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Treatment of cyanide bearing effluents by adsorption, biodegradation and combined processes: effect of process parameters

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ABSTRACT

The present investigation focused on a comparative study for the removal of cyanide from separate solutions of sodium, zinc and iron cyanide compounds by three different processes such as; adsorption, biodegradation and simultaneous adsorption and biodegradation (SAB) processes. Adsorption studies were carried out on commercial granular activated carbon (GAC). Biodegradation and SAB studies were conducted with suspended and immobilized cultures of *Pseudomonas putida*, respectively. Effect of pH, temperature and agitation time on percentage removal of cyanide was measured at an initial cyanide concentration of 100 mg/L and it was found that these parameters significantly affect the three process performances. The SAB process was found to have better removal efficiency as compared to adsorption and biodegradation processes. SAB process could achieve more than 95% removal efficiency for cyanide concentrations up to 100 mg/L in sodium, zinc and iron cyanide solutions. Higher percentage of cyanide removal and specific uptake was achieved in case of zinc–cyanide complexes as compared to other cyanide complexes. Adsorption isotherms evaluated the uptake and degradation on cyanide in SAB process.

Keywords: Pseudomonas putida; Zinc-cyanide; Iron-cyanide; Sodium-cyanide; SAB

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1. Introduction

Cyanide is a carbon–nitrogen radical, which may be found in a wide variety of organic and inorganic compounds [1]. The cyanide compounds are the strictly regulated compounds world-wide because of their extreme toxicity [2]. Although cyanides are present in small concentrations (1–4,000 mg HCN/kg of plant weight) in these plants and micro-organisms, their large-scale presence (10–10,000 mg CN^-/L) in the environment is attributed to the human activities as cyanide compounds are extensively used in industrial applications [3–5]. Cyanide is commonly found as a contaminant in wastewaters from various industries including metal cleaning, plating, electroplating, metal processing, automobile parts manufacture, steel tempering, mining, photography, pharmaceuticals, coal coking, ore leaching, plastics, etc. [3–7]. Cyanides

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are produced as wastes and emissions from these industries, along with ammonia, phenolic compounds, suspended particles, organic wastes and significant amount of heavy metals like copper, nickel, zinc, silver, gold, iron, etc. [1,5]. Cyanide, in some forms, is a very powerful and fast acting toxin. Cyanide compounds present in the environmental matrices and waste streams as free, simple and complex cyanides, cyanates and nitriles [2]. At short-term exposure, cyanide causes rapid breathing, tremors and other neurological effects and long-term exposure to cyanide causes weight loss, thyroid effects, nerve damage and death. Skin contact with liquids containing cyanide may produce irritation and sores [5]. To protect the environment and water bodies, wastewater containing cyanide must be treated before discharging into the environment. Typical cyanide consent to discharge standards to sewers range from 0.5 mg/L to 1.0 mg/L [8]. US EPA standards for drinking and aquatic-biota waters regarding total cyanide are 200 and 50 ppb [9], respectively. The German and Swiss regulations have set limit of 0.01 mg/L for cyanide for surface water and 0.5 mg/L for sewers. The Mexican Ministry of the Environment and Natural Resources (SEMARNAT) [10] and Central Pollution Control Board (CPCB), India [3] have set a limit for cyanide in effluent as 0.2 mg/L. Due to their toxic effects, cyanide-containing effluents cannot be discharged into the environment without detoxification. Cyanide and its related compounds such as ammonia, cyanate, nitrate and thiocyanate can be treated and removed by one of several processes such as alkaline breakpoint chlorination, INCO process (by SO_2/air), copper-catalyzed hydrogen peroxide, Caro's acid, natural attenuation, cyanide recovery, ozonation, electrolytic oxidation, ion exchange, acidification, AVR process, lime-sulfur, reverse osmosis, activated carbon adsorption, thermal hydrolysis and biological treatments etc. [3,6,10–13].

Adsorption and biodegradation are two significant methods for treatment of wastewater bearing cyanide compounds. Biological treatment is a cost-effective and environmentally acceptable method for cyanide removal compared with the other techniques currently in use [11]. Most reports demonstrated the ability of micro-organisms to utilize cyanide as a source of nitrogen or both carbon and nitrogen by specific enzymes and pathways [2]. Metabolism of cyanide by various strains of *Pseudomonas*, *Acinetobacter*, *Bacillus* and *Alcaligenes* has been reported [14–16]. Degradation of cyanide compounds by fungi such as *Fusarium solani*, *F. oxysporum*, *Gloeocercospora sorghi*, *Fusarium lateritum* and *Stemphylium loti* [17,18] has also been reported. Biodegradation is performed in the presence of microbes either in mobilized or immobilized phase [19]. The immobilization of living microbial cells on a suitable adsorbent improves the removal efficiency [20]. This improvement is due to the monolayer/biolayer formation on the adsorbent bed where adsorption and biodegradation occurs simultaneously (known as simultaneous adsorption and biodegradation process) [21,22]. It is also pointed out that attached growth processes and combined processes such as oxic/anoxic processes may prove advantageous for the cyanide detoxification [3,4]. Recently developed methods dealing with the removal of toxic compounds by adsorption [23] and biological treatment, either operated separately or simultaneously in one unit [24]. In most cases, the presence of both processes in one unit results in a better removal and process performance [19]. Microbial mass can, in some extent, adsorb the substances, but at the same time it also degrades them [24]. On the other hand, adsorption of the substances onto adsorbent reduces the inhibitory effect of the substances for microbial mass [9]. Accordingly, the process is expected to be more stable and the toxic compounds may be converted into less harmful substances [24]. Activated carbon is able to enrich dissolved oxygen; probably this oxygen can also be utilized by micro-organisms adsorbed on activated carbon [19,20].

