

Comparative evaluation of cyanide removal by adsorption, biodegradation, and simultaneous adsorption and biodegradation (SAB) process using *Bacillus cereus* and almond shell

Naveen Dwivedi^{1, 2*}, Chandrajit Balomajumder³ and Prasenjit Mondal³

¹Department of Biotechnology, S.D. College of Engineering and Technology, Muzaffarnagar-251 001, India

²Faculty of Biotechnology, Uttarakhand Technical University, Dehradun- 248 007, India

³Department of Chemical Engineering, Indian Institute of Technology Roorkee, Roorkee-247 667, India

*Corresponding Author E-mail: naveen.dwivedi@rediffmail.com

Abstract

The present study aimed to investigate the removal efficiency of cyanide from contaminated water by adsorption, biodegradation and simultaneous adsorption and biodegradation (SAB) process individually in a batch reactor. Adsorption was achieved by using almond shell granules and biodegradation was conducted with suspended cultures of *Bacillus cereus*, whereas SAB process was carried out using *Bacillus cereus* and almond shell in a batch reactor. The effect of agitation time, pH, and initial cyanide concentration on the % removal of cyanide has been discussed. Under experimental conditions, optimum removal was obtained at pH 7 with agitation time of 48 hrs and temperature of 35 °C. Cyanide was utilized by bacteria as sole source of nitrogen for growth. The removal efficiencies of cyanide by adsorption, biodegradation, and SAB were found to be 91.38%, 95.87%, and 99.63%, respectively, at initial cyanide concentration of 100 mg l⁻¹. The removal efficiency of SAB was found to be better as compared to that of biodegradation and adsorption alone.

Key words

Almond shell, *Bacillus cereus*, Biodegradation, Cyanide, Cyanidase, SAB process

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Introduction

Cyanide is one of the highly toxic compounds to humans as well as aquatic organisms. It enters into water through the effluents of various industries such as electroplating and metal finishing, steel tempering, mining (extraction of metals such as gold and silver), automobile parts manufacturing, photography, pharmaceuticals and coal processing units. (Monser and Adhoum, 2002; Z. Aksuet *et al.*, 1999; Patil and Paknikar, 2000). Several bacteria, fungi, algae, and various plants are able to produce cyanide as a defence mechanism against predation (Luque-Almagro *et al.*, 2005). Cyanide is a prominent metabolic inhibitor by inactivating respiration due to its tight binding to cytochrome C oxidase. In this process electron transport chain is blocked

by cyanide by binding with iron ion in terminal electron acceptor cytochrome C oxidase consequently rapidly falling respiration rates and ATP synthesis in mitochondria is inhibited (Gupta *et al.*, 2010). Various microorganisms and plants have shown resistivity against cyanide poisoning since they have developed alternate pathway for ATP production. Some of them have diverse oxidase instead of cytochrome C oxidase (Siedow and Umbach, 1995). In order to fulfill the environmental regulations, wastewater containing cyanide must be treated before discharging into the environment. Therefore, Central Pollution Control Board (CPCB), India has set a minimal national standard (MINAS) limit in effluent as 0.2 mg l⁻¹ (Dash *et al.*, 2009). USEPA (US Environmental Protection Agency) standard for drinking and aquatic-biota waters regarding total cyanide is 200 and 50 ppb respectively

(Young and Jordan, 1995; USEPA, 1985). There are several methods for treatment of cyanide contamination. Existing conventional wastewater treatments for cyanide removal employ expensive chemical methods. However, this method is disagreeable from both economic and environmental viewpoint because it requires the use of chemical compounds and does not degrade the complete range of cyanide compounds (Chen *et al.*, 2008). Adsorption is also used for cyanide removal and it is better to other wastewater treatment techniques in terms of efficiency and low cost (Bela and Malliga, 2015). Adsorption systems are not affected by the toxicity of target compound(s). It is simple to operate and no need to use hazardous chemicals in this process. The biodegradation method of cyanides removal is better to physical and chemical methods (Akcil *et al.*, 2003; Akcil and Mudder, 2003; Desai and Ramakrishna, 1998). Biodegradation is a faster, environmental friendly and quite inexpensive treatment method (Saarela and Kuokkanen, 2004). In cyanide biodegradation process; cyanide is converted to carbon and nitrogen source by various enzymes present in microorganism. Microorganisms are capable to degrade and assimilate cyanide into amino acids, thiocyanate, β -cyanoalanine and vitamins (Trapp *et al.*, 2003).

