58.25 and 50.73% for cyanide and 73.92, 72.99, 71.23, 60.13 and 51.55% for phenol respectively after 72 h of agitation. However SAB process was found to be better than biodegradation or adsorption alone in terms of both removal efficiency and time required for remediation with removal efficiencies > 94% for initial cyanide and phenol concentrations of 50 and 100 mg L^{-1}.

Highlights

- Biodegradation efficiency decreased with increasing concentations of phenol and cyanide
- simultaneous adsorption and biodegradation was found to be better than biodegradation or adsorption alone.

Keywords: *Adsorption, Biodegradation, Bioremediation, Simultaneous Adsorption and Biodegradation*

Compounds of cyanide and phenol are strictly regulated because of their toxicity (Busca *et al.,* 2008; Dash *et al.,* 2009). The MCL (Maximum Contaminant Limit) of phenol and cyanide in industrial discharge has been set as 0.5 mg L^{-1} and 0.2 mg L^{-1}, respectively by USEPA, WHO and CPCB, India. Slight presence of these compounds is seen

in the daily items like foods, cosmetics, microorganisms etc. In large concentrations these are found in the effluents of industries like coke plant, paint and dyes, automobiles, explosives, chemicals, pesticides, synthetic fiber and resin manufacture etc. Exposure to even low concentrations of cyanide can cause coma, heart pains, breathing disorders, thyroid gland enlargement, headaches and even death. On the other hand, phenol exposure can lead to skin and eyes injuries, headache, vomiting, gastrointestinal disorders, central nervous system depression, lung, kidney, liver and heart damage ultimately leading to death. Owing to their extreme toxicity efforts have been made for their remediation from industrial wastewater (Veeresh *et al.,* 2005; Dash *et al.,* 2008b).

Adsorption process is one such frequently used technology employing some common adsorbents like silica, alginate bead, activated carbon, polymeric material, etc. (Pazarlioglu and Telefoncu, 2005). There are several reports on the individual treatment of cyanide and phenols by adsorption on plain as well as metal-impregnated activated carbons as GAC as it has good adsorptive capacity (Srivastava *et al.,* 2006; Dash *et al.,* 2008b). However process of adsorption using GAC is marred by its high cost and difficulty in regeneration. Lately biodegradation has emerged as a technique of choice for wastewater treatment. It has been found that some microbes like *Pseudomonas putida* (El-Naas *et al.,* 2009, 2010), *Pseudomonas pesudomallei* (Afzal *et al.,* 2007), *Pseudomonas aeruginosa* (Afzal *et al.,* 2007), *Alcaligenes faecalis* (Jiang *et al.,* 2007) *etc.* utilize phenol like compounds as sole source of carbon and energy and strains such as *Pseudomonas putida*, *Pseudomonas pseudoalcaligenes* (Huertas *et al.,* 2010), *Pseudomonas fluorescens* (Dash *et al.,* 2008b), *Kelbseilla oxytoca* (Chen *et al.,* 2008) etc. consume cyanide as carbon and nitrogen source via specific enzymatic pathways (Gupta *et al.,* 2010).

Due to cost effectiveness of biodegradation and environmental favorability of adsorption, these process are extensively used for treatment of cyanide and/ or phenol laden wastewater. Few researchers have observed that combined application of these two techniques resulted in better removal efficiencies than individual processes (Mondal and Balomajumder, 2007; Dash *et al.,* 2008b). Process of simultaneous adsorption and biodegradation is essentially of surface interaction and is characterized by rapid uptake of ions by adsorbent surface followed by degradation by microorganisms. Rapidity of the process makes it a good candidate for use in effluent treatment on a large scale.

Keeping these facts in view the present study deals with simultaneous adsorption and biodegradation of cyanide and phenol. Optimization of process parameters like adsorbent dose, contact time and pH was carried out. The adsorption and biodegradation efficiency was calculated for both cyanide and phenol. Finally comparative study between adsorption, biodegradation and SAB was done and presented in this paper.