In the present study, cyanide was removed from sodium, zinc and iron cyanide contaminated synthetic solutions by adsorption, biodegradation and SAB in separate batch experiments. Granular Activated carbon (GAC) was used as adsorbent. Chapatwala et al. [25] reported that non-immobilized and immobilized cells of *P. putida* were able to degrade the cyanide to NH_3 and CO_2. A variety of enzymatic pathways for cyanide degradation has been reported. These hydrolytic pathways lead to the production of formamide or formate plus ammonia or the direct formation of bicarbonate plus ammonia via cyanide oxidase or by a dioxygenase [26]. *P. putida* could degrade cyanide into NH_3 and CO_2 by following one of the enzymatic pathways mentioned above. There are various reports which describe the cyanide degradation ability of *P. putida* for various cyanide compounds [25,27]. Chapatwala et al. (1998) [25] isolated *P. putida* capable of utilizing cyanide as the sole source of carbon, nitrogen and energy. Suspended and immobilized cultures of *Pseudomonas putida* (MTCC Code 1194) was used to evaluate the performance of the culture for removal of the three different cyanide compounds (sodium, zinc and iron cyanide) by biodegradation and SAB. The effect of pH, temperature, contact time and initial concentration of cyanide on removal of cyanide from the cyanide contaminated synthetic solutions were studied separately in batch experiments.

2. Materials and methods

All the chemicals were of analytical grade and solutions were prepared by Milli-Q water. The synthetic solutions of sodium cyanide (NaCN), iron cyanide (FeCN) were prepared by dissolving NaCN (1.88 g/l), $K_4Fe(CN)_6{\cdot}3H_2O$ (2.7 g/l) in Milli-Q water respectively, where as for zinc cyanide (ZnCN) solution sulfates of zinc (3.14 g/l) was added to KCN (3.25 g/l). The three cyanide compounds in the initial concentration ranges of 50–400 mg CN^-/L were used in the present study separately. The commercial GAC obtained from M/s. S. d. fine-chem, Ltd., India (bulk density 400 g/L, particle size of 2–4 mm, BET Surface area 583.35 m^2/g and micro-pore (<2 nm) volume 0.2112 cm^3/g) was used in the study after purification with Milli-Q water and dried at 110°C for 24 h. Adsorption studies were conducted in 250 ml conical flasks containing 100 ml of synthetic cyanide solution with 20 g/L of GAC.

Freeze-dried culture of *P. putida* (MTCC Code 1194) species was obtained from Institute of Microbial Technology (IMTECH), Chandigarh, India, was revived in the nutrient broth at 25°C and pH 7. The sterile phosphate buffer medium (pH 7.0) used for growth study of *P. putida* contained: K_2HPO_4 (4.3 g/L), KH_2PO_4 (4.3 g/L) and $MgSO_4{\cdot}7H_2O$ (0.35 g/L) [25]. The medium was amended with 0.5 ml of a trace element solution containing the following: $MnSO_4{\cdot}4H_2O$ (1.52 mg/L), $FeSO_4{\cdot}7H_2O$ (0.6 mg/L), $CaCl_2{\cdot}2H_2O$ (3.0 mg/L) and $Na_2MoO_4{\cdot}2H_2O$ (6.0 mg/L) [25]. For adaptation of the microbe, cyanide was added stepwise in 10–50 mg CN^-/L as only source of carbon and nitrogen. After the culture was inoculated into 100 ml of growth medium (in 1:100 ratio) in a 250 ml conical flask, it was incubated at 25°C in a agitated shaker (150 rpm) for 24 h [9]. Sterilization of the GAC, glassware and medium was performed in an autoclave at 121°C for at least 20 min. All biodegradation studies were performed separately in 250 ml conical flasks containing *P. putida* culture (1:100) in 100 ml of the biodegradation medium. For immobilization of microbes on GAC, a known volume of thick slurry of *P. putida* cells, whose concentration was determined by drying a sample to a constant weight, was added to purified and sterilized GAC. The GAC was shaken in an incubator shaker for 6 h at 120 rpm and left for one day. The supernatant liquor was taken out, and the biologically activated GAC on which the cells of *P. putida* were immobilized were used for SAB study. *P. putida* immobilized GAC was added to the biodegradation medium (containing 50–400 mg CN^-/L) to maintain 20 g/L biological activated carbon (BAC) concentration for SAB study. Effect of initial pH, temperature, cyanide concentration and agitation time on adsorption, biodegradation and SAB for removal of cyanide was studied. The pH and temperature was optimized for the cyanide compounds with initial the concentration of 100 mg/L from a range of pH 4–11 and temperature 20–45°C keeping one parameter constant at a time. All studies were conducted in 250 ml conical flasks in rotary incubator shaker at 150 rpm for an agitation period of 120 h [9]. Fig. 1 presents the photomicrographs of GAC and BAC by scanning electron microscope (SEM) (LEO® 435 VP, UK).

