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Treatment of metal cyanide bearing wastewater by simultaneous adsorption and biodegradation (SAB)

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Abstract

This paper presents process review and comparative study of biodegradation and adsorption alone with simultaneous adsorption and biodegradation (SAB) process using *Pseudomonas fluorescens*. Ferrocyanide solution was used for all studies with initial CN^- concentrations of 50, 100, 200 and 300 mg/L, and initial pH of 6. *Pseudomonas fluorescens* used ferrocyanide as sole source of nitrogen and biodegradation efficiency was observed as 96.4, 94.1, 86.2 and 69.3%, respectively after 60 h of agitation. Whereas in adsorption process with granular activated carbon (GAC) as adsorbent, CN^- removal efficiency was found to be 85.6, 80.1, 70.2 and 50.2%, respectively. But in SAB process the removal efficiency could be more than 70% for all concentrations only at 36 h of agitation and achieved removal efficiency of 99.9% for 50 and 100 mg CN^-/L. It was found that SAB process is more effective than biodegradation and adsorption alone.

Keywords: Adsorption; Biodegradation; Ferrocyanide; *Pseudomonas fluorescens*; SAB

1. Introduction

Cyanide compounds are the strictly regulated compounds worldwide because of their extreme toxicity [1]. Although cyanides are present in small concentrations in a number of plants, foods and microorganisms, their large-scale presence in the environment is attributed to the human activities as cyanide compounds are extensively used in various industries [2]. Bulk of cyanide occurrence in environment is due to metal finishing and mining (extraction of gold, silver, etc.) industries. Cyanide is also used and produced as waste with varying concentrations in effluents from industries like, coke plant, paint and ink formulation, petroleum refining, explosive manufacturing, case hardening, automobile manufacturing, printed circuit board manufacturing, chemicals, pesticides industries and synthetic fiber production units, etc. [2–6]. The release of cyanide from these industries worldwide has been estimated to be more than 14 million kg/year [1]. Generally cyanide compounds present in environmental matrices and waste streams as simple and complex cyanides, cyanates and nitriles [1,7]. The stability of cyanide salts and complexes is pH dependent, and therefore, their potential environmental impacts and interactions (i.e. their acute or chronic effects, attenuation and re-release) can vary [1,2]. 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 [2]. Cyanide complexes with iron are very stable even under mildly acidic conditions, however, both ferro- and ferricyanides decompose to release free cyanide when exposed to direct ultraviolet light in aqueous solutions [2,8].

Due to their toxic effects, cyanide-containing effluents cannot be discharged without detoxification to the environment. United States (U.S.) health service cites 0.01 mg/L as guideline and 0.2 mg/L as permissible limit for cyanide in effluent [2]. U.S. Environmental Protection Agency standard for drinking and aquatic-biota waters regarding total cyanide are 200 and 50 ppb, respectively [6,9]. In India Central Pollution Control Board (CPCB) has set a minimal national standard (MINAS) limit for

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Nomenclature

C_e	equilibrium concentration of adsorbate in aqueous solution (mg/L)
C_f	initial ferrocyanide concentration (mg CN^-/L)
D_c	dose/mass of GAC in aqueous solution (g/L)
K_f	Freundlich constant (mg/g)/(mg/L)$^{1/n}$
K_l	Langmuir constant related to enthalpy (energy/intensity) of adsorption
M	weight of GAC taken
n	Freundlich constant
P_c	particle size of GAC (mm)
Q_e	mass of pollutant adsorbed on unit mass of GAC at equilibrium (mg/g)
Q_m	Langmuir constants related to adsorption capacity (mg/g)
S_a	agitation speed (rpm)
t_a	agitation time (h)
X	amount of cyanide adsorbed

cyanide in effluent as 0.2 mg/L [2,10]. 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 (acidification, volatilization and reneutralization) process, lime–sulfur, reverse osmosis, activated carbon adsorption, thermal hydrolysis and biological treatments, etc. [2–6,10–12]. Adsorption and biodegradation are two significant methods for treatment of wastewater bearing cyanide compounds, were used separately and together for present study.

1.1. Background of these methods

Adsorption is a widely used technology for removal of cyanide [13]. There are several reports on the treatment cyanide compounds by adsorption on plain and metal-impregnated activated carbons [14–19]. Huff et al. examined the feasibility of removing cyanide from refinery wastewater with powdered activated carbon [14]. The mining operations mostly use adsorbents for recovery and removal of cyanide from effluents [14,15]. Granular/powdered carbon is the most widely used adsorbent, as it has a good capacity for the adsorption of cyanide compounds [16]. However, high cost of activated carbon and 10–15% loss during conventional regeneration has been the disadvantages in the utilization of activated carbon in the developing countries [20].

Although the physical and chemical processes are frequently used for cyanide removal, biological methods are gaining support as potentially inexpensive and environmental friendly alternatives [5]. Many cyanogenic and non-cyanogenic microorganisms were found to have specific enzymes and pathways for the cyanide degradation [1]. Most reports described that metabolism of cyanides by strains of *Pseudomonas*, *Acinetobacter*, *Bacillus*, and *Alcaligenes* [21–24]. In addition, some fungi such as *Fusarium solani*, *Fusarium oxysporum*, *Gloeocercospora sorghi*, *Fusarium lateritum*, *Stemphylium loti* has demonstrated the ability to utilize cyanide as a nutrient for growth [25–29]. Several other bacteria including *P. fluorescens* NCIMB 11764 utilized moderately strong metal complexed cyanides at neutral pH [30].