Adsorption and biodegradation are the significant methods for the treatment of cyanide containing wastewater. Therefore, in present study potential of adsorption, biodegradation, and simultaneous adsorption and biodegradation (SAB) process have been explored individually for removal of cyanide from aqueous solution. The effect of agitation time, solution pH and initial cyanide concentration on removal of cyanide has been studied.

Materials and Methods

Adsorbent and standard solution preparation : Almond shell (*Prunus amygdalus*) a waste product of almond fruit to be used as bioadsorbent, which were obtained from the local market of Muzaffarnagar, UP, India. The shells were cut into small pieces and, after drying and crushing, washed thoroughly with double-distilled water to remove adhering dirt. Then, they were dried in oven at 100°C for 24 hrs and were sieved (Reza *et al.*, 2009). After screen analysis of the grinded product the fraction having average particle size of ~600 μ m was used. The stock solution containing 1000 mg l⁻¹ of cyanide was prepared by dissolving 2.51 g KCN in double distilled water with NaOH pellet (APHA, 2005). The standard solutions of desired concentration range were obtained from stock solution by successive dilutions with double-distilled water. All chemicals used were analytical grade and purchased from Merck Co and Qualigens Fine Chemical Company (Glaxo Smithkline).

Microorganism and growth medium : In present study cyanide-degrading bacterial strain was isolated from soil near to effluent treatment plant of electroplating industry by an enrichment culture technique. After repeated enrichment and purification practices, the bacterial isolate was identified as *Bacillus cereus* on the basis of their morphological, physiological and biochemical characteristics. The microorganism was maintained in a standard nutrient agar medium for growth.

Acclimatization and preparation of inoculum : The acclimatization of isolated culture in cyanide environment was performed as follows. The revived culture was first grown in nutrient broth (NB) media (13 gl⁻¹) in a 250 ml conical flask. Significant bacterial growth was observed in the flask after 36 hrs. Appropriate amount of potassium cyanide (KCN) was added into the flask containing nutrient broth to get a concentration of 10 mg l⁻¹ of cyanide. The flask was inoculated (10% v/v) by above stated revived culture of bacteria and incubated in an orbital incubator shaker at 35 °C with agitation speed of 150 rpm. Thereafter, the cyanide was intermittently added in increments of 10 mg l⁻¹ in a series of 250 ml flasks till the cyanide concentration in the growth media reached 100 mg l⁻¹. For inoculum preparation, a further sub culturing was done and all the inoculum transfers were completed in exponential phase in the media. The temperature was maintained at 35±1 °C.

Experimental procedure

Adsorption study : Batch experiments were carried out for adsorption studies in a 250 ml conical flask using 100 ml solution of different concentration ranges of cyanide from 100 to 1000 mg l⁻¹ along with adsorbent dose 20 g l⁻¹ at pH range 4 to 12 and kept in the incubator shaker at 150 rpm. The solutions pH were maintained by measuring it intermittently each hour and controlled by drop wise addition of N/10 HCL or NaOH solutions. The samples were collected at definite intervals, filtered and analyzed for cyanide ion concentration using picric acid method at 520 nm wavelength using double beam UV/visible spectrophotometer (Microprocessor UV/VIS EI Spectrophotometer model 1371).