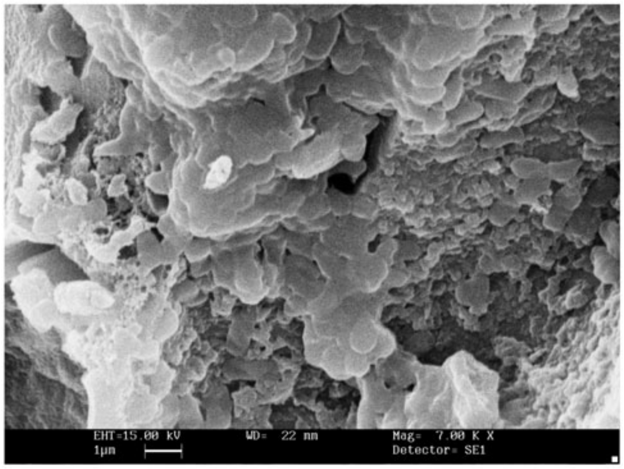

Fig. 1. Photomicrograph of BAC immobilized with *P. putida*.

Parameters such as pH, total cyanide content, optical density (550 nm) were checked periodically. Total cyanide was determined by pyridine–barbituric acid colourimetric method (578 nm) after distillation as described in Standard Methods with a precision up to 0.001 mg/L [28]. The pH was measured using pH meter as specified by standard methods [28].

3. Results and discussion

3.1. Effect of pH

pH plays an important role for the adsorption of metal cyanides on GAC [29]. Effect of pH on removal of cyanide from aqueous solutions by adsorption, biodegradation and SAB processes was investigated. The effect of initial pH of the sample solution on cyanide removal has been given in Table 1. The significant increase in adsorption of cyanide was above pH 7 in NaCN solution. Maximum adsorption occurred in pH 9–10. Again there was decrease in adsorption above pH 10. In case of ZnCN solutions, the adsorption was optimum in the pH range of 6–7. At higher or lower pH values there was decrease in adsorption.

Table 1
Effect of pH on removal of cyanides by adsorption, biodegradation and SAB

pH	% Removal of cyanide								
	Adsorption			Biodegradation			SAB		
	NaCN	ZnCN	FeCN	NaCN	ZnCN	FeCN	NaCN	ZnCN	FeCN
4	45.2	71.6	81.7	–	–	51.5	45.1	58.2	84.1
5	50.2	80.2	83.3	30.2	52.8	74.3	68.1	84.7	91.4
6	52.4	84.2	82.6	75.8	80.4	78.2	92.9	94.8	96.7
7	53.7	84.4	79.6	86.5	82.6	78.0	97.9	97.3	96.5
8	59.9	82.5	72.8	88.5	82.5	72.8	98.6	94.7	81.8
9	63.2	79.4	64.2	88.3	65.7	46.7	98.3	89.4	76.5
10	63.2	66.0	43.3	81.4	46.5	18.2	89.5	68.5	62.9
11	61.7	42.4	31.2	31.0	09.0	–	87.6	43.6	51.7

The FeCN complexes showed adsorption was greater at lower pH values and decreased as pH increased. Above pH 10, ferrocyanide adsorption was negligible. From the results it was observed that, percentage removal of metal–cyanide complexes were maximum at neutral and slight acidic pH. Whereas, there was increase in percentage removal for sodium cyanide in alkaline conditions.

At pK_a 9.39 value of cyanide, pH had a marked effect on the stability of sodium cyanide [30,31]. ZnCN is a weak-acid dissociable (WAD) cyanide complex as it is easily dissolved under mildly acidic conditions ($pH = 4–6$) [5]. Hence in lower pH ranges ($pH < 6$), there is a possibility of dissociation of ZnCN, which reduced the adsorption efficiency. Hence, the optimum removal was found at a pH above 6. In 1997 Chank studied the adsorption of ferrocyanide on activated carbon as a function of pH and found greater adsorption at low pH [32], but below pH 3 there was possibility of volatilization of ferrocyanide [5,31]. At these pH values, cyanide ion exists as HCN which was a weak acid and is highly soluble in water [30,33]. In 1979 Davidson and Veronese related the effect of pH on the adsorption of metal–cyanide complexes to the strong adsorption of both hydroxide and hydronium ions [30], whereas Adams et al. [34] and Adams and Fleming [35] favoured a mechanism in which OH^- reacts with the functional groups on the surface of the carbon. Huang and Ostovic proposed that hydration of the activated carbon may result in the formation of reactive surface functional groups [36]. The relative proportion of the surface functional groups may vary with the method of preparation of the activated carbon, thereby causing a difference in the value of the surface acidity constants (equilibrium constants). The cyanide ion is a nucleophile, and in contact with the surface of activated carbon, could replace the OH^- present in various surface functional groups. Both the processes would be equivalent to CN^- uptake by ion exchange. In acidic medium ($pH < 3$), complexes such as hexacyano-iron (II) or (III) probably absorbs as the protonated anion [37].

The optimum pH for maximum percentage of removal of cyanide in the presence of suspended cultures of *P. putida* from NaCN, ZnCN and FeCN solutions was found to be 8, 7 and 6, respectively, from Table 1. At pH more than 9 there was a sudden decrease of cyanide removal for all the three compounds. However, below pH 5, no biological activity was found for NaCN and ZnCN, but there was possibility of degradation of FeCN at pH less than 5. However, above pH 10 there is no biological activity in the presence of FeCN solution. From Table 1, it was also observed that, maximum percentage of removal of cyanide by immobilized *P. putida* on BAC was found in the pH 8–9, 7 and 6–7 from NaCN, ZnCN and FeCN solutions, respectively. Above pH 9, there was decrease in percentage cyanide removal in all cyanide solutions. However, percentage cyanide removal from FeCN solution decreased significantly above pH 7. The reduction in percentage removal from ZnCN and FeCN solutions was more as compared to cyanide removal from NaCN solution at $pH > 9$. At acidic pH conditions the percentage removal of cyanide was higher in FeCN solution as compared to NaCN and ZnCN solutions.