Biodegradation is performed in presence of microbes either in mobilized or immobilized phase [31]. The immobilization of living microbial cells on a suitable adsorbent improves the removal efficiency [32]. This improvement is due to the bio-layer formation on the adsorbent bed where adsorption and biodegradation occurs simultaneously [18,33,34]. Unlike adsorption, there is a continuous diffusion of adsorbate onto the solid surface and back diffusion of solute into the solution phase. The solute remaining in solution exists in dynamic equilibrium with that of in the surface of biofilm [35]. Adsorption and the biodegradation successfully supplement each other in the various schemes of wastewater treatment. Microbial mass can, in some extent, adsorb the substances, but at the same time it also degrades them [36]. On the other hand, adsorption of the substances onto adsorbent reduces the inhibitory effect of the substances for microbial mass. Accordingly, simultaneous adsorption and biodegradation (SAB) is expected to be more stable and the toxic compounds may be converted into less harmful substances [20,37]. There are several reports on the treatment of cyanide compounds by adsorption, biodegradation and bio-adsorption processes. Patil and Paknikar got encouraging results using a combination of biosorption and biodegradation processes for removal of metal cyanides [36]. Dursun and Aksu [11], Bose et al. [38] and Papirer et al. [39] investigated the effectiveness of biodegradation process by immobilized microbes for removal of cyanide compounds. Granular activated carbon (GAC) has good adsorption capacity as well as bio-layer formation capacity [40], however; the use of GAC for the immobilization of cells has rarely been reported for the removal of cyanide compounds. Although the fate of SAB using activated carbon is not clear, but this process has been successfully used for many other chemicals and toxic substances.

1.2. Present study

In the present study biodegradation, adsorption and SAB of ferrocyanide bearing synthetic solutions were performed separately in absence or in presence of GAC in a batch reactor without or with immobilized *Pseudomonas fluorescens* (MTCC Code103) on GAC bed. The process parameters like adsorbent dose, particle size of GAC and pH were optimized in batch experiments by studying the effect of process parameters on percentage removal. All studies were carried out in 250 mL conical flasks in incubator shaker at temperature of 26 °C through out the experiment. Equal initial concentrations of pollutant were maintained for these three processes. Percentage removal and specific uptake were studied and compared in both cases of adsorption and SAB. The microbes adopted to grow at maximum cyanide concentration were harvested and its

ability to degrade cyanide was measured in biodegradation and SAB.

2. Materials and methods

2.1. Preparation of ferrocyanide solution

Cyanide solution was prepared by dissolving 2.7 g of $K_4[Fe(CN)_6]\cdot 3H_2O$ in 1 L of double distilled water to yield a stock ferrocyanide solution of 1000 mg CN^-/L. Initial ferrocyanide concentrations (C_f) of 50, 100, 200 and 300 mg CN^-/L were used for adsorption, biodegradation and SAB study.

2.2. Adsorption studies

GAC particles of analytical grade purchased from S.d. fine-chem. limited, India in the size range of 2–5 mm, were used as adsorbent and as the support medium for bio-layer formation. The bulk density of this carbon was found to be 400 g/L. It was sieved to various fractions of particle sizes (P_c) of 1.2–2 mm, 2–4 mm and 4–5 mm with standard testing sieves. Purification was carried out by soxhlet extraction with acetone/*n*-heptane (50:50 v/v) for 24 h and dried at 110 °C. The elemental analysis of the GAC, surface area and the porosity of various fractions have been listed in Table 1. The GAC particle size and dose had been optimized from a particle size range of 1.2–2 mm, 2–4 mm and 4–5 v mm and GAC dose (D_c) of 5–50 g/L. For optimization of GAC, studies were carried out in a 250 mL conical flask at 26 °C and initial pH 6 in an incubator shaker at 150 rpm with initial ferrocyanide concentration of 100 mg CN^-/L. All adsorption studies were carried out for various concentrations of ferrocyanide with optimum size and dose of sterilized GAC in a 250 mL conical flask at 26 °C and initial pH 6 in an incubator shaker at 150 rpm. Sterilization of GAC was performed in an autoclave at 121 °C for at least 20 min.

2.3. Microorganisms and growth conditions

Freeze-dried culture/lyophilized culture of *P. fluorescens* (MTCC Code 103) species were obtained from Institute of Microbial Technology (IMTECH), Chandigarh, India. Culture was revived in the growth media as per the guidelines of IMTECH at 26 °C. The bacterium was grown in an enrichment medium prepared by adding following ingredients; glucose (5 g), peptone (1 g), yeast extract (1 g), KH_2PO_4 (0.5 g), K_2HPO_4 (0.5 g), NH_4SO_4 (0.5 g), $MgSO_4$ (0.05 g) in 1 L distilled water and pH was adjusted to 7.5 [13]. The pH was adjusted with concentrated and diluted H_2SO_4 and NaOH. After the culture was inoculated into 100 mL enrichment medium (in 1:100 ratio) in a 250 mL conical flask, it was incubated at 26 °C in incubator shaker (120 rpm) for 24 h [12,20]. Figs. 1 and 2 show the growth of *P. fluorescens* on Petri dish after revival of the culture.

