Jr. of Industrial Pollution Control 21 (2)(2005) pp 247- 254

BIODEGRADATION OF CYANIDE USING *BACILLUS MEGATERIUM*

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Key words : Electroplating, Cyanide, Isolation, Biodegradation, Bacillus megaterium

ABSTRACT

The cyanide-utilizing bacteria from municipal sewage, bearing gold plating industrial wastewater was isolated and identified as *Bacillus megaterium*. The batch reaction process has been tried for various concentrations of cyanide with varying pH values, additional co-substrate, biomass concentration and also with inhibitory compound. From the experimental studies, it was found that cyanide concentration of about 150 ppm at pH 6.5 with biomass 4 % (v/v) was completely degraded using the isolated bacterial organism at a residence time of 5 days. It was also found that the same organism degrades cyanide completely in the presence of phenol, as inhibitory substance. There was no influence of co-substrate in the degradation process.

INTRODUCTION

Large amounts of cyanide are used in industries involved in the metal-plating, pharmaceuticals, synthetic fibers, plastics, coal gasification, coal coking, ore leaching, gold mining, and electroplating (Knowles and Bunch, 1986; White *et al*., 1988). Cyanide is highly toxic to living organisms (Chena and Liu, 1999), particularly in inactivating the respiration system by tightly binding to terminal oxidase (Porter *et al*., 1983). To protect the environment and the receiving water bodies, wastewater-containing cyanide must be treated before discharging into the environment. Currently, wastewater contained cyanide is treated by chemical oxidation methods (alkaline chlorination, ozonization,

wet-air oxidation) (Palmer *et al.,* 1988; Watanabe *et al.,* 1998). However, these methods are expensive and hazardous chemicals are used as the reagents (chlorine and sodium hypochlorite) (Watanabe *et al*., 1998). Moreover, these techniques cannot completely degrade all cyanide complexes in many cases (Figueira *et al*., 1996). Based on the above discussions, biological treatment would be a cost-effective and environmentally acceptable method for cyanide removal compared with the other techniques currently in use (Raybuck, 1992; Dubey and Holmes, 1995). To develop the biological technology for the treatment of cyanide-containing wastewater, screening of the Cyanide-degrading microorganisms have been carried out.

Most reports described that metabolism of cyanides by strains of *Pseudomonas, Acinetobacter, Bacillus,* and *Alcaligenes* (Harris and Knowles, 1983; Finnegan *et al*., 1991; Ingvorsen *et al*., 1991; Meyers *et al*., 1991). *Pseudomonas fluorescens* NCIMB 11764 is able to convert cyanide to ammonia and carbon dioxide via the action of an oxygenase enzyme which may also proceed by means of NAD (P)-dependent oxygenase and cyanase (Dorr and Knowles, 1989). *Pseudomonas putida* can use cyanide as the nitrogen source and metabolize it to ammonia (Babu *et al.,* 1996). Kunz *et al.* (1994) found that *P. fluorescens* strain NCIMB degraded cyanide via the activities of several cyanide-degrading enzymes such as oxygenase, cyanide nitrilase, and cyanide hydratase. *Pseudomonas* species degraded cyanide through a pathway where cyanide is converted to ammonium and formate under both aerobic and anaerobic conditions (Watanabe *et al*., 1998). Like the cyanide degrading pathway of *Pseudomonas, Alcaligenes xylosoxidans subsp*. denitrificans DF3 (Ingvorsen *et al*., 1991) and *Bacillus pumilus* C1 (Meyers *et al*., 1991) converted cyanide to ammonium and formate via the activity of cyanidase. In addition, some fungi such as *Gloeocerocospora sorghi* (Fry and Munch, 1975; Wang *et al.,* 1992), *Fusarium lateritum* (Cluness *et al*., 1993), and *Stemphylium loti* (Fry and Millar, 1972) have also been reported to be able to degrade cyanide.

The present study was aimed to determine the feasibility of *Bacillus megaterium,* isolated from Chidambaram municipal sewage bearing gold plating industrial wastewater for cyanide biodegradation under various experimental conditions.