There was a decrease in CN^- removal efficiency above and below pH 6–7 for all three processes. At higher pH conditions the efficiency of adsorption decreased, and below pH 6, there was possibility of volatilization of zinc cyanide [5]. In WAD complexes, cyanide is readily released from the complexes when the pH is lowered to 4.5–6. Weakly complexed metal-cyanides decompose at pH values lower than 4 and evolve HCN [38,39]. Therefore, WAD refers to any free cyanide already present and cyanide released from nickel, zinc, copper, and cadmium complexes (but not from iron or cobalt complexes) [40]. The iron complexes are very stable and for this reason less toxic and more recalcitrant. The stability of the complexes depends on the pH; they are, in general, more stable as the pH increases [9]. The zinc-cyanide containing wastewaters emanating from plating industries have pH values in the alkaline range (7.5–10) [4]. These results are conducive for the practical use of the process, as very little pH adjustment of the effluents may be required. It is known that the relationship between simple and complex cyanides in water is dependent on factors such as the pH and the heavy metal concentrations capable of forming metal–cyanide complexes [41]. Under alkaline conditions, free cyanide (HCN, CN^-) is completely ionized and stable metal complexes are formed. In neutral and acidic conditions, the free cyanide is weakly ionized and the formation and partial liberation of HCN is favoured. The growth and biological activity of the microbes depend on the pH conditions of the medium. Probably, the optimum pH for degrading cyanide complexes is the result of a balance between the stability of the complex and the optimum pH for growth. Patil and Paknikar [4] obtained an optimum pH 7 for cyanide biodegradation by *Pseudomonas* sp. Degradation of cyanide compounds by bacteria and fungal species was commonly observed at neutral and alkaline pH values. In any case, any bacterium is able to use both free cyanide and its metal complexes at alkaline pHs, thus providing a clear advantage since both cyanide volatilization and precipitation of its metal complexes are prevented. In the present study, pH 7 was taken as the optimum pH value for the three processes for the three cyanide solutions.

3.2. Effect of temperature

The influence of temperature on removal of cyanide compounds by the three processes has been presented in Table 2. It was observed that the difference was not great at different temperatures, but adsorption increased slightly with rise in temperature in FeCN solution. Increased adsorption at higher temperature is difficult to explain as it is against the general adsorption behaviour. However, such adsorption nature may be explained carefully by examining the mode and type of the adsorption process. Diffusion of adsorbate species from the bulk phase into pores of adsorbent have been observed in some of the adsorption processes of endothermic nature [42]. Here the rise of temperature favours the adsorbate transport within the pores of the adsorbent. The increase in adsorption with temperature was mainly due to an increase in number of adsorption sites caused by breaking of some of the internal bonds near the edge of the active surface sites of the adsorbent [42]. With increasing temperature chemisorption predominate physical sorption of cyanide onto activated carbon [31]. Hydrolytic decomposition reaction was more important than oxidation to cyanate at high temperatures in presence of activated carbon [43]. Increase in adsorption behaviour at higher temperatures for FeCN may be due to the dissociation of iron–cyanide complexes.

In case of biodegradation and SAB maximum growth and removal of CN^- was observed at temperature 25–30°C and growth of *P. pudia* ceased above 40° C. The percentage removal of cyanide by biodegradation in the presence of the microbes mainly depends on the optimum growth condition of the microbes. The optimum temperature for removal of cyanide complexes was the result of a balance between the stability of the complex and the optimum temperature for growth. The dissociation of cyanide from metal

Table 2
Effect of temperature on removal of cyanides by adsorption, biodegradation and SAB

Temp. (°C)	% Removal of cyanide								
	Adsorption			Biodegradation			SAB		
	NaCN	ZnCN	FeCN	NaCN	ZnCN	FeCN	NaCN	ZnCN	FeCN
20	62.6	81.6	78.9	86.7	81.7	75.5	92.3	93.7	89.9
25	65.8	83.7	80.8	88.5	82.5	78.2	98.4	97.0	96.3
30	67.0	83.9	82.6	88.0	82.5	78.2	98.6	97.3	96.7
35	67.2	83.9	82.6	84.1	79.8	75.4	96.3	96.7	96.6
40	67.0	84.1	83.5	80.2	76.3	70.3	92.3	93.6	95.6
45	67.3	84.2	83.5	72.7	69.8	67	86.4	88.2	87.3

complexes increases with the increase in temperature. The dissociation rate varies depending on the stability of the complexes. The dissociation constant of ZnCN complexes are higher than FeCN complexes. 30˚C was taken as the optimal temperature for all the studies.