Table 1
Properties of GAC particles

Particle size (mm)	BET surface area (m^2/g)	Micropore (<2nm) volume (cm^3/g)	Elemental analysis (%)
1.2–2.0	778.23	0.2381	C, 75.11
2.0–4.0	583.35	0.2112	H, 1.913
4.0–5.0	393.72	0.1701	N, S, 0.0

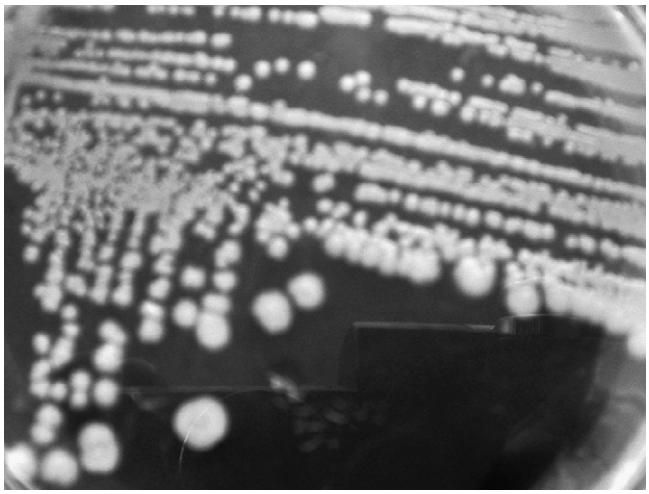

Fig. 1. Photograph of *P. fluorescens* colonies grown on Petri dish.

Microorganisms were transferred (in 1:100 ratio) into the growth medium containing ferrocyanide ions as only source of carbon and nitrogen replacing glucose, peptone yeast extract and NH_4SO_4 from the enrichment medium. Ferrocyanide was first used as both carbon and nitrogen source with the mineral salts. But no growth was observed in media containing ferrocyanide as the sole source of carbon and nitrogen. Addition of glucose into the medium had major influence on degradation of ferrocyanide and led to bacterial growth and biodegradation of ferrocyanide. Hence, glucose was used as the carbon and energy source in the biodegradation medium. Again, addition of NH_4SO_4 to the medium reduced the utilization of ferrocyanide as nitrogen source. The biodegradation medium was prepared by mixing the ferrocyanide solution (as the only source of nitrogen) autoclaved separately and the sterilized solution

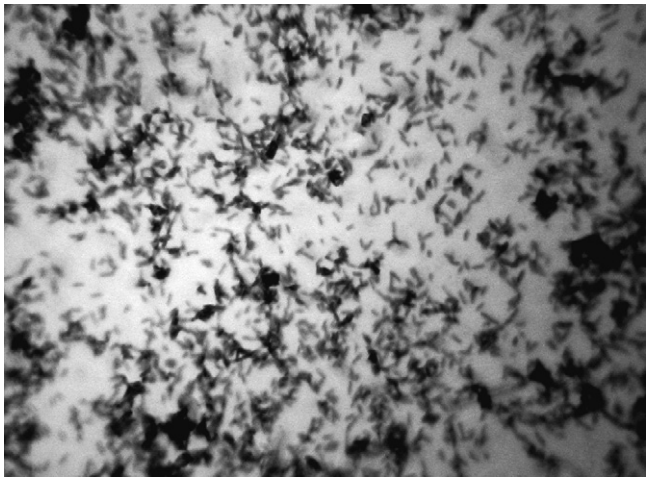

Fig. 2. Photomicrograph of *P. fluorescens* cultures (observed in microscope with 100× magnifications).

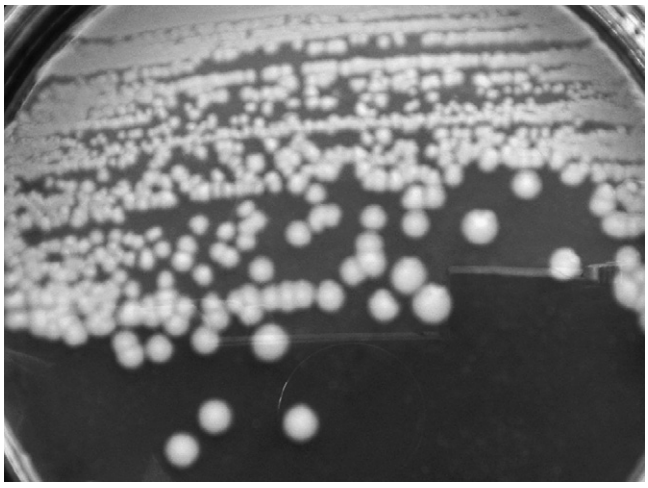

Fig. 3. Photograph of *P. fluorescens* colonies adapted to ferrocyanide grown on Petri dish.

containing other ingredients such as glucose (5 g/L), KH_2PO_4 (0.5 g/L), K_2HPO_4 (0.5 g/L), $MgSO_4$ (0.05 g/L). The microorganisms were adapted to ferrocyanide in the medium starting from a lower concentration of 5–50 mg CN^-/L. The initial pH of the solution was adjusted to the desired value by using sterile dilute and concentrated H_2SO_4 or NaOH. Sterilization of the medium was performed in an autoclave at 121 °C for at least 20 min. Figs. 3 and 4 show the growth of ferrocyanide-adapted culture of *P. fluorescens* on Petri dish.

2.4. Biodegradation and SAB studies

All batch experiments for biodegradation and SAB processes were performed in 250 mL conical flasks containing 100 mL of the growth medium (microbial mass 1:100) at 26 °C in a rotary incubator shaker at 150 rpm for 60 h [3,12]. The effect of pH on rate of degradation of ferrocyanide for both biodegradation and SAB was observed for 100 mg CN^-/L. 20 g/L sterilized GAC was added to the growth medium in conical flask for SAB study. Separate studies were conducted for various initial ferrocyanide concentrations. During agitation in incubator shaker, the contents of the flasks were analyzed periodically for residual cyanide concentration.