MATERIAL AND METHOD

The sewage wastewater samples were collected from the gold plating indutrial zones from under-ground drainage in Chidambaram muncipal area *viz.,* Chinna chetty street (sampling point I), Gold plating industrial area (sampling point II).The wastewater samples were analysed for various cyanide concentration as perAPHA (1992) .

Isolation of bacteria

The cyanide contaminated soil and wastewater samples were collected. One milli litre of the watewater (soil suspension) was inoculated into sterile test

tubes containing 9 ml of the minimal medium and supplemented with different concentrations of potassium cyanide (5 to 40 mg/L). The minimal media used for the isolation of cyanide utilising bacteria contained the following , KH_2PO_4 - 3.3 g/L,K_2HPO_4 – 4.3 g/L,$MgCL_2$. H_2O - 0.3 g/L The medium was amended with 0.5 ml of the trace elements solution containing the following (in mg/L) MnCl2 - 1.0; Fe SO_4. 7 H_2O - 0.8; $CaCl_2$ H_2O - 2.4 and Na_2 MnO_4 . 2H2O - 10.0. The pH was adjusted to 7.0 and the medium was autoclaved. The minimal medium plates were prepared by adding 15gm of agar to one litre of the medium.The contents of the tubes were incubated at 25°C. After 6 days of incubation the inoculum was streaked out on plates containing cyanide as a sole source. Colonies that grew on the plates were selected for identificaiton . The morphological and physiological test results show that the organism isolated from the gold plating industrial wastewater (sediments of Chidambaram town) is gram positive, homogenous, rod shaped *Bacillus megaterium.*

Acclimatized inoculum preparation

About 90 ml of Nitrogen free Glucose (NFG) medium ($Na_2HPO_4.2H_2O$ [50mM]7.098g/L,KH_2PO_4 [100 mM]-13.609 g/L, $MgSO_4.7H_2O$[1 mM]-0.246 g/L, $CaCl_2$[0.1 mM]-0.11 g/L, Dextose[0.8%]-8 g/L) with pH adjusted to required level was taken and sterilized at 1.5 kg/cm^2 gauge pressure for 30 minutes and cooled to room temperature. To this, 1 ml of 1000 ppm cyanide was added and final volume was adjusted to 100ml in order to get an initial cyanide substrate concentration 100 ppm. A loop full of the test organism from the freshly subculture slant was inoculated into the above medium. The culture was incubated in a shaker at 100 rpm and room temperature for three days. They formed the Primary culture. The secondary acclimated inoculum was prepared in the same way wherein 1 ml of primary culture was used instead of the subculture from slant to inoculate the medium. The total volume of NFG medium, stock phenol solution and primary culture was adjusted to set the initial substrate concentration of 100 mg/L .Tertiary culture was used for batch studies.

Screening of microorganisms

23 flasks of organism, which consists of the original and the duplicate set were prepared. In each flask about 100 mL of the original and the duplicate were prepared. In each flask about 100 ml of NFG whose initial pH was adjusted to 6.5 was taken. All the contents in the flasks were sterilized at 1.5 kg/cm^2 gauge pressure for 30 minutes. After cooling to room temperature, 4 %(v/v) of inoculum and 1 mL of 1000 mg/L cyanide stack were added to get a final concentration 100 mg/L (except 1-6 flask).In the medium while keeping the total volume of medium, inoculum and cyanide was 100 mL. Experimental flask and sterile control were placed on a shaker running at 100 rpm. The flasks were removed at predetermined time intervals 0 to 12 hours from starting and the samples were analysed for residual cyanide.

Effect of Initial substrate concentration

To determine the effect of substrate concentration, 1 to 6 flasks with different

initial substrate concentration were taken while all other parameters given previously were kept the same. As before, each run of 6 flasks along with their duplicate are drawn at predetermined timing. 100 mL medium was added to each of the flask in the first set and kept for sterilization after adjusting pH 6.5. To each sterilized and cooled flask 4%(v/v) of inoculum was added. The initial cyanide concentration was maintained by adding 1 mL (1mg) from the stock solution whose concentration was 1000 mg/L. Similarly, the other flasks were prepared to a final volume of each 100mL with initial cyanide concentration of 0, 50,100,150,200 and 250 ppm in the NFG medium along with 4 %(v/v) of Inoculum.