3.3. *Effect of contact/agitation time*

Fig. 2(a–c) represents the percentage removal of 100 mg/L of sodium, zinc and iron cyanide complexes, respectively, with increase in agitation time by adsorption, biodegradation and SAB at 30˚C and pH 7. The rate of increase in the percentage removal of cyanide species with the increase in agitation time is appreciably fast at the initial stage. However, after a period of ~42 h in case of NaCN solutions, ~30 h in case of ZnCN solutions and ~36 h in case of FeCN solutions, rate of increase in percentage removal of cyanide species by adsorption with increase in agitation is less. It was observed that after 84 h, 72 h and 78 h in case of NaCN, ZnCN and FeCN solutions, respectively, there was no further increase in percentage removal of cyanide by GAC and a steady state had arrived. The highest percentage of cyanide removal was achieved in case of ZnCN solutions as compared to NaCN and FeCN solutions. Further, it was also observed that, the steady state and equilibrium condition arrived earlier in case of cyanide adsorption from ZnCN solutions as compared to NaCN and FeCN solutions. Cyanide removal from NaCN solution was found to be the lowest and it takes more time to reach the equilibrium condition. The slow step in the NaCN solution was considered to be the diffusion of CN^- from the bulk solution to the active surface sites. This process would be influenced by the concentration gradient between those two points and the thickness of the diffusion layer which was a function of agitation process. Available adsorption results revealed that the uptake of cyanide species were fast at the initial stage of contact period, and thereafter, it becomes slower near the equilibrium. During the initial stage of the experiment a large number of active surface sites of GAC are available for adsorption [44]. Thus, the concentration of cyanide in the solutions as well as the driving force for adsorption of cyanide on the GAC surface is maximum. Further, the resistance to mass transfer between bulk phase and adsorbent is overcome by the energy provided by agitation to bring the cyanide species from bulk of the solutions to the active sites of the adsorbent. In physical adsorption, most of the adsorbate species are adsorbed within a short interval of contact time [42]. GAC contains both positive as well as negative sites on its surface, with negative charges predominating over positive charges [45]. The presence of positive charge of the metal ions get attracted towards the negatively charged GAC surfaces sites [46] neutralizing some negative charges of GAC surface after their adsorption on the negative sites of GAC and will create some additional positive sites on the GAC surface. The positive sites created on the GAC surface make the cyanide ion adsorbed on the surface of the GAC. Here chemisorption predominates over physical adsorption for adsorption of cyanides on GAC. Hence, strong chemical binding of adsorbates with adsorbent required longer contact time for the attainment of equilibrium.

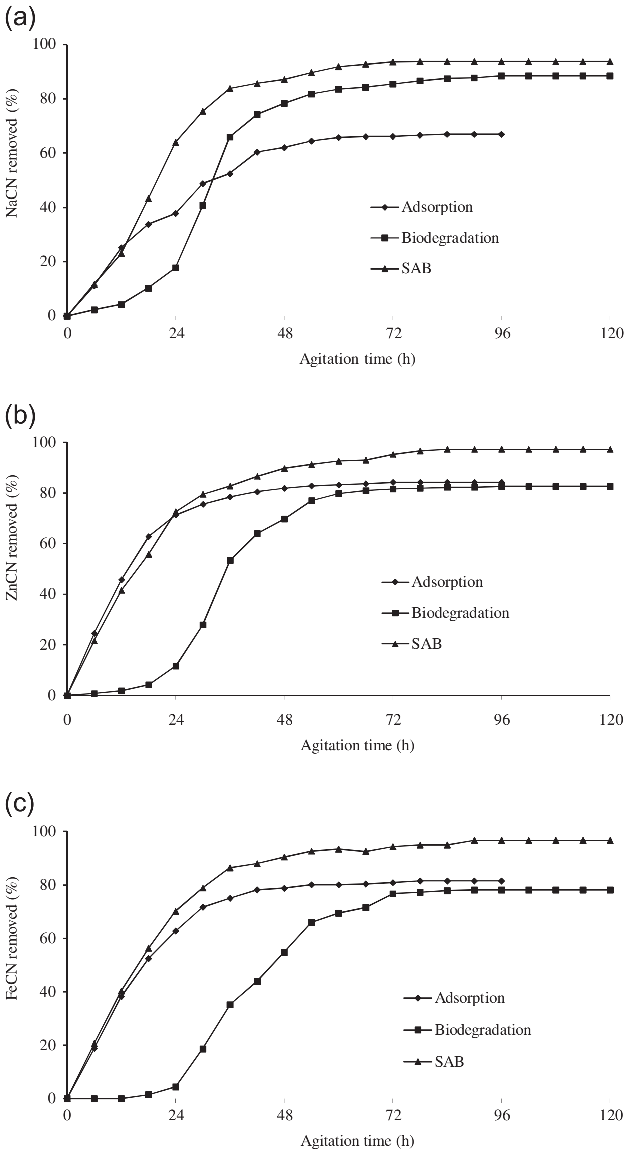

Fig. 2. (a) Effect of agitation time on removal of NaCN, (b) effect of agitation time on removal of ZnCN and (c) effect of agitation time on removal of FeCN.

From the figures it was evident that no biodegradation or growth of microbe was found in the staring 12–18 h of agitation. This may be due the lag phase of microbe. Degradation started at 24 h and after 72 h of agitation there was no significant increase in the percentage removal of cyanide. Removal of cyanide from the medium occurred during the initial stages of growth. This suggested that the complex was taken up by the biomass prior to utilization. The maximum percentage removal was found in the log phase of growth of microbes. From Fig. 2 it was evident that biodegradation delayed for 12–18 h due to delayed growth of microbes in the presence of cyanide ions, but in SAB process the percentage removal of cyanide was started earlier. This may be due to adsorption occurred in the first phase followed by biodegradation. With the increase in agitation period, greater numbers of cyanide ions were moved to the surface of BAC; as a result the percentage removal of cyanide increased with the increase in agitation time. The maximum percentage of removal of cyanides from the three cyanide solutions was faster in SAB process as compared to its corresponding adsorption and biodegradation processes. The combined process was more effective and less time consuming. The cyanide adsorbed on the biologically active GAC surface could be easily biodegraded by microbes as GAC acted as an enrichment surface and attached growth gave better efficiency [9,23]. Moreover, during SAB there was the possibility of bio-regeneration of BAC, which increased the adsorption capacity and prolonged the time of adsorption process. The continuous bio-regeneration of GAC and biodegradation of cyanide adsorbed on GAC surface increase the possibility of higher efficiency and the increase in percentage removal [9,47]. SAB process took a long duration to reach the equilibrium condition after 96–108 h of agitation for all the three cyanide solutions. Hence, SAB is more efficient and prolonged process for the removal of cyanide as compared to adsorption and biodegradation alone.