Materials and Methods

Preparation of Cyanide and Phenol solution

Cyanide solution was prepared by dissolving 1.89 g of NaCN in 1 L of distilled water whose pH was pre-adjusted to 10, to yield a stock cyanide solution of 1000 mg (cyanide) L^{-1}. Phenol solution (1000 mg L^{-1}) was prepared by dissolving 1 g of pure phenol crystals in 1 L distilled water. Synthetic simulated solution with initial cyanide and phenol concentrations ($C_{i,CN}$ and $C_{i,Ph}$) of 50, 100, 200, 300 and 350 mg L^{-1} with cyanide and phenol in 1:1 ratio, were prepared by appropriate dilutions of stock solutions with distilled water and used for adsorption, biodegradation and SAB study.

Adsorption Studies

GAC particles of analytical grade, purchased from HiMedia Laboratories Pvt. Ltd., India, were used as adsorbent as well as the support medium for bio-layer formation. The bulk density of activated carbon was found to be 400 g L^{-1}. It was sieved to various fractions of particle sizes using standard sieves and particle size of 4-5 mm were used in the experiment. The adsorbent was treated with 0.5 M H_2SO_4 in 1:2 ratio of adsorbent to acid for 24 h. It was then dried in hot air over at 110°C for 2 h, after which it was stored in air tight bags. The elemental analysis, surface area, pore volume etc. of GAC used are listed in Table 1. All the studies were carried out in

250 mL conical flasks in incubator shaker at temperature of 30 °C and pH 7.

Micro organisms and growth conditions

Freeze-dried/lyophilized culture of *P. putida* (MTCC 1194) and *A. chroococcum* (MTCC 446) were obtained from Institute of Microbial Technology (IMTECH), Chandigarh, India. The microorganisms were grown in the standard Nutrient medium, purchased from HiMedia Laboratories Pvt. Ltd., India, at pH 7 and temperature 30 °C in an incubator shaker at 120 rpm for 24 h. After their complete growth, about 0.5 g microbial cells were collected by centrifuging the medium at 10000 rpm for 10 min and added to 50 mL of cyanide and phenol simulated solution. For biodegradation studies, simulated solutions of cyanide and phenol were prepared in modified enrichment medium whose composition is given in Table 2. For the preparation of simulated solutions enrichment media was sterilized in autoclave at 121 °C for 20 min after which cyanide and phenol from stock solutions were added in appropriate amounts. Addition of 2% glucose solution and 0.5 g L^{-1} NH_4Cl to the enrichment medium was found to be necessary for the initial growth of micro-organisms which on later stages were capable of utilizing cyanide and phenol for their growth. Excess addition of the glucose and NH_4Cl resulted in decreased amount of cyanide and phenol degradation since the bacteria preferentially utilized easily available sources instead of the toxic compounds.

Biodegradation and SAB studies

All batch experiments for biodegradation and SAB processes were performed in 250 mL conical flasks containing 50 mL of growth medium, 0.5 g microbial mass of each *P. putida* and *A. chroococcum*, at 30 ^oC in rotary incubator shaker at 120 rpm for 72 h. The effect of pH on the amount of degradation of cyanide and phenol for both biodegradation and SAB was studied for 100 mg L^{-1} initial concentration of cyanide and phenol each. 30 g L^{-1} sterilized GAC was added to the enrichment medium in conical flask for SAB study. Separate studies were conducted for various initial cyanide and phenol (in 1:1 ratio) concentrations. Samples were drawn periodically for determination of residual cyanide and phenol concentration.

Analysis

Parameters such as pH, total cyanide content, total phenol content and optical density (at 600 nm) were determined using standard procedures. Total cyanide concentration was determined by picric acid colorimetric method (at 520 nm wavelength) as described in standard methods (ASTM, 1987). Total phenol concentration was determined by colorimetric 4-aminoantipyrene method (at 510 nm wave length) (Ettinger *et al.,* 1951). For the detection of microbial growth, the biomass concentration was determined by measuring the absorbance of the broth at 600 nm using a standard curve of absorbance against dry cell weight. All spectrometric measurements were carried out using DR-4000 UV-Vis Spectrophotometer (Hach® USA). pH was measured as specified by standard methods (APHA, 2001) using pH meter supplied by Toschon pH/ Redox electrode, India. The CHNS analysis was done by CE-440 Elemental analyser, Exeter Analytical Inc. The pore volume and surface area were measured by nitrogen adsorption isotherm using Micrometrics Chemisorb 2720 instrument by Brunauer-Emmett-Teller (BET) method.