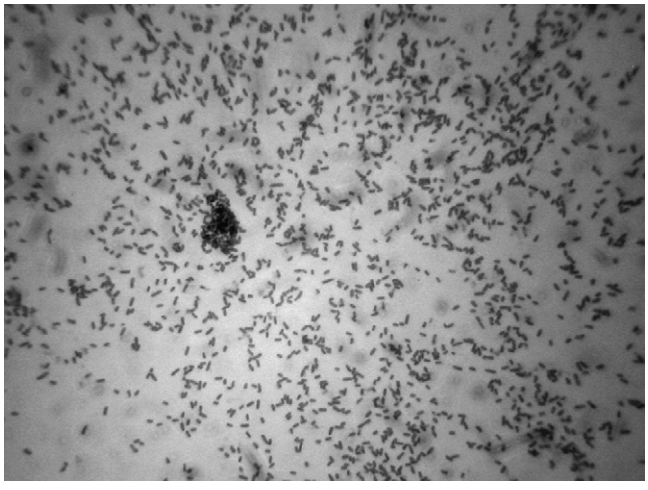

Fig. 4. Photomicrographs of *P. fluorescens* cultures adapted to ferrocyanide (observed in microscope with 100× magnifications).

2.5. Analyses

Parameters such as pH, total cyanide content, optical density (at 550 nm wavelength) and/or bacterial cell count were checked periodically. Total cyanide concentration was determined by pyridine–barbituric acid colorimetric method (at 578 nm wavelength), after distillation as described in standard methods [41] up to a minimum concentration of 0.01 mg CN^-/L. For the detection of bacterial growth, the biomass concentration was determined by measuring the absorbance of the broth at 550 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 [41] using pH meter supplied by WTW® Germany (makes pH 720). The CHNS analysis was done by elemental analyzer system GmbH, model Vario-EL V3.00 and the pore volume (micropore <2 nm) of the samples were measured by N_2 adsorption isotherm using an ASAP 2010 Micromeritics instrument by Brunauer–Emmett–Teller (BET) method, using the software of Micromeritics [37]. Nitrogen was used as cold bath (77.15 K) [37]. Photomicrographs of bacterial colonies and GAC were studied by scanning electron microscope (SEM) (LEO® 435 VP). Photomicrographs of bacterial cultures were studied by light microscope and digital camera (Nikon®)

3. Results and discussions

3.1. Effect of initial pH

pH played an important role for the removal of ferrocyanide from aqueous solutions by adsorption, biodegradation and SAB processes. The effect of initial pH on the rate of ferrocyanide removal has been illustrated in Fig. 5. Dursun et al. has obtained an optimum pH 5 for ferrocyanide biodegradation by *P. fluorescens* [12]. Chank [42] and Dzombak et al. [43] reported that adsorption of ferrocyanide was greatest at low pH and decreased as pH increased. From Fig. 5 it was clear that, for adsorption study the rate of ferrocyanide removal was higher at lower pH values. The effect of pH on the adsorption could be attributed to several mechanisms such as electrostatic interaction, complexation, ion exchange and surface charge on carbon [15,18,44]. Although the removal rate increased for lower pH value, it was not significant below pH 5.5. During biodegradation and SAB process the maximum rate of ferrocyanide removal was observed at pH 5.5–6.5. But no significant efficiency was observed at lower or higher pH values in case of biodegradation. Although the removal efficiency achieved maximum in between pH 5.5 and 6.5, but to avoid high acidic condition and to maintain a similar comparative study, pH 6 was taken as the optimum value for the three processes. Also, as *P. fluorescens* grow optimally at pH 7.5 [13], the pH value was taken closer to the neutral side.

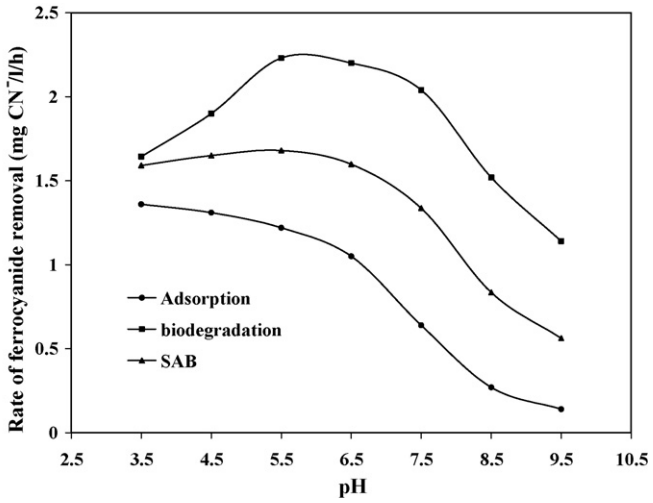

Fig. 5. The effect of initial pH on ferrocyanide removal by adsorption, biodegradation and SAB (T = 26 °C, C_f = 100 mg CN^-/L, D_c = 20 g/L, t_a = 60 h, S_a = 150 rpm).

3.2. Adsorption efficiency 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. During sieving of the GAC particles it was found that more than 80% of particles were of 2–4 mm size. Fig. 6 shows the percentage of ferrocyanide removal with agitation time (t_a) on various GAC particle sizes (P_c) of 1.2–2 mm, 2–4 mm and 4–5 mm for initial ferrocyanide concentration of 100 mg CN^-/L. Although not much variation in removal efficiencies was found on these particles sizes, results indicated a better performance in the particle range of 2–4 mm. Hence 2–4 mm size particles (optimum size) of GAC were used for adsorption studies. Adsorbent doses of 5–50 g/L were used and optimum dose was decided to be 20 g/L, as 75–80% of ferrocyanide was removed with 20 g/L GAC at 36 h agitations