3.4. Effectiveness of adsorption, biodegradation and SAB process at various initial cyanide concentrations

Fig. 3(a–c) represents the percentage removal of cyanide from of sodium, zinc and iron cyanides solutions, respectively, at various initial cyanide concentrations by adsorption, biodegradation and SAB process. From the experimental results it was observed that, the removal efficiency decreased with increase in initial cyanide concentration for all processes. It can be noted that the increase in percentage removal of cyanide compounds with decrease in initial cyanide concentration was less for higher value of initial concentration of cyanide than that for lower value. Removal of higher concentrations of cyanide could be achieved with immobilized cultures in SAB process as compared to adsorption and biodegradation. Better removal efficiency was found for zinc–cyanide complex with higher concentrations by adsorption and combined process of adsorption and biodegradation. However, biodegradation of zinc cyanide was less as at higher concentrations, but in the presence of BAC, there was possibility of biological growth and degradation at high concentrated cyanide

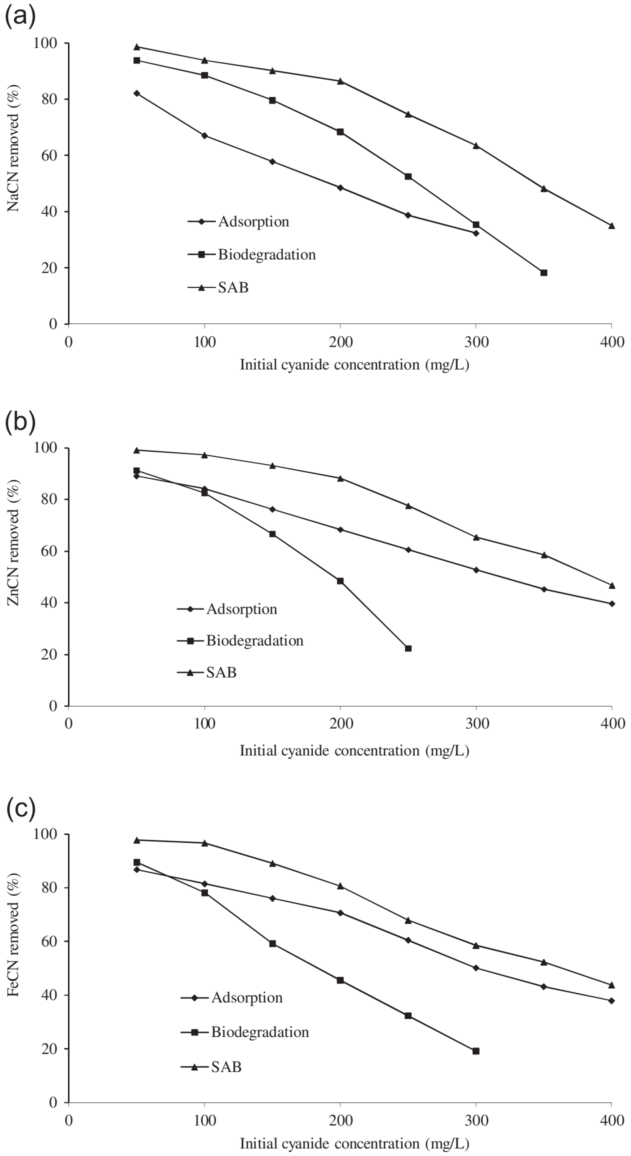

Fig. 3. (a) Effect of initial cyanide concentration on removal of NaCN, (b) effect of initial cyanide concentration on removal of ZnCN and (c) effect of initial cyanide concentration on removal of FeCN.

contaminated solutions. Biodegradation of sodium cyanide was maximum as compared to the other cyanide complexes, may be it was available readily to microbes. For zinc and iron cyanide complexes, removal due to adsorption was more as compared to biodegradation at higher concentrations of cyanide. It is a well-known fact of an adsorption process that at a particular environment the percentage removal depends upon the ratio of the number of adsorbate moiety to the available active sites of adsorbent. This ratio is also related to the surface coverage of the adsorbent (number of active sites occupied/number of active sites available) that increases with increase in the number of adsorbate moiety per unit volume of solution at a fixed dose of adsorbent. Less is the value of this ratio more is the percentage removal. At higher cyanide concentration, this ratio was high and decreased gradually with the decrease in cyanide concentration as a result the percentage removal was increased with decrease in cyanide concentration [31].

