Studies on Biodegradation Kinetics of Cyanide by using an isolated microorganism from steel plant wastewater

Rahul Singh, Anuj Kumar, Dalia Dasgupta, Tamal Mandal
National Institute of Technology, Durgapur, West Bengal, India
rahulsingh12988@gmail.com

Siddartha Datta
Jadavpur University, Kolkata 32, India

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ABSTRACT:
Large amounts of cyanides are released into the aquatic system due to effluents of numerous industrial activities such as mineral processing, gold and silver extraction (from their ores), steel plating, coal coking, chemical fertilizer, automobile industry and mining. Cyanides are highly toxic in nature due to its metabolic inhibition potential particularly in terminal cytochromes of electron transport chain. Due to their toxic effects flora and fauna are greatly affected and our food chain getting contaminated, therefore cyanide containing wastewater cannot be discharged without detoxification to the environment. Therefore it is necessary to develop an effective, eco-friendly and economic treatment process. Microbial treatment (biodegradation and bioremediation) can effectively degrade cyanide as carbon and/or nitrogen source for their growth. Experimental investigations were conducted in the batch mode for studying the biodegradation of cyanide using isolated monoculture bacteria CB-1(name given in laboratory) from cyanide contaminated coke-oven wastewater isolated from cyanide contaminated coke-oven wastewater. This paper presents the study on biodegradation process using isolated bacteria CB-1 from steel plant wastewater of IISCO, Burnpur (SAIL) for the treatment of simulated cyanide containing wastewater. Bacterial strain CB-1 was isolated from cyanide contaminated coke-oven wastewater by serial dilution method and used for biodegradation of cyanide. Bioremediation of cyanide by monoculture of bacteria CB-1 in basal salt medium (BSM) was observed in shake-flask experiments at 35 ˚C and pH 7. Synthetic wastewater containing cyanide solution of $[K_2Zn(CN)_4]$ was used for all studies with initial cyanide concentrations of 30,50,100,150,200,250,300 and 350 mg/L , and initial pH of 7 at different hours. From our results it seems that CB-1 used potassium tetracyanozincate as a source of nitrogen and carbon. During metabolism process this microbe use cyanide as a nitrogen and carbon source converting it to ammonia and carbonate. A maximum growth rate was observed at 200 mg/L, cyanide has been shown to have a significant inhibitory effect on growth of microorganism at higher concentration (>200 mg/L). No significant growth was observed for initial cyanide concentration of 350 mg/L and beyond. The kinetics of bacterial growth was studied with varying concentrations of cyanide between 30 to 350 mg/L,above concentration of 200 mg/L cyanide was found to exercise inhibition effect. Kinetic parameters were determined using both Monod's and Haldane's model.

1. Introduction

Cyanide is a carbon/nitrogen compound that is highly toxic and poses serious threat to the environment as well as water bodies in the receiving waters (Barclay et al., 1998; Adjei and Ohta, 2000; Raybuck, S. A., 1992; Dursan et al., 1999). Due to their toxic effects, cyanide containing effluents cannot be discharged without detoxification to the environment. To protect the environment and water bodies, wastewater containing cyanide must be treated before discharging into the environment (Sax and Lewis, 1989; USEPA, 1979). Cyanide, in some forms, is a very powerful toxin. When combined with metals and organic compounds it forms simple and complex salts and compounds, the most commonly used forms being hydrogen cyanide, sodium cyanide, and potassium cyanide. At present time industries such as photo-processing, electroplating, gold mining, steel plants and chemical- fertilizer generate large amount of cyanide and cyanate containing waste water (Wilson, D.C., 1981; White et al., 2000; White and Schnabel, 1998; Shivaraman et al., 1985; Goncalves et al., 1998). Cyanide is a serious pollutant for environment due to its high potential of genotoxic effect. Cyanides are considered as serious hazardous substances due to their strong affects on both the environment and human. Cyanide is highly toxic to living organisms, particularly in inactivating the respiratory system by tightly binding to terminal oxidase (Porter et al., 1983). Hence many countries and environmental protection agencies have imposed limiting standards for discharge of cyanide bearing wastewater to rivers/sea. The US Environmental Protection Agency (USEPA) has proposed a

