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Conversion of sodium cyanide to carbon dioxide and ammonia by immobilized cells of *Pseudomonas putida*

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Key words: Biodegradation; *Pseudomonas putida*; Immobilization; Sodium cyanide

SUMMARY

Pseudomonas putida, isolated from contaminated industrial wastewaters and soil sites, was found to utilize sodium cyanide (NaCN) as a sole source of carbon and nitrogen. Cells, immobilized in calcium alginate beads (1–2 mm diameter) were aerated in air-uplift-type fluidized batch bioreactor containing 100–400 ppm of NaCN. Degradation of NaCN was monitored for 168 h by analyzing gaseous and dissolved ammonia (NH_3), CO_2, pH and optical density. The results indicated that the alginate-immobilized cells of *P. putida* were able to degrade NaCN into NH_3 and CO_2 in a time-dependent manner.

INTRODUCTION

It is well known that bacteria can degrade and thereby detoxify a wide variety of inorganic and organic cyanides [10,16]. The recent methods to detoxify these toxic chemicals include application of immobilized cell technology [16]. The cells which are in an immobilized state have advantages over either free cells or immobilized enzymes because it prevents the cell washing [6]. Also the cell immobilization process allows a high cell density to be maintained in a bioreactor at any flow rate. Since the immobilized cells also have higher catalytic stability, they can tolerate higher concentrations of toxic compounds than in the free cell state [1,3].

Cyanide is a highly toxic substance and a metabolic inhibitor. Considerable amounts of cyanide are found in the wastewaters of industries involved in metal plating, pharmaceuticals, synthetic fibers, plastics, coal gasification, coal coking and ore leaching [7,17]. Since high concentrations of cyanide are too toxic for biodegradation, the cyanides generally are removed by expensive chemical or physical means prior to biological treatment [2,8]. However, the application of biological treatments to degrade cyanides has been reported earlier [11,12,14]. *Pseudomonas* sp. has been shown to grow utilizing cyanide as a source of carbon and nitrogen [4,17]. The present study deals with the ability of the calcium alginate-immobilized *P. putida* cells to degrade various concentrations of NaCN in an air-uplift-type fluidized bed batch bioreactor.

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MATERIALS AND METHODS

Chemicals. Sodium cyanide was purchased from EM Science, Division of EM Industries, Inc., Gibbstown, NJ. All other chemicals were of ACS reagent grade or approximately 98–99% purity and were purchased from Sigma Chemical Company, St. Louis, MO.

Isolation of cyanide-degrading microorganisms. The method for the preparation of sterile minimal medium and isolation of bacteria from the contaminated soil and water samples collected from industrial sites was described earlier [9]. In the present study the samples were supplemented with NaCN (50–400 ppm) and the colonies were isolated after 7 days of incubation at 25 °C.

Identification of organism and determination of optimal growth conditions. The isolates were characterized by use of rapid identification kits (Flow Laboratory kits for Gram-negative microorganisms, Flow Laboratories Inc., McLean, VA and the API 20E test kit, Analytab Products, Plainview, NY). The results were also confirmed by a computer survey available from these two suppliers. Subsequently, the specific substrate utilization tests were performed as described in the Bergey's Manual of Systematic Bacteriology [13] and as described by Smibert and Krieg [15].

Optimal pH and temperature for the growth of the microorganisms were determined by measuring the growth in the minimal medium containing 100 ppm NaCN at 5–55 °C, with increments of 10 °C, and pH 3.0 to 9.0 with increments of 1.0 after 72 h of incubation.

Immobilization of P. putida in alginate. 3–4 g (wet weight) of cells were mixed (1 : 1 (v/v)) with sterile 2% sodium alginate (Type VII: Sigma) and adjusted to a final volume of 200 ml with cold 4% sodium alginate. The alginate–cell mixture was added dropwise to cold 0.2 M $CaCl_2$ solution and each drop hardened into a bead containing entrapped *P. putida* cells. The beads (1–2 mm in diameter) were allowed to harden further in $CaCl_2$ solution for 24 h in a refrigerator.

Batch bioreactor experiment. Batch bioreactor experiment was performed using an air-uplift-type reactor (800 ml) (Fig. 1). The reactor was filled with 750 ml of normal saline (0.85%) containing beads (50% bead volume) and NaCN (100–400 ppm). For the circulation of beads, the bioreactor was aerated with CO_2 and NH_3-free air (200 ml/min) at 25 °C. Batch bioreactor experiments were repeated three times with duplicates and the mean values are reported.