In biodegradation process, the toxicity of cyanide compounds exerts difficulties in bacteria capable of using these as a carbon source for growth [16]. The growth of *P. putida* was found in the absence of any external carbon source. However, the removal efficiency was increased with the addition of external glucose source. It could be easier to utilize cyanide as a source of nitrogen in the presence of another source of carbon and energy, as the amount of nitrogen needed for the growth is less than the requirement for carbon. The decrease in removal of cyanide with increase in initial concentration may be due to the toxicity of cyanide compounds to *P. putida* at higher concentration. No significant biological activity was found in the medium above cyanide concentrations of 350, 250 and 300 mg/L in sodium, zinc and iron cyanides solutions, respectively. The initial concentration up to which biodegradation was possible is less in zinc cyanide as compared to other compounds may be due to the presence of free cyanide ions and zinc ions. Although metal–cyanide complexes by themselves are much less toxic than free cyanide, their dissociation releases free cyanide as well as the metal cation, which can also be toxic [3].

It was also evident from Fig. 3 that in SAB process, due to attach growth and combined process performance, resistance to cyanide toxicity by *P. putida* was more and could achieved good efficiency even at higher concentrations. SAB process has been used successfully for degradation of various compounds such as phenol, toxic metals, dye, etc., however its fate was not known for removal of cyanide compounds.

In the present case, removal of cyanide was due to physical and chemical adsorption on GAC surface as well as due to the bioadsorption and biodegradation. The adsorption of cyanide ions was mostly due to monolayer adsorption. Hence in case of SAB process, due to the monolayer adsorption of cyanide on the GAC, there was a possibility of the reduction in toxicity of cyanide and metal ions to microbes, which increase the possibility of biodegradation rather than bioadsorption. It took some time for the immobilized biomass to be active in the biodegradation medium with cyanide solution. Hence, at the initial stage of agitation, the adsorption on GAC surface predominates over the biodegradation. Due to this no lag phase was found in case of the SAB process as compared to biodegradation. The initial rapid increase in the percentage removal of cyanide may be attributed to the dominating role of only adsorption process at the initial stage of SAB process. The increase in percentage removal in adsorption process was due to the presence of active sites on the GAC surface. However, in case of SAB process the adsorption decreased up to some extent due to the formation of biofilms on the active sites of adsorbent. But in that case, biodegradation predominated over adsorption process for removal of cyanide from various cyanide solutions. The toxicity of the cyanide in the solution was reduced due to adsorption on monolayer and cyanide was easily available for degradation. This made the combined process more effective than the single process and gave better efficiency. Although, the process was continuous theoretically, due to the formation of biofilm on the active sites of GAC, equilibrium condition arrived. However, it had taken a long duration to reach the equilibrium condition as compared to adsorption process.

3.5. Isotherms for adsorption and SAB

The efficiency of an adsorptive removal process is described either by specific uptake or percentage removal. Specific uptake is used to describe the total removal of adsorbate per unit mass of adsorbent, whereas percentage removal gives a comparison between the concentrations of adsorbate in the solution before and after treatment. Table 3 represents the specific uptake of cyanide from the three cyanide (NaCN, ZnCN and FeCN) solutions by GAC and BAC. The specific uptake of cyanide was found to be more by BAC, as compared to GAC for all the three cyanide solutions. It was also observed that, specific uptake of cyanide from ZnCN solution was found more in both adsorption and SAB process, may be due its easy dissociation as compared to cyanide in FeCN solutions. From Table 3 it was observed that, for adsorption process the equilibrium condition was

Table 3
Specific uptake of cyanide by GAC and BAC

Cyanide concentration (mg/L)	Specific uptake (mg CN^-/g GAC)			Specific uptake (mg CN^-/g BAC)		
	NaCN	ZnCN	FeCN	NaCN	ZnCN	FeCN
50	2.052	2.227	2.170	2.465	2.477	2.445
100	3.350	4.210	4.075	4.690	4.865	4.835
150	4.115	5.722	5.572	6.757	6.990	6.682
200	4.850	6.840	7.070	8.640	8.820	8.070
250	4.837	7.575	7.530	9.325	9.700	8.487
300	4.845	7.920	7.560	9.525	9.810	8.775
350		7.930	7.580	8.435	10.255	9.152
400		7.940		7.000	8.190	8.760

arrived at an initial cyanide concentration of 200–250 mg/L, however in case of SAB process specific uptake increased up to higher concentrations. However, there is a decrease in specific uptake in SAB process at cyanide concentrations of 400 mg/L due the toxicity of cyanide to immobilized microbes, and adsorption predominated biodegradation at such a high concentration.

The experimental adsorption and bio-adsorption equilibrium data for removal of cyanide from solutions of NaCN, ZnCN and FeCN on GAC and BAC were fitted with three adsorption isotherm models. Large numbers of researchers in the field of environmental engineering have used Freundlich and Langmuir isotherm equations to represent equilibrium adsorption data using plain and biological activated carbon for adsorption and SAB processes [9,48,49]. In the present study along with the two isotherm models, Redlich and Peterson (R–P) [50] isotherm model has also been used to evaluate the adsorption and SAB isotherms. Table 4 represents the Freundlich, Langmuir and R–P isotherm constants and MPSD for GAC and BAC.