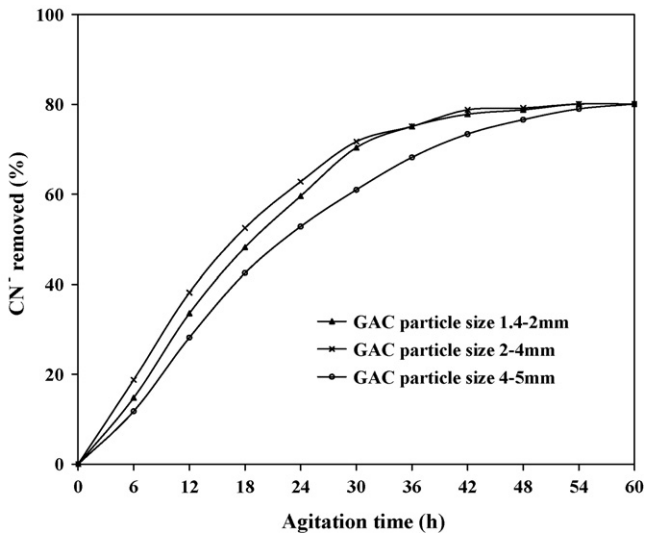

Fig. 6. Effect of particle size on adsorption of cyanide (T = 26 °C, C_f = 100 mg CN^-/L, D_c = 20 g/L, concentration of CN^- = 100 mg/L, initial pH 6, S_a = 150 rpm).

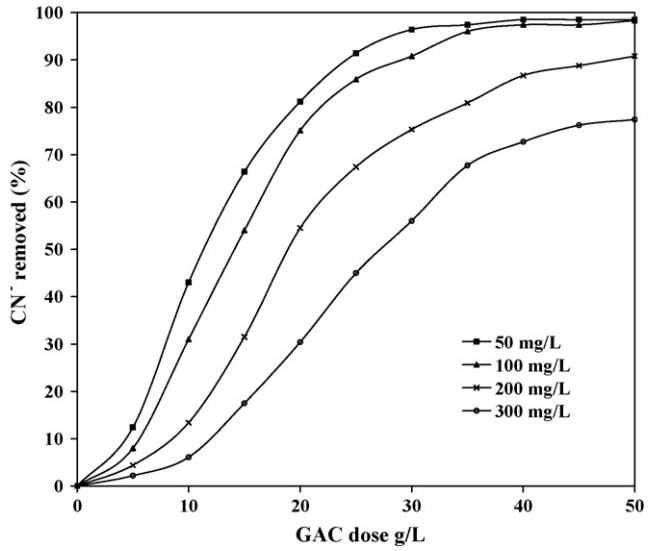

Fig. 7. Effect of GAC dose on ferrocyanide removal (%) by adsorption for various initial ferrocyanide concentrations (T = 26 °C, initial pH 6, P_c = 2–4 mm, t_a = 60 h, S_a = 150 rpm).

for initial ferrocyanide concentrations of 50 and 100 mg CN^-/L (Fig. 7). Guo et al. (1993) presented that diffusion of CN^- from the bulk solution to the active surface sites was appeared to be the slowest step [15]. This process was dependent on the concentration gradient between those two points and the thickness of the diffusion layer, which was a function of the agitation process [18,44]. Although there are reports on adsorption of cyanide in 24 h agitation is adequate, but equilibrium (90–95% uptake) has been attained only after 24 h [15]. Fig. 8 shows the percentage of cyanide removal with the increase in duration of shaking for various concentrations of ferrocyanide. In the present study the maximum adsorption efficiency for various concentrations of ferrocyanide was achieved only after 36–42 h of shaking.

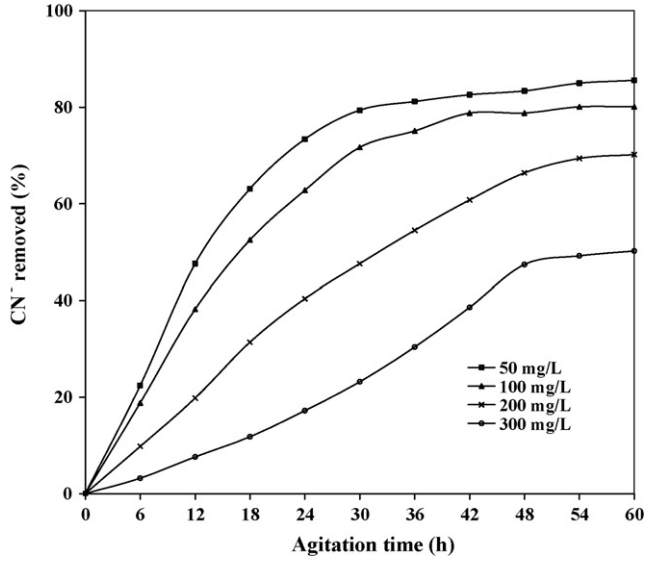

Fig. 8. Effect of agitation time on ferrocyanide removal (%) by adsorption for various initial concentrations (mg CN^-/L) (T = 26 °C, initial pH 6, P_c = 2–4 mm, S_a = 150 rpm).

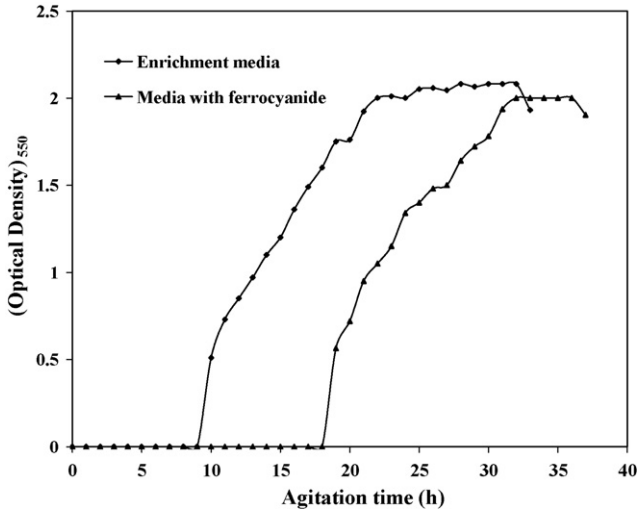

Fig. 9. Growth of non-adapted and adapted cultures of *P. fluorescens* (T = 26 °C, initial pH 7.5, S_a = 120 rpm).