Effect of pH

pH ranges from 4.5 to 8.5 were maintained to determine the effect of pH on degradation of cyanide. 100 ml of NFG medium were prepared at a cyanide concentration of 150 ppm in different flasks(7-9) with pH ranging 4.5, 5.5, 6.5, 7.5 and 8.5 and all the flasks were subjected to sterilization before adding the 4%(v/v) of inoculums.

Effect of co-substrate

To determine the effect of co-substrate in the degradation process of cyanide, another 5 nos. of flasks (10-14) were prepared with different 0.4%(v/w), 0.6%(v/w), 0.8%(v/w), 1.0%(v/w) and 1.2%(v/w), of Dextrose as co-substrate. The same NFG medium with pH 6.5 and 4%(v/v)of inoculum was used for the experiment setup maintaining the initial cyanide concentration of 150 ppm.

Effect of inoculum size

To study the effect of inoculum variation in degrading the cyanide, another set of flasks (15-18) were prepared with different inoculum loadings (2 %(v/v), 3 %(v/v), 4 %(v/v), 5 %(v/v), and 6%(v/v)) of the same species adjusted to a final volume of 100ml of the same medium. The pH of the solution was maintained at 6.5 and the initial concentration of cyanide as 150 ppm.

Effect of Interference of Phenol

The NFG medium with pH 6.5 taken in each of 5 flasks(19-23). After sterilization and cooling, the contents were inoculated with 4 %(v/v) of culture and added with 50, 100, 150, 200 & 250 mg/L of phenol concentration. Maintaining cyanide concentration as 150 mg/L, experiments were carried out.

RESULT AND DISCUSSION

The samples collected from the two sampling points, were analyzed for cyanide concentration and the concentrations at sampling point I and II were 10.5 mg/L and 6350 mg/L respectively.

Degradation of cyanide by *Bacillus megaterium* with different substrate concentration (50 to 250 mg/L) was investigated in order to find out whether

the substrate inhibition is exhibited. The results of the experiments were given Fig 1.

It was found that for a cyanide concentration of 50 mg/L, the maximum degradation was achieved (100%) at a residence time of 3 days. When the concentration was increased further the degradation process takes more time. This could be seen from the figure 1 and up to a concentration of 200 mg/L, 100 % degradation was achieved and at concentration of 250 mg/L, the maximum degradation was 97.72 % at a residence time of 6 days. This may be due to cell lysis, because of the concentration of cyanide can be beyond the permissible limit at which the organisms cannot survive. A further increase in concentration would cause a reduction in % degradation. These concentrations can only the maximum concentration up to which organism can work.

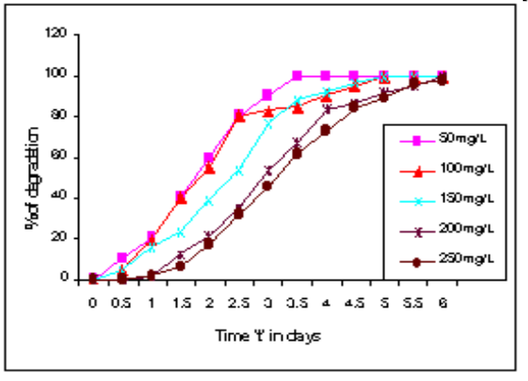

Fig 1 .Effect of initial substrate concentration

The pH concentration also plays a major role in the biological activity. With an increase in pH there was an increase of degradation of cyanide. At pH of 4.5 the degradation was 92.59 % and for pH 6.5 it was 100 %. As per the observations made in the batch reaction process it was found that the maximum of 100% degradation was achieved at pH 7.5 and 8.5. From this, it can be concluded that the organism works well in both the neutral, slightly acidic and alkaline condition. The results were presented in graphically represented in Fig 2.