The Freundlich isotherm model is given in Eq. (1)

$$q_e = X/M = \left(q_\mathrm{e} = K_\mathrm{F} C_\mathrm{e}^{\frac{1}{n}}\right) \tag{1}$$

where K_F is the Freundlich constant (mg/g)/(mg/L)$^{1/n}$, the heterogeneity factor is $1/n$, Langmuir isotherm model, given in Eq. (2)

$$q_\mathrm{e} = \frac{q_\mathrm{m} K_\mathrm{L} C_\mathrm{e}}{1 + K_\mathrm{L} C_\mathrm{e}} \tag{2}$$

where K_L is the Langmuir adsorption constant (l/mg) related to the energy of adsorption and q_m signifies adsorption capacity (mg/g). The essential characteristics of a Langmuir isotherm could be expressed in terms of a dimensionless separation factor, R_L which describes the type of isotherm and is defined by Eq. (3) [50]:

$$R_\mathrm{L} = 1/(1 + K_\mathrm{L} C_\mathrm{o}) \tag{3}$$

If $R_\mathrm{L} > 1$, unfavourable; $R_\mathrm{L} = 1$, linear; $0 < R_\mathrm{L} < 1$, favourable; $R_\mathrm{L} = 0$, irreversible.

The values of R_L for $C_0 = 100$ mg/L are given in Table 4.

R–P equation incorporates three parameters into an empirical isotherm, and therefore, can be applied either in homogenous or heterogeneous systems due to the high versatility of the equation. It can be described as follows in Eq. (4):

$$q_\mathrm{e} = \frac{K_\mathrm{R} C_\mathrm{e}}{1 + a_\mathrm{R} C_\mathrm{e}^{\beta}} \tag{4}$$

where K_R is R–P isotherm constant (L/g), a_R is R–P isotherm constant (L/mg)$^{1/\beta}$ and β is the exponent which lies between 0 and 1, C_e is the equilibrium liquid phase concentration (mg/L).

Due to the inherent bias resulting from linearization, Marquardt's percent standard deviation (MPSD) error function was used from a number of different error functions of non-linear regression basin to find out the best-fit isotherm model to the experimental equilibrium data. To determine the best-fit isotherm, Marquardt's percent standard deviation (MPSD) error function may be generated as follows [50,51]:

$$\mathrm{MPSD} = 100\sqrt{\frac{1}{n_\mathrm{m} - n_\mathrm{p}} \sum_{i=1}^{n} \left[\frac{q_\mathrm{e,cal} - q_\mathrm{e,exp}}{q_\mathrm{e,exp}}\right]_i^2} \tag{5}$$

Table 4
Isotherm constants for GAC and BAC

Cyanide compounds	n	K_F	R^2	MPSD	
Freundlich isotherm model constants					
Adsorption on GAC					
NaCN	3.48432	1.15718	0.9544	9.2913	
ZnCN	3.06185	1.55668	0.9132	14.7587	
FeCN	2.99133	1.38388	0.8920	18.3558	
SAB on *P. putida* immobilized BAC					
NaCN	4.84027	3.19520	0.7723	27.1759	
ZnCN	4.56830	3.63580	0.8989	17.6247	
FeCN	4.52489	3.11100	0.8806	17.7356	
Langmuir isotherm model constants					
Cyanide compounds	q_m	K_L	R_L	R^2	MPSD
Adsorption on GAC					
NaCN	5.15730	0.07262	0.12103	0.9867	6.29131
ZnCN	8.56898	0.06410	0.13495	0.9993	2.52841
FeCN	8.53242	0.05158	0.16238	0.9967	5.43892
SAB on *P. putida* immobilized BAC					
NaCN	7.99361	0.62456	0.01576	0.9346	20.9986
ZnCN	8.86525	0.83991	0.01177	0.9634	15.0962
FeCN	8.74891	0.35365	0.02750	0.9955	5.5821
R–P isotherm model constants					
Cyanide compounds	K_r	a_r	β	R^2	MPSD
Adsorption on GAC					
NaCN	0.47492	0.14946	0.9030	0.9982	6.17291
ZnCN	0.57474	0.07933	0.9667	0.9996	2.45786
FeCN	0.42054	0.03988	1.0418	0.9982	5.22377
SAB on *P. putida* immobilized BAC					
NaCN	5.65906	0.89056	0.9403	0.9915	21.94940
ZnCN	10.04420	1.71224	0.8899	0.9984	8.57022
FeCN	3.26429	0.41745	0.9738	0.9992	4.84039

here, $q_{e,cal}$ and $q_{e,exp}$ are the calculated and the experimental value of the equilibrium adsorbate solid concentration in the solid phase (mg/g) and n_m is the number of data points, where n_p is the number of parameters in the isotherm equation.

From Table 4, it was found that, similar to the adsorptive removal of cyanide from various cyanide solutions, various isotherm models were fitted to the SAB removal of cyanide. The correlation coefficient for R–P isotherm model was found closer to unity as compared to other isotherm models. The MPSD values for R–P isotherm models were found to be lower comparative to the other isotherm models for the three cyanide compounds for SAB studies immobilized BAC. Hence R–P isotherm model was found to be best fitted to the experimental results.

4. Conclusion

The process parameters such as pH and agitation time have significant effect on the removal of cyanide. It was found that SAB process is more effective and less time consuming. The cyanide removal is much higher in SAB process as compared to biodegradation. It was observed from the biodegradation process that biodegradation delayed due to increase in lag phase of *P. putida* in higher concentration of cyanide. In case of the SAB process due to adsorption, a certain amount of cyanide ions was removed, and then the biodegradation process was started along with the adsorption. The toxicity of the cyanide in the solution was reduced due to adsorption on monolayer and cyanide was easily available for degradation. The cyanide adsorbed on the biologically active GAC surface could be easily biodegraded by microbes as GAC acts as enrichment surface and attached growth on it has shown better efficiency. This made the attach growth process more effective than the suspended growth process and has given better efficiency.

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