Results and Discussion

Effect of Initial pH

pH plays an important role in the removal of cyanide and phenol from aqueous solutions by adsorption, biodegradation and SAB processes. For various biological processes the optimum pH is generally designated as the near neutral pH i.e. in ranges of 6-8 (Busca *et al.,* 2008; Dash *et al.,* 2009). Dash *et al.,* 2008a and Srivastava *et al.,* 2006 reported the optimum initial pH for adsorption of cyanide (NaCN) and phenol as 9.2 and 6.5 respectively. Figure 1 and 2 shows the effect of pH on cyanide and phenol removal respectively by adsorption, biodegradation and SAB process. As is evident adsorption of cyanide was favored in alkaline conditions while that of phenol in acidic conditions. Adsorption of both cyanide and phenol was found optimum at near neutral pH of 8. The results are in compliance with the findings of Agarwal *et al.,* 2013. The effect of pH onto adsorption could be attributed to several mechanisms such as electrostatic interaction, complexation, ion exchange and surface charge on carbon (Blanco-Martínez *et al.,*

2009). Since cyanide and phenol behave in antagonistic manners, so the process is extremely sensitive to pH. During biodegradation and SAB process the maximum rate of cyanide and phenol removal was observed in the ranges of 7-8 and 5-7 respectively. At lower and higher pH values, biodegradation and SAB were found to be less effective. Thus to maintain a similarity in comparative studies of adsorption, biodegradation and SAB, pH of 7 (± 0.1), was taken as optimum value for three processes.

Table 1. Properties of GAC

S.No	Parameter	Value
1	Particle Size (mm)	4-5
2	Bulk Density (g L^{-1})	400
3	Surface Area (m^2 g^{-1})	228.6375
4	Total pore Volume (m^3 g^{-1})	0.1151
5	CHNS content (%)	C : 75.11 H : 1.913 N,S : 0, 0

Table 2. Composition of enrichment media for biodegradation of cyanide and phenol

S.No	Component	Concentration (g L^{-1})
1	K_2HPO_4	1
2	KH_2PO_4	1
3	$MgSO_4.7\ H_2O$	0.1
4	$CaCl_2.2H_2O$	0.02
5	NaCl	0.01
6	NH_4Cl	1
7	Sucrose	5

Adsorption capacity of GAC

Activated carbon has been used as an inert porous carrier material for distributing chemicals on the large internal surface thus making them accessible to reactants (Srivastava *et al.,* 2006). Various experiments were conducted for optimizing the adsorption process parameters like GAC dose, contact time etc. Adsorbent doses of 10-50 g L^{-1} were used and optimum dose was decided to be 30 g L^{-1} as 75-80% of cyanide and phenol was removed at this GAC dose, after 36 h of agitation at 50 mg L^{-1} initial concentration of cyanide and phenol (Figures 3 and 4 respectively). On an optimum value of 30 g L^{-1}, the removal efficiency was evaluated for various initial concentrations. It was observed that there was no significant removal of either cyanide or phenol at high initial concentrations i.e. 300 mg L^{-1} and above.

Figure 5 shows the effect of initial concentration on the percentage removal of cyanide and phenol. It was observed that removal efficiency of both phenol and cyanide decreases markedly on increasing their initial concentration. The effect could be attributed to the involvement of less favorable adsorption sites in adsorption of phenol and cyanide (Mishra and Bhattacharya, 2006).