Biodegradation study : Experiments on the removal of cyanide by *Bacillus cereus* in bulk phase were carried out in a 250 ml conical flask containing 100 ml of sterilized biodegradation media (BM). The biodegradation media prepared by adding following ingredients(gl⁻¹): KH₂PO₄: 0.5, K₂HPO₄: 0.5, MgSO₄: 0.05 and 0.5 ml of a trace elements solution which containing (mg l⁻¹): MnSO₄.4H₂O: 1.52, Fe SO₄.7H₂O: 0.6, CaCl₂.2H₂O: 3.0, Na₂MOO₄.2H₂O: 6.0. potassium cyanide (KCN) was sterilized by passing it through 0.45 μ m filter and required volume of the cyanide solution was added to the steam sterilized biodegradation

media in laminar hood under aseptic condition through sterile pipette. The biodegradation capacity of *Bacillus cereus* was investigated at different pH range (4-12), agitation time (6-60 hrs) and cyanide concentration ($100\text{-}1000\text{ mg l}^{-1}$). 10% (v/v) acclimatized culture of *Bacillus cereus* was inoculated in each flask containing biodegradation media (BM) with known concentration of KCN under aseptic condition. The pH was maintained at 7 and flasks were kept at $35\text{ }^{\circ}\text{C}$ in an orbital incubator shaker at 150 rpm. After the completion of incubation periods, the solution was centrifuged at $8000 \times g$ for 10 min. The collected liquid from the centrifuge tube was filtered and analyzed spectrophotometrically at suitable wavelength for remaining concentration of cyanide (Dash *et al.*, 2008; Ullhyan and Ghosh, 2014).

Simultaneous adsorption and biodegradation (SAB) study : For simultaneous adsorption and biodegradation (SAB) study, 20 g l^{-1} fresh and sterilized bioadsorbent was added in a 250 ml conical flask having 100 ml of biodegradation medium with 10% (v/v) inoculum of acclimatized *Bacillus cereus* with known concentration of KCN at pH 7 and temperature $35\text{ }^{\circ}\text{C}$. The flask was kept in a rotary incubator shaker at 150 rpm for 60 hrs. The effect of agitation time and initial cyanide concentration on cyanide removal for SAB was observed. Initial cyanide concentration and agitation time was varied between 100 to 1000 mg l^{-1} and 6-60 hrs, respectively.

Results and Discussion

Acclimatization of isolated culture in cyanide environment was performed in $10\text{-}100\text{ mg l}^{-1}$ potassium cyanide concentration. Growth pattern of bacteria in cyanide environment was observed by measuring the optical density (OD) of the incubated media by UV-Visible spectrophotometer at 600 nm (Fig. 1). The effect of varying cyanide concentration in growth medium was observed from Fig. 1 and depicts that the bacterial cell mass decreased with increase in cyanide concentration. However, for low concentration of cyanide not much variation in the growth pattern is found, but for higher cyanide concentration the death phase comes faster and consequently the bacterial autolysis will start (Reyes *et al.*, 2000). Besides this the cells reached stationary phase after 36 hrs and the bacterial population decreased gradually in numbers due to depletion of carbon source and energy supply. It is a well-known fact that the carbon source in the media increases the cell numbers, whereas nitrogen source increases the cell mass of bacteria (Lengeler *et al.*, 1999). It is interesting to note that the lag phase is absent in Fig. 1, as inoculum transfer in present study was performed in log phase. It is reported that if an inoculum is taken from an exponential phase culture subsequently the lag phase will not be noticeable (Raina, 2009). It suggests that the length of lag phase depends on

initial inoculum size as well as the type of medium.