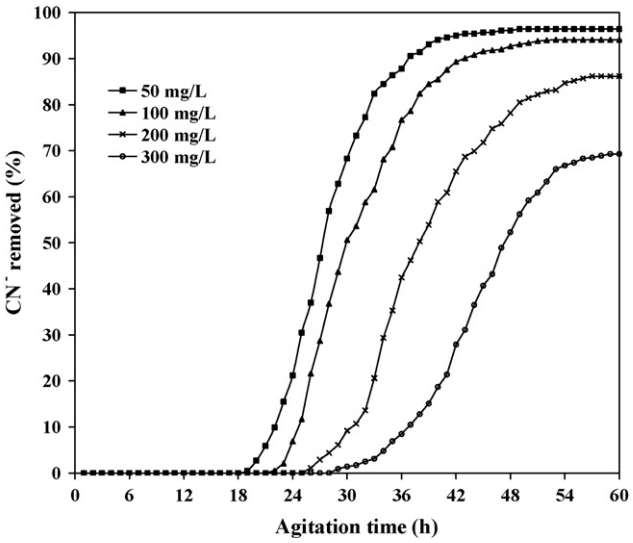

Fig. 10. Biodegradation efficiency (%) for removal of ferrocyanide by *P. fluorescens* for various initial concentrations (mg CN^-/L) (T = 26 °C, initial pH 6, S_a = 150 rpm).

3.3. Growth of microbes in culture media

Fig. 9 shows the growth of non-adapted and adapted culture (with 50 mg CN^-/L) in nutrient medium and medium with ferrocyanide, respectively. From Fig. 9 it was observed that growth of *P. fluorescens* starts after 8–9 h of agitation in enrichment medium. But in the medium without any other nitrogen source, in the presence of ferrocyanide the growth was delayed for about 10–12 h. In the presence of cyanide ions the death phase of the microbe comes faster as compared to the growth in enrichment medium. The log growth phase in both the culture medium was lasted for 12–15 h.

3.4. Biodegradation study

The effects of biodegradation parameters including pH, primary (glucose) and secondary (ferrocyanide) substrate concentrations on the growth of *P. fluorescens* and biodegradation of ferrocyanide were investigated. For biodegradation study in the presence of ferrocyanide, initial pH was adjusted to 6.0. The toxicity of cyanide compounds exerts difficulties in bacteria capable of using these as a carbon source for growth [12]. Cyanide was found to be not utilized as a carbon source by *P. fluorescens*, as no growth was observed in the absence of glucose. Since the amount of nitrogen needed for the growth is less than the requirement for carbon, it could be easier to utilize ferrocyanide as a source of nitrogen in the presence of another source of carbon and energy [3,11]. It was also reported that, microorganism degraded ferrocyanide only in the absence of another nitrogen source and therefore, ferrocyanide was used as the sole source of nitrogen [3,13,20]. Initially NH_4SO_4 (0.5 g/L) (with other compounds as given in Section 2.3.) was added to the biodegradation medium with ferrocyanide. The presence of separate nitrogen source (NH_4SO_4) in the biodegradation medium, highly affected the ferrocyanide degrading ability of the *P. fluorescens* cells. The bacteria utilized the easily degradable nitrogen source (NH_4SO_4) and ferrocyanide remained in the medium. NH_4SO_4 was not used in the biodegradation medium and ferrocyanide was therefore used as sole nitrogen source in these experiments. Fig. 10 shows the percentage removal of ferrocyanide with agitation time (t_a). Ferrocyanide biodegradation rate was determined from the slope of ferrocyanide consumption versus time plot. From the results it was observed that after 60 h of agitation, ferrocyanide removal efficiency of 96.4, 94.1, 86.2 and 69.3% were achieved for initial ferrocyanide concentrations of 50, 100, 200 and 300 mg CN^-/L, respectively.

3.5. Simultaneous adsorption and biodegradation study

Simultaneous adsorption and biodegradation study was conducted to evaluate the performance of the combine process of ferrocyanide removal and to compare its data to adsorption

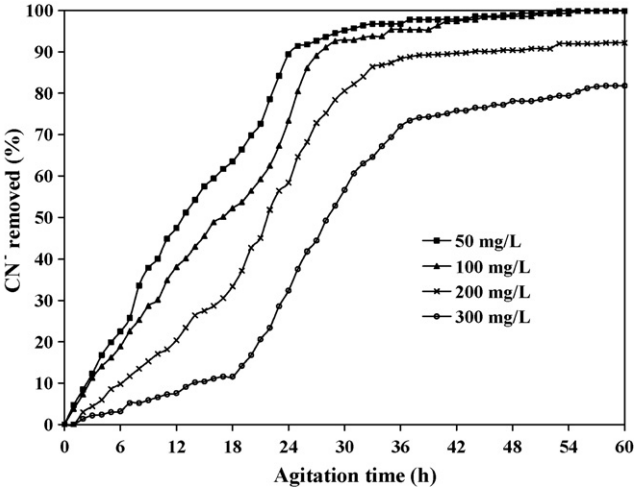

Fig. 11. SAB efficiency (%) for removal of ferrocyanide by *P. fluorescens* immobilized on GAC for various initial concentrations (mg CN^-/L) (T = 26 °C, initial pH 6, P_c = 2–4 mm, D_c = 20 g/L, S_a = 150 rpm).