Biodegradation studies of Cyanide and Phenol

The effects of parameters like pH and initial concentrations of substrates (glucose, NH_4Cl, cyanide, phenol) on the biodegradation of cyanide and phenol were investigated. For biodegradation study in the presence of cyanide and phenol, initial pH was adjusted to 7.0. Researchers have suggested that *P. putida* has the ability to utilize phenol as a carbon source for its growth (El-Naas *et al.,* 2009, 2010) and cyanide as nitrogen and carbon source (Babu *et al.,* 1992). But it was observed that when inoculated singly, *P. putida* cells were not completely able to utilize cyanide, though the degradation of phenol was more than 80%. Thus the second microbial strain *A. chroococcum* was also inoculated to aid the cyanide degradation. *A. chroococcum* is a nitrogen fixing bacteria similar to *Klebsiella oxytoca*, which utilizes nitrogenase enzyme to convert cyanide into methane and ammonia (Chen *et al.,* 2008; Dash *et al.,* 2009). It was seen that no degradation of either cyanide or phenol was seen in complete absence of additional carbon or nitrogen source. But when glucose and NH_4Cl were added, the degradation of cyanide and phenol increased indicating that initially the microorganisms propagated utilizing the carbon and nitrogen source and utilized cyanide and phenol as nitrogen and carbon sources in the later stages. Figure 6 shows the plot of percentage removal of cyanide and phenol at different initial pollutant concentrations. From the results it was observed that after 72 h of agitation, cyanide and phenol removal efficiencies were 99.99, 92.45, 86.12, 75.21 and 60.34% and 99.61, 85.62, 79.15, 64.21 and 56.63% respectively for initial cyanide and phenol concentrations of 50, 100, 200, 300 and 350 mg L^{-1} respectively.

Table 3. Comparison of removal efficiencies of adsorption, biodegradation and SAB

Initial Conc. (mg L^{-1})	Removal Efficiency% (Adsorption)		Removal Efficiency% (Biodegradation)		Removal Efficiency% (SAB)	
	Cyanide	Phenol	Cyanide	Phenol	Cyanide	Phenol
50	85.8	73.92	99.99	99.61	99.99	99.99
100	77.67	72.99	92.45	85.62	99.64	94.03
200	75.51	71.23	86.12	79.15	97.26	85.49
300	58.25	60.13	75.21	64.12	86.21	76.3
350	50.73	51.55	60.34	56.63	74.74	71.12

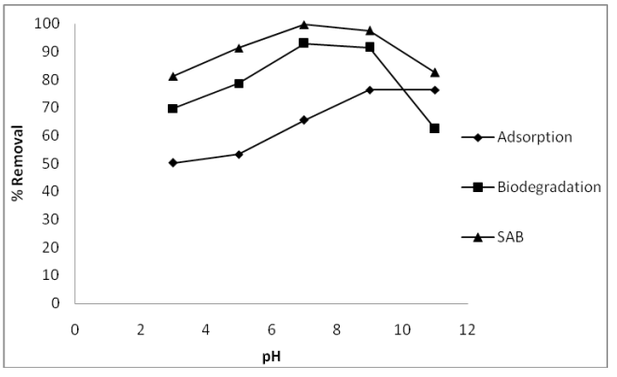

Fig 1. The effect of initial pH on the removal of cyanide by adsorption, biodegradation and SAB (T= 30°C, $C_{i,CN}$=$C_{i,PH}$= 100 mg L^{-1}, D_c= 30 g L^{-1}, t_a= 72 h, S_a= 120 rpm)

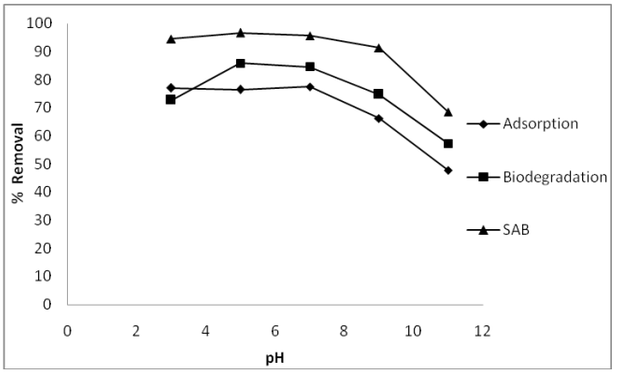

Fig 2. The effect of initial pH on the removal of Phenol by adsorption, biodegradation and SAB (T= 30°C, $C_{i,CN}$=$C_{i,PH}$= 100 mg L^{-1}, D_c= 30 g L^{-1}, t_a= 72 h, S_a= 120 rpm)