The solution pH plays a significant role in adsorption, biodegradation and SAB process. Fig. 2 shows that, the rate of cyanide removal was higher at lower pH values and it increased with increase in solution pH in adsorption study. For biodegradation study, the percent removal of cyanide increased with increase in solution pH. Significant upturn in percentage removal of cyanide was observed at pH 6-7 for biodegradation. Highest cyanide removal of 91.38% and 95.87% was found for adsorption and biodegradation respectively, at pH 7 and then decreased considerably with increase in solution pH up to 12. Overall pH_{zpc} of almond shell is 6.20. Thus at pH 7 the surface of almond shell contains more negative charge than positive charge. Number of positive charges on bioadsorbent surface gradually decreases with increase in solution pH. On the other hand KCN in aqueous solution produce HCN, which may exist as HCN (gas), HCN (aqueous) or CN^{-} is depending upon the solution pH. It is reported that at lower pH (~ 4) HCN exist predominantly as HCN (gas). At neutral pH it predominantly exists as HCN (aqueous) along with considerable amount of cyanide (Gupta *et al.*, 2013), which increases solution pH. Due to above reason maximum cyanide removal is obtained at around 7 pH. However, the removal efficiency of cyanide in biodegradation process was maximum at pH 8, but to avoid high basic condition, neutral pH was considered optimum pH for all the further biodegradation experiments. Barclay *et al.*, (1998) reported that biodegradation of cyanide compounds by fungal and bacterial species was generally observed at neutral pH.

The effect of contact time on adsorption was investigated at agitation time 15-120 min at optimum pH 7 and adsorbent dose 20 g l^{-1} , whereas for biodegradation and SAB study at 6-60 hrs. Fig. 3 shows that 84.7% cyanide removal was achieved at 100 mg l^{-1} cyanide in a very short contact time of 15 min. The equilibrium removal efficiency of cyanide was 91.38% at 90 min. Adsorption results depicted that cyanide uptake was fast at initial stage of contact time, and after that it became slower near equilibrium. The adsorption rate was found to be almost constant between these two phases of cyanide uptake. It is clear from the fact that enormous amount of unoccupied surface sites exists on the adsorbent for adsorption of cyanide during the initial stage, and after a lapse of time the remaining vacant surface sites were difficult to occupy owing to repulsive forces between the solute molecules of the solid and bulk phase (Mall *et al.*, 1996; Agrawal and Majumder, 2015). From Fig 3 it was observed that the adsorptive removal of cyanide decreased and remained constant after 90 min.

In case of biodegradation, the experimental observation was made for the degradation of cyanide against contact time for 100 mg l^{-1} cyanide concentration up to 60 hrs

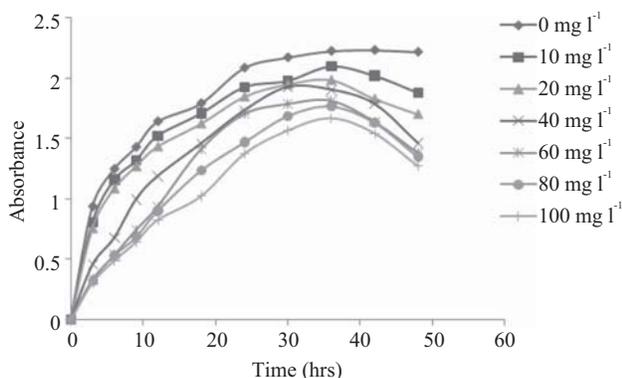


Fig. 1 : Growth pattern of *Bacillus cereus* in nutrient broth (NB) media under various cyanide (KCN) concentration. (Process conditions: Temp.: $35 \pm 1^\circ\text{C}$, pH: 7.0, NB concentration: 13 gl^{-1})

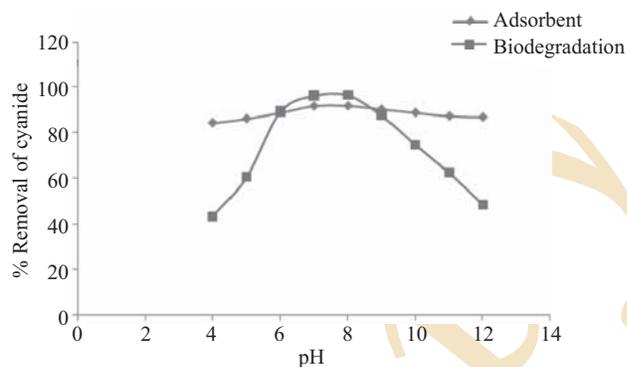


Fig. 2 : Effect of pH on the removal of cyanide by adsorption and biodegradation (at initial concentration of cyanide: 100 mg l^{-1} and concentration of adsorbents: 20 gl^{-1})