and biodegradation processes alone. Fig. 11 shows the percentage of ferrocyanide removal with agitation time (t_a). Aksu and Gülen (2002) reported binary biosorption of iron (II) and iron (III)-cyanide complex ions on *Rhizopus arrhizus* [45]. Patil and Paknikar (1999) reported that residual cyanide in the solution subjected to biodegradation after bio-adsorption [36]. From Fig. 10 it was observed that biodegradation delayed for a few hours due to delayed growth of microbes in the presence of cyanide ions. But in Fig. 11, in SAB process cyanide removal was started at an earlier time than that observed in biodegradation alone in Fig. 10. Due to the delayed growth of microbes in the culture media, adsorption might have been occurred initially in the first phase followed by biodegradation. Removal efficiency was varied linearly in the first phase and then parabolically after 18–24 h of agitation. It was observed from the results that in SAB process nearly 99.9% removal efficiency of cyanide was achieved only at 30–36 h of agitation, where as maximum efficiency achieved by biodegradation was 91.4 and 96.4% and by adsorption was 80.1 and 85.6% for initial ferrocyanide concentrations of 50 and 100 mg CN^-/L. For ferrocyanide concentrations of 200 and 300 mg CN^-/L, the removal efficiency was found to be 92.2 and 81.8%, respectively. The immobilization of living microbial cells on adsorbent improves the removal efficiency [32]. In the presence of microbial film, the removal of substances is mechanistically complex involving (i) transport of substances from the bulk liquid to the surface of microbial film, (ii) simultaneous mass transfer, adsorption, and biochemical reaction within microbial film, and (iii) simultaneous mass transfer and adsorption within adsorbent [35,37]. The complexity increases due to dynamic nature of the microbial film. As biochemical reaction of substances may occur on the adsorbent and in bulk suspension, the presence of biomass in adsorption process could also be expected, in some extent, to regenerate the adsorbent and thus long life operation of adsorption process may be expected. Activated carbon can be partially regenerated by microorganisms while the carbon bed is in operation [46,47]. In SAB the presence of activated carbon increases the liquid–solid surfaces, on which microbial cells, enzymes, organic materials and oxygen are adsorbed providing an enriched environment for microbial metabolism [32,35,37]. Surface catalysis of physicochemical reactions is also possible on the surface of activated carbon. The carbon adsorption capacity, controlled by the bioregeneration, is highly increased and the carbon adsorption column cycle is prolonged as compared to pure adsorption system alone [48,49]. The removal efficiency for higher concentrations of ferrocyanide, SAB showed better performance than adsorption and biodegradation alone. The SAB process was found to be more effective than other processes used alone.

3.6. Scanning electron microscope (SEM) analysis

Photomicrography of bacteria and GAC was carried out in SEM. SEM shows the structure of biofilm formation on the surface of GAC. Fig. 12 shows the SEM photograph of plain GAC used in adsorption study. Figs. 13 and 14 show the SEM photograph of *P. fluorescens* in biodegradation medium and on GAC, respectively. The formation of biofilm on the surface of GAC not

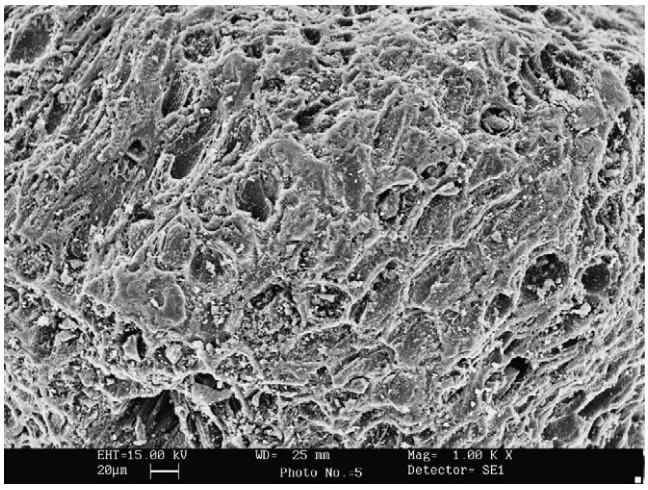

Fig. 12. Photomicrograph of Plain GAC during adsorption (observed in SEM at 1000× magnifications).

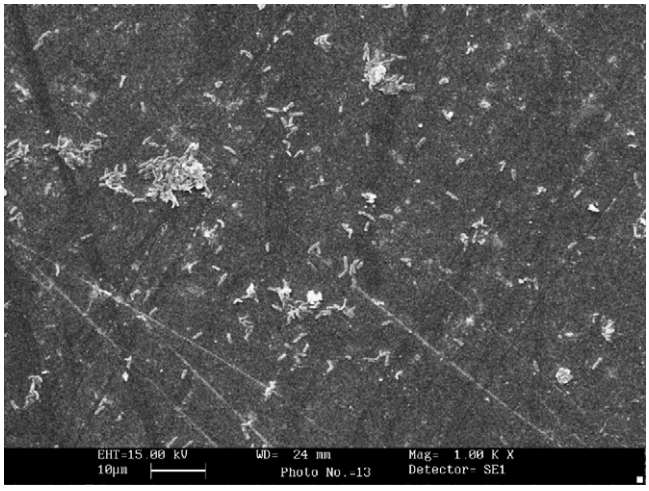

Fig. 13. Photomicrograph of *P. fluorescens* in biodegradation medium (observed in SEM at 1000× magnifications).