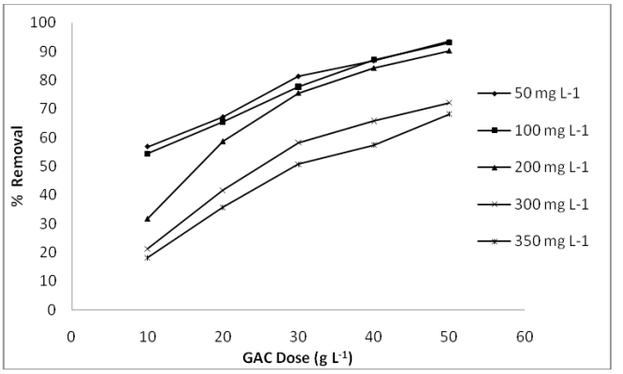

Fig 3. Effect of GAC dose on cyanide removal (%) by adsorption for various initial concentrations (pH =7, T= 30 °C, t_a= 72 h, S_a= 120 rpm)

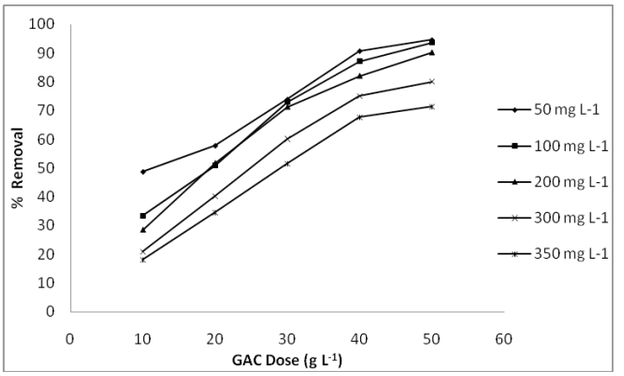

Fig 4. Effect of GAC dose on phenol removal (%) by adsorption for various initial concentrations (pH =7, T= 30 °C, t_a= 72 h, S_a= 120 rpm)

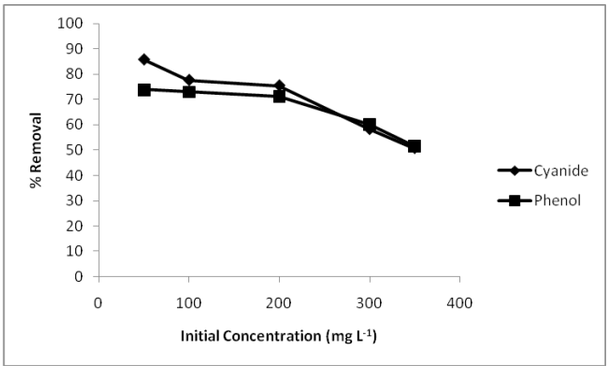

Fig 5. Effect of initial pollutant concentration on adsorption efficiency of GAC (pH =7, T= 30 °C, t_a= 72 h, S_a= 120 rpm)

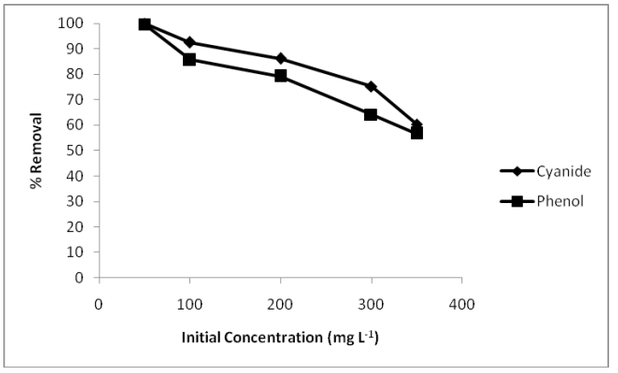

Fig 6. Effect of initial pollutant concentration of biodegradation of cyanide and phenol (pH =7, T= 30 °C, t_a= 72 h, S_a= 120 rpm)