limit for drinking and aquatic-biota waters regarding total cyanide are 200 and 50 ppb, respectively, where total cyanide refers to free and metal-complexed cyanides (Dash et al., 2008; USEPA, 1985). The German and Swiss regulations have set limit of 0.01mg/L for cyanide for surface water and 0.5 mg/L (Parga et al., 2003) for sewers. In Mexico, disposal limit for cyanide has been set as 0.2 mg/L (Parga et al., 2003). In India Central Pollution Control Board (CPCB) has set a minimal national standard (MINAS) limit for cyanide in effluent as 0.2 mg/L (Dash et al., 2008). In view of these considerations cyanide recovery and/or removal is necessary for lowering the concentrations of cyanides to below regulatory limits. Various physical, chemical, catalytic, photo catalytic, electrolytic, ultrasonic and biological methods have been reported for the removal of cyanide (Raybuck, S.A., 1992; Finnegan et al., 1991; Baxter and Cummings, 2006). The chemical treatment processes, especially, chemical oxidation and coagulation are common use and suitable to treat above wastewater due to high concentration of cyanide whereas the chemical methods, such as alkaline chlorination using NaOCl, HOCl, or Cl_2 are the most widely used methods, in which there is oxidation of CN to CO_2 and N_2. However, these methods are expensive and hazardous chemicals are used as the reagents like chlorine and sodium hypochlorite [Kim et al., 1998]. Moreover, these techniques cannot completely degrade all cyanide complexes in many cases and there is also complexicity of secondary by product. On the other side, biodegradation that exploits the ability of microorganism to convert pollutant to carbon dioxide, water and biomass under aerobic or anaerobic condition (Eddy Metcalf, 1991; Stephen, E., 2004; Pereira et al., 1999; Keller et al., 1997; Kao et al., 2003). Biological methods are economical than physicochemical and environmentally acceptable for cyanide removal. This paper presents the study on biodegradation process using isolated bacteria CB-1 from steel plant wastewater of IISCO, Burnpur (SAIL) for the treatment of simulated cyanide containing wastewater.

2. MATERIALS AND METHODS

2.1. Microorganism

Bacterial strain CB-1 was used in this study. This strain was isolated by serial dilution method from IISCO steel plant, Burnpur, West Bengal and the strain was subcultured regularly in nutrient agar and liquid medium for reuse.

2.2. Biochemical test of CB-1: IMVIC Test

Each IMVIC test kit (KB001HIMViC^{TM}) is a standardized colorimetric identification system utilizing four conventional biochemical tests and eight carbohydrate utilization tests. The tests are based on the principle of pH change and substrate utilization. On incubation organisms undergo metabolic changes which are indicated as a color change in the media that can be either interpreted visually or after addition of the reagent.

2.3. Chemicals and Culture Medium

All the chemical reagents were of reagent grade and no further purification was done. Cyanide standard solution (100 mg/L) was procured from Merck (Germany). Sodium hydroxide pellets and HCl used during analysis were procured from Merck. The culture medium used is inorganic medium which contained 0.43 g/L dipotassium hydrogen phosphate (K_2HPO_4), 0.43 g/L potassium dihydrogen phosphate (KH_2PO_4), 0.42 g/L ferrous sulfate ($FeSO_4.7H_2O$), 0.10 g/L calcium chloride ($CaCl_2.2H_2O$), 0.10 g/L magnesium chloride ($MgCl_2$), 0.5 g/L NaCl, 0.01 g/L MnSO4, 0.01 g/L Na_2MoO_4. The medium was autoclaved at 121ºC for 15 min before use. The growth media were prepared by adding phenol as carbon source of required concentration (500 mg/L) and cyanide (35-300 mg/L) as nitrogen source to the inorganic medium. Initially for isolation the pH of the medium was set at 7 and the working volume was kept 100 ml in all experiments.

2.4. Analysis of Cell Concentration

Cell concentrations in the samples were analyzed by measuring the optical density (OD) at 600nm using spectrophotometer. A measurement of cyanide was done by UV–vis spectrophotometer UV. For measuring biomass, the samples were centrifuged at approximately 8000 rpm for 10 min. The biomasses attached to the walls of tubes were resuspended in distilled water and optical density of

this suspension was measured against distilled water as reference at 600 nm using UV–vis double beam spectrophotometer.