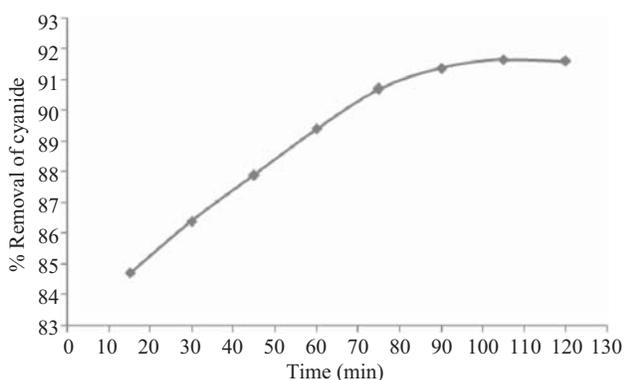


Fig. 3 : Effect of contact time (15-120 min) on cyanide removal by almond shell. (Process conditions: pH: 7; temp: 35°C ; adsorbent dose: 20 gl^{-1} ; initial concentration of cyanide: 100 mg l^{-1} ; rpm: 150)

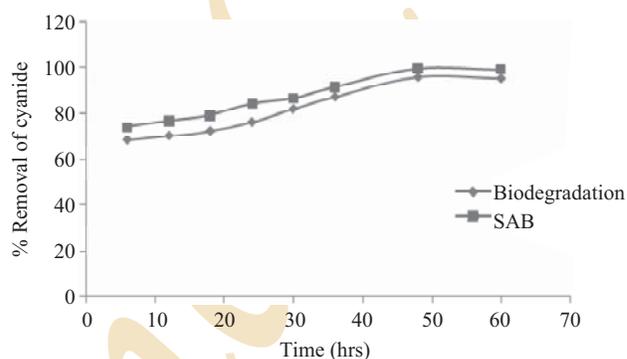


Fig. 4 : Effect of agitation time on biodegradation and SAB of cyanide by *Bacillus cereus* and almond shell (Temp: 35°C ; CN concentration: 100 mg l^{-1} ; pH: 7; rpm: 150)

agitation time. Fig. 4 shows that increase in % removal of cyanide with agitation time in biodegradation process by *Bacillus cereus*. 68.4% cyanide degradation was observed in short period i.e. only 6 hrs. The maximum % removal of cyanide achieved by *Bacillus cereus* was 95.87% at 48 hrs agitation time. After about 48 hrs of agitation time the increase in % removal of cyanide remained almost constant. The percent removal of cyanide was 99.63% at 48 hrs for SAB study; it was higher than adsorption and biodegradation. The results showed that, the percent removal of cyanide by SAB process was not satisfactory in early stage, however, the percent removal of cyanide increased and reached maximum value at 48 hrs. This development was due to formation of bio-layer on the adsorbent surface where adsorption and biodegradation occurred simultaneously (Thompson *et al.*, 2001; Ullhyan and Ghosh, 2014). Formation of bio-layer, at initial stage, is dependent on the porosity of solid support. More porosity gives more layer formation. However, bio-film structure is mainly influenced by substrate concentration (Mondal *et al.*, 2008).

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 on the surface of biofilm (Dash *et al.*, 2008). Combination of both processes i.e., adsorption and the biodegradation are effectively accompaniment to each other in the many schemes of wastewater treatment. Microbial biomass can adsorb materials up to some extent; however, simultaneously it is also degraded by the microorganism (Patil and Paknikar, 1999). Besides this, the inhibitory effect of toxic material for microorganism also decreased by adsorption of cyanide onto adsorbent surface. Hence, simultaneous adsorption and biodegradation (SAB) is more stable and more compatible process to remove pollutants from waste water than adsorption and biodegradation alone.