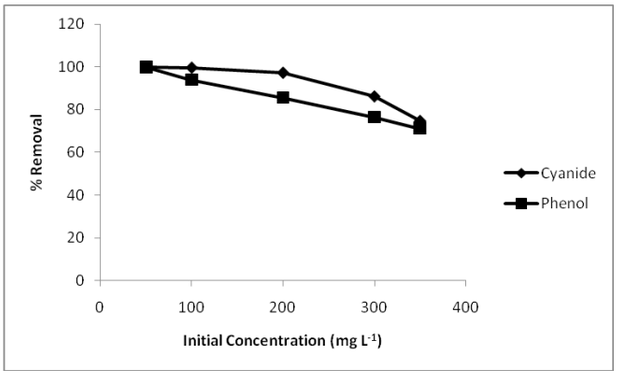

Fig 7. Effect of initial pollutant concentration of SAB of cyanide and phenol (pH =7, T= 30 ° C, t_a= 72 h, S_a= 120 rpm)

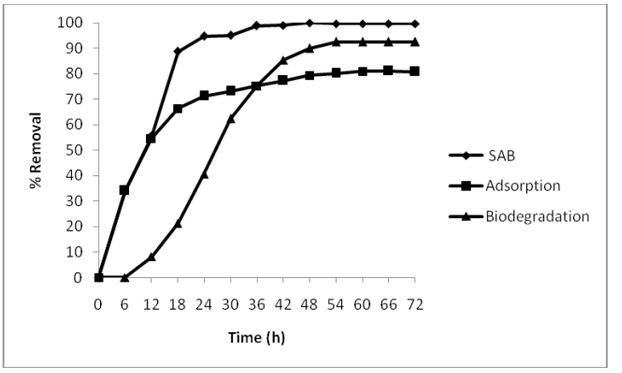

Fig 8. Comparison of cyanide removal efficiency of SAB, adsorption and biodegradation at various time intervals (pH =7, $C_{i,CN}$=$C_{i.Ph}$= 100 mg L^{-1}, T= 30 ° C, t_a= 72 h, S_a= 120 rpm)

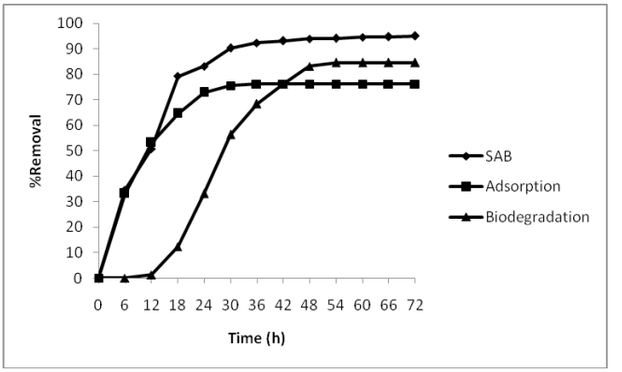

Fig 9. Comparison of phenol removal efficiency of SAB, adsorption and biodegradation at various time intervals (pH =7, $C_{i,CN}$=$C_{i.Ph}$= 100 mg L^{-1}, T= 30 ° C, t_a= 72 h, S_a= 120 rpm)

Simultaneous Adsorption and Biodegradation (SAB) study

Simultaneous adsorption and biodegradation study was conducted to evaluate the performance of the process for cyanide and phenol removal and was compared to adsorption and biodegradation processes. Figure 7 shows the percentage removal of cyanide and phenol by SAB at various initial concentrations. It was observed that for low concentrations i.e. 50 mg L^{-1}, the removal efficiency achieved for SAB and biodegradation were comparative (almost 99.99% for both cyanide and phenol), while the maximum removal achieved for adsorption process

was only 85.8 and 73.92% for cyanide and phenol respectively. However the removal efficiency of SAB was found to be appreciably higher than biodegradation or adsorption alone for moderate (100, 200 mg L^{-1}) and high initial pollutant concentrations (300, 350 mg L^{-1}). Table 3 provides the comparison of removal efficiencies of the three processes. As is evident the SAB process was found to be more effective than the other processes when used alone.