2.5. Experimental design to study the biodegradation of cyanide

Experimental studies were carried out with shake flasks as batch reactors. A sample volume of 100 ml was taken in each 250 ml conical flask. Each sample contained the minimal medium with cyanide of varying concentrations as the carbon and nitrogen source. The flasks were maintained at 37 °C and the shaker speed was maintained at 150 rpm.

2.6. Analytics

Measurement of Cyanide Concentration
Cyanide electrode (Orion 94-06) was used for detection of cyanide concentration in wastewater samples. The cyanide electrode was calibrated by their standard solutions. Afterwards, cyanide electrode was used for direct cyanide detection.

3. RESULTS AND DISCUSSIONS

3.1. IMVIC test:

Biochemical identification of the bacterial isolate was carried out following tests: Indole test, methyl red test, voges-poskauer test, citrate utilization test and carbohydrate utilization test (table 1).

Table 1. Results of IMVIC test of CBREL1

S.N.	Test	Reagents to be added after incubation	Principle	Results
1.	Indole	1-2 drops of kovacs's reagent	Deamination of tryptophan	Negative
2.	Methyl red	1-2 drops of methyl red reagent	Detects acid production	Negative
3.	voges-prokauer's	1-2 drops of Baritt reagent A and 1-2 drops of Baritt reagent B	Detects acetoin production	Negative
4.	Citrate utilization		Detects capability of organism to utilize citrate as a sole carbon source	negative
5.	glucose		Glucose utilization	positive
6.	Adonitol		Adonitol utilization	Negative
7.	Arabinose		Arabinose utilization	Negative
8.	lactose		Lactose utilization	Negative
9.	sorbitol		Sorbitol utilization	Negative
10.	Mannitol		Mannitol utilization	Negative
11.	Ramnose		Ramnose utilization	Negative
12.	Sucrose		Melbiose utilization	Negative

3.2. Effect of initial concentrations

Investigations were carried out to examine action of this bacteria on cyanide at different initial cyanide concentrations from 35 mg/L to 300 mg/L at 37^0C and at a pH of 7.0. The initial concentration of cyanide was varied from 35 to 300 mg/L (Fig. 1). The bacteria utilizes cyanide as carbon and nitrogen source upto 200 mg/L and after this level the inhibitory effect of cyanide comes into play, thereby reducing its degradation potential (Fig. 2). At lower concentration this substrate removal rate was also found to get reduced towards the end of substrate consumption curve. This might be due to availability of less substrate source for the bacteria. But at higher concentration the same substrate acts as an

inhibitory compound. For the bacteria, 200 mg/L has been observed to be optimum concentration of substrate. Different cyanide removal percentage has been observed from 35-100 mg/L in 96 hrs (Fig. 3). Cyanide degradation data at initial concentrations of 60 mg/L is given in Fig. 4. As is evident from Fig. 4, bacterial biomass increases as cyanide degrades.