A series of batch experiments were conducted to determine the effect of initial cyanide concentrations on percentage removal by adsorption, biodegradation and SAB process. Initial cyanide concentrations varied in the range of

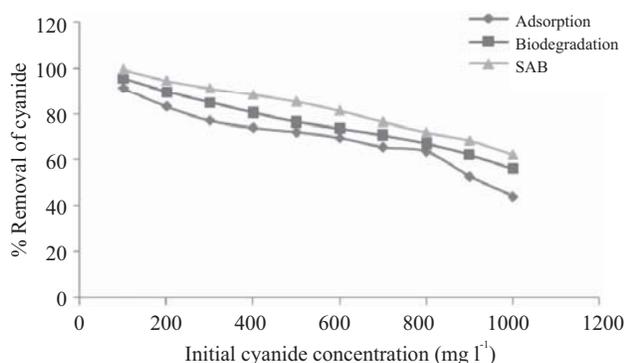


Fig. 5 : Effect of initial cyanide concentration on percentage removal of cyanide (Process conditions: temp.: 35 ± 1 °C, pH: 7.0, rpm: 150)

100-1000 mg l⁻¹ for all the processes. Fig. 5 depicts the results of influence of varying initial cyanide concentration on adsorption, biodegradation and SAB. It was noted that the removal efficiency of cyanide by adsorption decreased with increase in initial cyanide concentration. The results showed that 91.38% cyanide removal was achieved at 100 mg l⁻¹ cyanide and 44.12% at 1000 mg CN l⁻¹ respectively. The reduction of cyanide removal as an efficacy of its concentration can be explained by the restriction of available free sites for adsorption of cyanide with increased cyanide concentration in bulk solution for a fixed mass of adsorbent (Asgari *et al.*, 2014).

Maximum cyanide removal efficiency of *Bacillus cereus* was achieved as 95.87% for concentration as low as 100 mg l⁻¹ cyanide and 56.12% cyanide removal was achieved for concentrations as high as 1000 mg l⁻¹ at 48 hrs agitation time. Microbial growth was highly dependent on initial cyanide concentration. Cyanide degradation rate decreased with increase in initial cyanide concentration in the medium which might be due to toxicity of cyanide compounds to *Bacillus cereus* at higher cyanide concentration. Cyanide inhibits not only cytochrome oxidases but also an extensive range of other enzymes at high concentrations (Dixon and Webb 1964). Cyanide degradation ability of *Bacillus species* has described in literature but there are no reports on cyanide biodegradation by *Bacillus cereus*. The biodegradation efficiency was found to be 89.87, 85.16, 80.62, 76.51, 73.68, 70.75, 67.23 and 62.36% at 200, 300, 400, 500, 600, 700, 800 and 900 mg l⁻¹ cyanide concentrations respectively. *Bacillus cereus* was capable of degrading cyanide compounds without adding any carbon and nitrogen sources in the biodegradation media. From the results it was evident that bacteria were capable of degrading high level of potassium cyanide. SAB process has been used effectively for degradation of several toxic metals and compounds. Fig. 5 represents maximum cyanide removal efficiency of SAB process for initial cyanide

concentrations of 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000mg CN l⁻¹ was 99.63, 94.76, 91.40, 88.55, 85.44, 81.48, 76.53, 72.02, 68.51 and 62.29%. The % removal efficiency was found better in SAB process as compared to adsorption and biodegradation alone. Therefore, the above results suggested that high concentration of cyanide in water and wastewater can be successfully removed by SAB process. As a result, simultaneous adsorption and biodegradation is a more competent and protracted process for removal of cyanide as compared to adsorption and biodegradation alone.

All experiments were conducted at optimum pH 7, adsorbent dose 20g l⁻¹ and temperature 35 °C. *Bacillus cereus* was found to be capable of degrading cyanide compound (KCN) without adding any carbon and nitrogen sources in biodegradation media. Present study has proved that combined process (SAB) was more effective than single process and gave better removal efficiency of cyanide. Therefore, it can be used fruitfully for cyanide removal and various contaminants from industrial waste waters.

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