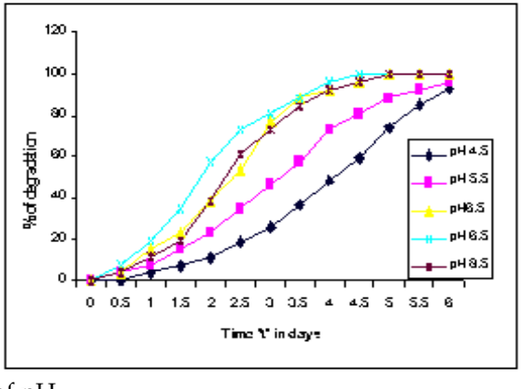

Fig 2. Effect of pH

The co-substrate variation was done by varying the dextrose concentration from 0.4 % (v/w) to1.2 %(v/w). The degradation was attained to a maximum 100 % for 0.4 %(v/w) dextrose concentration at a residence time of 5 days. When the co-substrate concentration was increased the degradation of 100 % was achieved with increased residence time. Also, at the co-substrate concentration 1.2 %(v/w) the degradation was only 96 %. From this, it can be concluded that increased co-substrate will inhibit the degradation process. The details were also presented in **Fig.3**

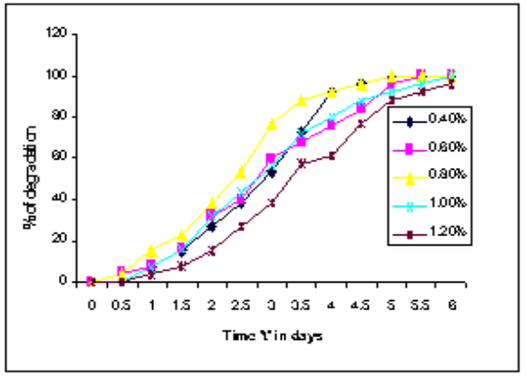

Fig 3. Effect of co substrate

It was found that the degradation percentage increased when the biomass concentration was increased. Except for the concentration of 2 %(v/v) all other concentrations have shown a complete degradation (100%). With an increase in biomass concentration the residence time have also been reduced considerably. At a concentration of 6 ml, the residence time was 4.5 days. The details of the results were also presented graphically in **Fig 4.**

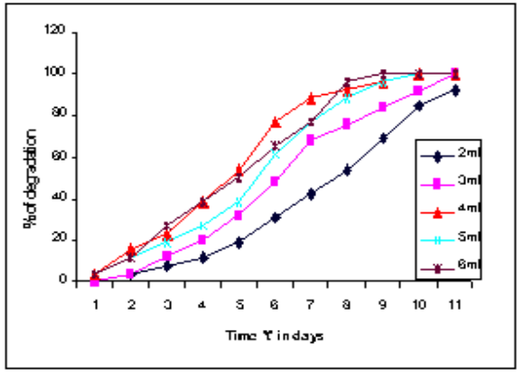

Fig 4.Effect of inoculums size

It was found that the with increase of phenol concentration in the cyanide wastewater the degradation of cyanide reduces due to inhibition of phenol and the residence time increases with interference of phenol (Fig 5). As per the observation made during the experiment, it was found that the organism was more active in degrading the phenol rather degrading the cyanide.

CONCLUSION

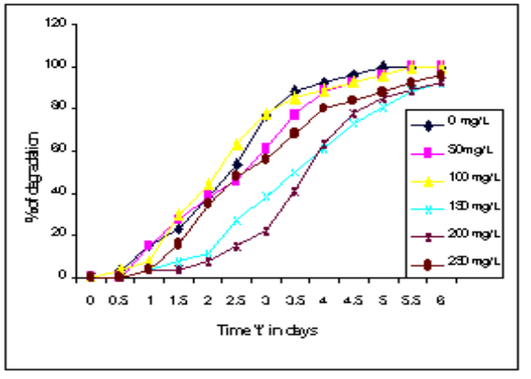

Fig 5. Effect of interferences phenol

The isolated and identified species *Bacillus megaterium* was found to be very active in degrading the wastewater bearing cyanide. The organism metabolizes the waste both in acidic and alkaline range, which can be utilized for such type of wastes. The present study shows that the co-substrate will not have any significant part in degradation. So in the real plants the cost of co-substrate can be avoided. It is also observed that there is an increase in the degradation of cyanide with increase in quantity of inoculum and there is no substantial effect of inoculum size on the degradation at the completion of degradation s o that minimum MLSS can be maintained in the bioreactor.

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