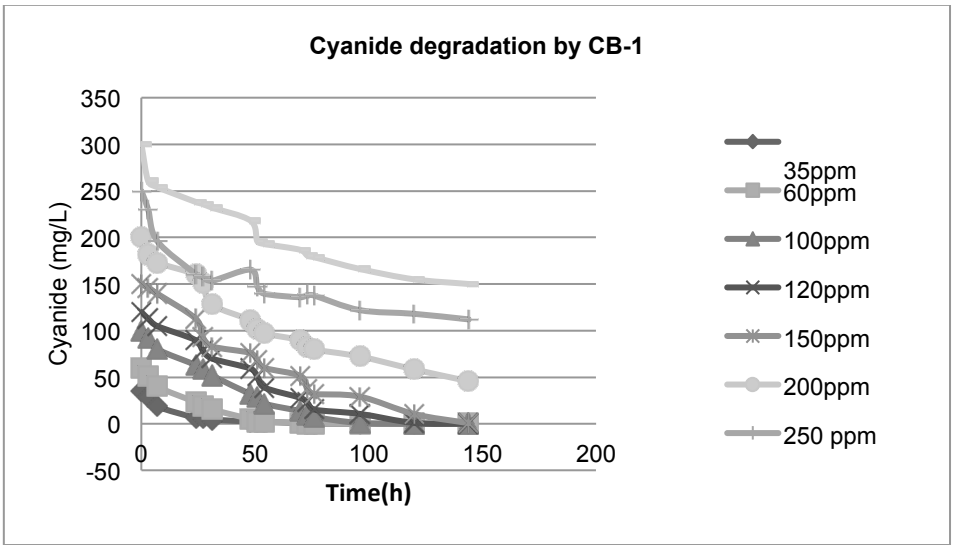

Fig.1. Cyanide degradation by CB-1 in batch reactor

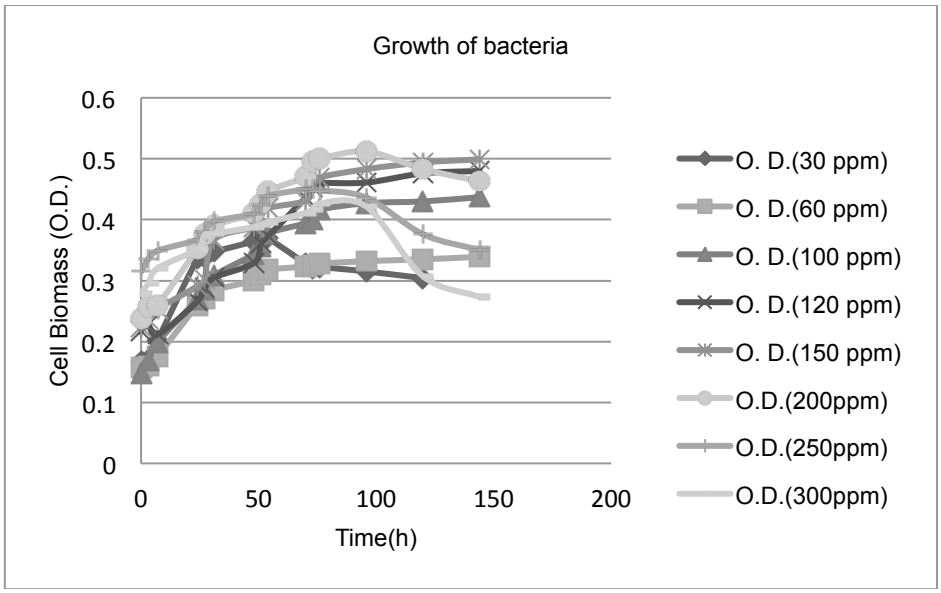

Fig. 2. Growth of CB-1 in Cyanide

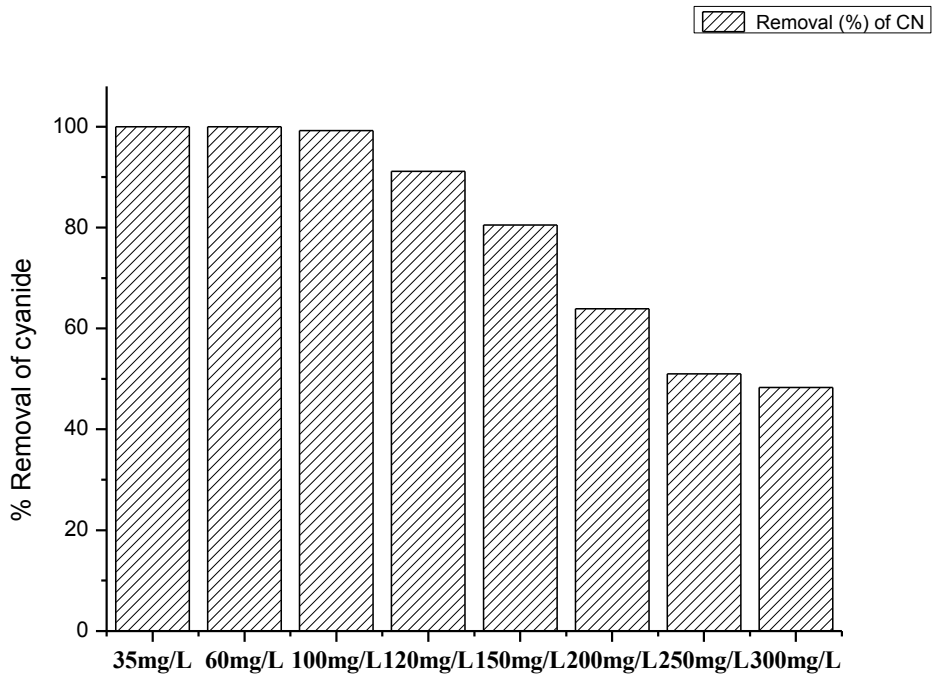

Fig.3. % Removal of Cyanide

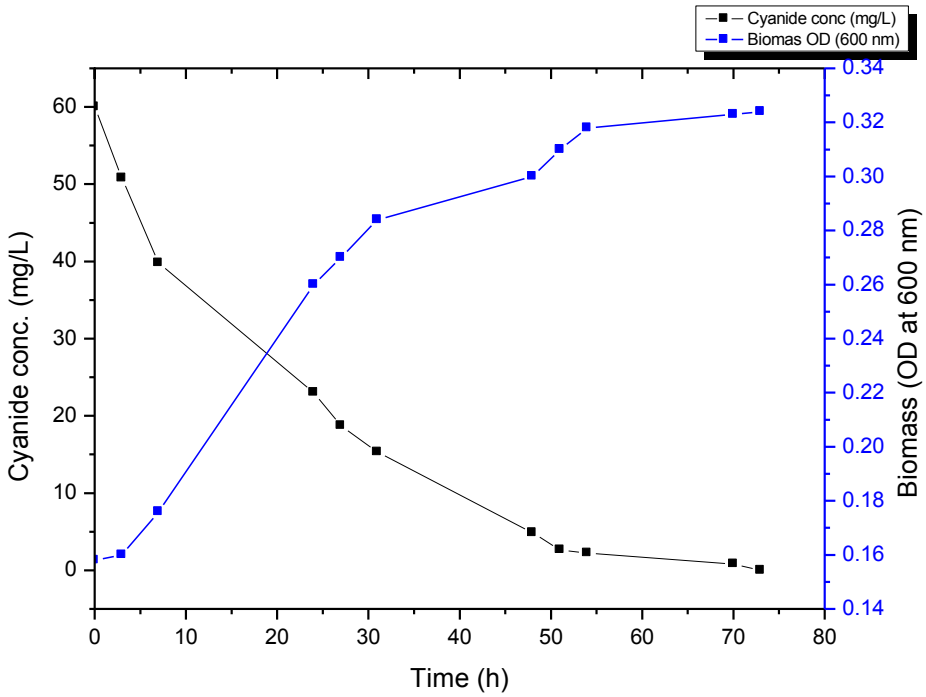

Fig.4. Cyanide degradation by CB-1 in batch reactor [initial conc. = 60 mg/L, temperature = 35.0±2 ºC, pH = 7.0].

3.3 Growth kinetic parameters

The kinetic parameters μ_{max}, Monods coefficient (k_S) and inhibition constant (k_I) were determined. We obtained the values of μ_{max} and k_s as are 0 .015(hr^{-1}) and 21.47 mg/L for Monod's model and μ_{max} and

K_I of 0.1344(hr^{-1}) and 1.66 mg/L for Haldane's model(Table2). The coefficient of correlation R^2 was found to be 0.986 in Monod's model and 0.614 in Haldane's model. The growth of these bacteria thus reasonably is in conformity with Monod's model.

Table 2. Growth kinetics parameter values of Monod and liberalized Haldane's model for biodegradation of cyanide using CB-1

Compound	Monod's Model		Linearized Haldane model	
	μ_{max} (h^{-1})	K_s (mg/l)	μ_{max} (h^{-1})	K_I (mg/l)
cyanide	0.015	21.47	0.1344/hr	1.66 mg/L

4. Conclusions

The isolated strain CB-1 degraded cyanide up to 200 mg/L quite comfortably. The degradation rate thereafter was found declining. The results of shake-flask experiments have shown that the duration taken up by the bacteria to degrade cyanide of concentration 200 mg/L was about 144 hrs (Fig1). The degradation potential can be increased by acclimitization process. The organism shows a short lag phase at high substrate concentration, whereas in the low concentrations the lag phase was absent. Cyanide exhibited inhibitory behaviour and their growth kinetics could be correlated well by the simple Haldane's inhibitory growth kinetics model. It is our view that above information would be useful in modeling and designing of units treating wastewater.

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