Effect of Agitation/Contact time

Figures 8 and 9 show the effect of agitation time (t_a) on percentage removal of cyanide and phenol respectively. As could be seen the percentage removal increases with agitation time for all the three processes. As is evident in the initial period (0-12 h) the removal efficiency of both phenol and cyanide is comparable and fast by SAB and adsorption. Biodegradation process was found to be delayed for few hours which may be caused by delayed growth (longer lag phase) of microbes in the presence of cyanide and phenol ions as well as due to initial dominance by adsorption phenomena followed by biodegradation (Mondal and Balomajumder, 2007; Dash *et al.,* 2008b). In SAB, removal efficiency varied linearly in the first phase (0-18 h) and parabolically in the second phase i.e. after 18-30 h of agitation. Also it was observed that with SAB equilibrium removal conditions was achieved only after 36 h for cyanide (99.99%) and 30 h of agitation for phenol (96%) while adsorption and biodegradation took longer to reach equilibrium i.e. after 36 h and 48 h respectively. The results are in accordance with the studies of Mordocco *et al.,* 1999 that immobilization of living microbial cells on adsorbent improves removal efficiency. The removal of substances in the presence of microbial film is mechanistically complex which involve (i) transport of substances from bulk liquid to the surface of the microbial film, (ii) simultaneous mass transfer and adsorption within the adsorbent and,(iii) simultaneous mass transfer, adsorption and biochemical reaction within the microbial film (Mondal and Balomajumder, 2007). Since the biochemical reaction could occur in either biological suspension or on adsorbent, active presence of biomass in adsorption could also be expected, which could partially regenerate the adsorbent and ensure a long life operation of adsorption process. Thus activated carbon can be partially regenerated by microorganisms while carbon bed is in operation. Also the active sites on GAC could adsorb the enzymes, minerals, organic material and oxygen thus providing enriched environment for microbial metabolism. The carbon adsorption capacity, controlled by the bio-regeneration, is highly increased and the carbon adsorption column cycle is thus prolonged as compared to pure adsorption system alone. SAB thus provides higher removal efficiency for removal of cyanide and phenol than adsorption or biodegradation alone and reaches equilibrium much earlier than either of the processes.

Conclusion

The capabilities of two microbes (*P. putida* and *A. chroococcum*) to co-ferment and degrade cyanide and phenol simultaneously from simulated solutions was successfully examined by biodegradation alone as well as by simultaneous adsorption and biodegradation. Removal efficiencies of up to 74.14% and 71.22% were achieved for cyanide and phenol respectively by SAB at high initial concentrations of 350 mg L^{-1} of cyanide and phenol. However removal efficiencies of only 60.34% and 56.63% were achieved for cyanide and phenol respectively with biodegradation alone. It was also observed that the removal of cyanide and phenol started earlier in SAB process and the equilibrium was achieved in lesser time as compared to biodegradation alone. When compared to SAB the removal efficiencies of only 50.73% and 51.55% for cyanide and phenol were achieved with adsorption. Better removal efficiencies with SAB, as compared to adsorption or biodegradation alone, is due to easy biodegradation of adsorbed cyanide and phenol since the GAC surface acts as enrichment surface for the microorganisms providing all the nutrients on its active sites. This proves SAB to be a polishing step and more effective process for removal of pollutants.

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Nomenclature

$C_{i,CN}$: initial cyanide concentration (mg L^{-1})

$C_{i,Ph}$: initial phenol concentration (mg L^{-1})

t_a : agitation time (h)

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