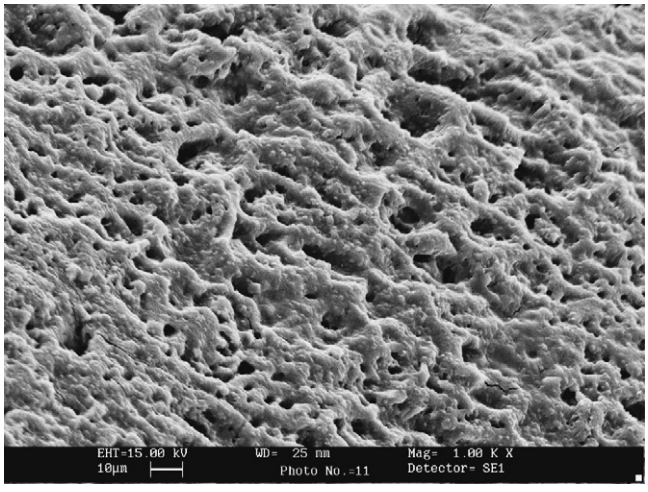

Fig. 14. Photomicrograph of *P. fluorescens* on GAC (observed in SEM at 1000× magnifications).

Table 2
Freundlich and Langmuir isotherm constants for adsorption and SAB

Process	Freundlich isotherm constant			Langmuir isotherm constants		
	K_f (mg/g)/(mg/l)$^{1/n}$	$1/n$	R^2	Q_m (mg/g)	K_l(l/mg)	R^2
Adsorption of cyanide	1.023	0.428	0.9306	9.02	0.043	0.9966
SAB of cyanide	5.72	0.189	0.8927	11.65	5.882	0.9686

only depends on GAC, but also on nutrient provided [50]. From SEM study it was observed that GAC was a good adsorbent, which could be used as a good biological attachment medium for SAB.

3.7. Adsorption isotherm study

The ferrocyanide removal by adsorption and SAB values were fitted to Freundlich and Langmuir models as validity of these models were reported for adsorption and SAB process for various compounds [37,51–55]. Freundlich isotherm may be developed on the basis of formation of monolayer due to the adsorption onto a rough heterogeneous surface (multisites) [15]. For calculating the Freundlich adsorption isotherm, the data had to be plotted in terms of equilibrium concentration (C_e) as per Eq. (1) [15]:

$$Q_e = \frac{X}{M} = K_f(C_e)^{1/n} \quad (1)$$

where Q_e is the specific uptake per gram of GAC, X the amount of cyanide adsorbed, M the weight of GAC taken, and K_f and n are the adsorption isotherm constants. The equation for Freundlich adsorption isotherm can be rewritten as [37]:

$$\log Q_e = \frac{1}{n}(\log C_e) + \log K_f \quad (2)$$

The cyanide uptake probably corresponds to the amount of cyanide adsorbed in forming complete monolayer coverage (Q_m) in Langmuir isotherm. To arrive at the adsorption capacity at the GAC surface, the adsorption data were analyzed using Langmuir isotherm given by Eq. (3) [17,19]:

$$Q_e = \frac{Q_m K_l C_e}{1 + K_l C_e} \quad (3)$$

where Q_m is the mass of solute adsorbed/mass of adsorbent for complete monolayer and K_l is the constant related to enthalpy (energy/intensity) of adsorption. Eq. (3) can be rearranged in following linear form as [37]:

$$\frac{1}{Q_e} = \frac{1}{Q_m K_l}\frac{1}{C_e} + \frac{1}{Q_m} \quad (4)$$

Table 2 represents the Freundlich and Langmuir isotherm constants for adsorption and SAB studies. Linear transformation of the adsorption data using Freundlich and Langmuir isotherm models allowed computation of metal cyanide adsorption capacities. Experimental data obtained in the study were found to obey basic principles underlying the models, that is, heterogeneous surface adsorption and monolayer adsorption at constant adsorption energy respectively for Freundlich and Langmuir isotherm [36]. From Table 2 it is evident that R value lies within 0–1 for all the cases and n value is also greater than 1 and lies within 1–6. Therefore both Langmuir and Freundlich isotherms can explain the adsorption as well as SAB processes [37]. It is also evident that both for adsorption and SAB of cyanide the Q_m values in Langmuir isotherms are greater than the corresponding K_f values in Freundlich isotherms. This indicates that Langmuir isotherm is better fit for the description of adsorption process than the Freundlich isotherm [37,52]. For SAB of cyanide Q_m and K_l values are more than those of adsorption in Langmuir isotherms. This indicates more adsorption capacity of the adsorbents and more energy of adsorption in SAB process. This may be due to the dominating role of SAB over adsorption.

4. Conclusion

In the present study, the capability of the microbe to remove ferrocyanide complex individually and simultaneously by adsorption and biodegradation from synthetic wastewater was examined. The removal efficiency was achieved up to 81.8% for ferrocyanide with initial concentration of 300 mg CN^-/L by SAB. Growth of microbe and reduction in cyanide concentration started earlier by this process. This may be due to the reduction of certain amount of cyanide in the solution due to adsorption on GAC in initial phase. It was observed from the results that biodegradation delayed due to presence of higher concentration of CN^- and only about 69.3% removal efficiency was achieved for 300 mg CN^-/L. Also, for adsorption the efficiency of removal reduced to 50.2% for 300 mg CN^-/L. Although there is a decrease in removal efficiency for higher concentrations of ferrocyanide in SAB process, but it was clear from the results that SAB was effective for higher concentrations as compared to biodegradation and adsorption alone. The cyanide adsorbed on the GAC surface could be easily biodegraded by microbes as GAC act as enrichment surface and attached growth gives better removal efficiency. This makes the SAB process more effective than the single process and gives a better efficiency.

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