Analytical methods. Growth in liquid culture was estimated from the A_{546} using 10 mm cuvettes (Gilford Spectrophotometer, Ciba-Corning, U.S.A.). The Beer-Lambert law was followed for an absorbance of 1.0 contained (653 mg dry wt. ml^{-1}) [4]. Samples (5 ml) were taken from effluent port and bacteria were removed by centrifuging at 15000 × *g* for 10 min at 5 °C (Beckman Instruments, U.S.A.). Dissolved NH_3 was determined colorimetrically by the Berthelot's procedure as described by Kaplan [5]. The gaseous NH_3 was determined by back titration of boric acid (known concentration) with 0.5 M KOH. The gaseous CO_2 was first dissolved in known volume of 0.5 M KOH and back titrated for free KOH using 0.5 M HCl.

RESULTS AND DISCUSSION

The bacterial species were isolated from contaminated soil and water samples supplemented with 100 ppm of NaCN. The colonies obtained were small (1–3 mm in diameter), circular, convex with entire margin, beige and creamy in color and fluorescent. Each isolated colony was able to grow aerobically in minimal medium containing cyanide as a sole source of carbon and nitrogen. The cellular morphology of the bacteria was Gram-negative, small rod-shaped, motile, non-spore forming and non-capsulated. Oxidase, catalase and arginine dihydrolase reactions were positive. Growth was observed on sodium benzoate, MacConkey, and glucose plates but not on xylose and maltose. The isolate failed to hydrolyze gelatin. On the basis of these characteristics the organism was identified and confirmed as *P. putida*.

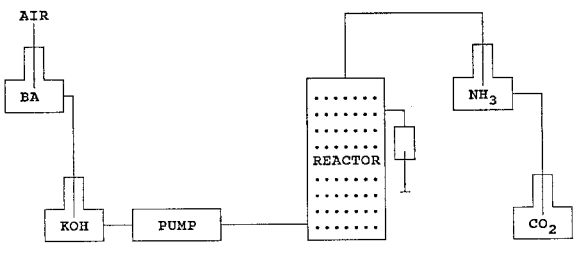

Fig. 1. Schematic diagram of the batch bioreactor.

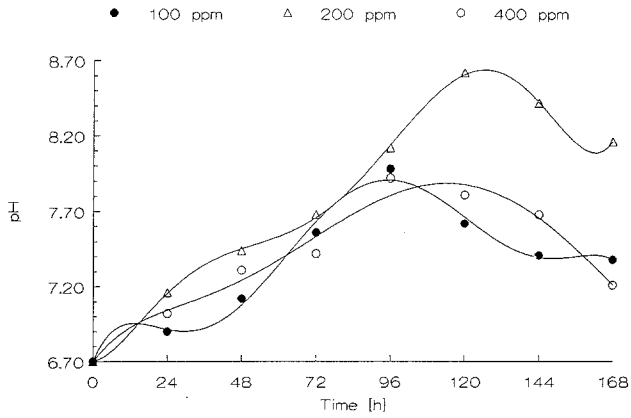

Fig. 2. Changes in the pH of the medium containing NaCN (100–400 ppm) as a function of incubation-time.

Optimal conditions for the growth of *P. putida* were pH 6.7 and 25 °C. No growth was observed below 5.0 or above 8.5 pH, and at 10 °C or 55 °C.

In the presence of NaCN, the pH of the growth medium was increased in a time-dependent manner up to 96–120 h (Fig. 2). Maximum pH of 7.98, 8.62, and 7.92

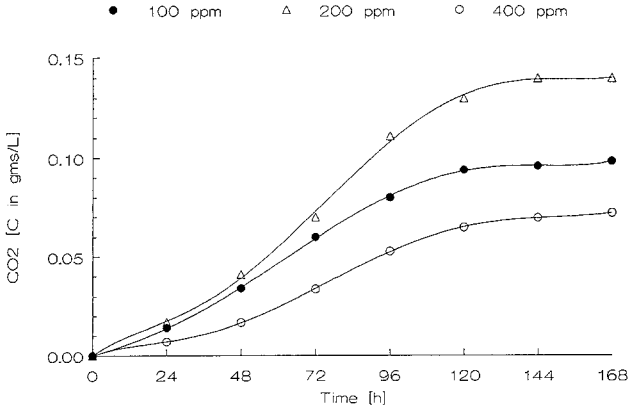

Fig. 3. Formation of CO_2 by *P. putida* in presence of NaCN as a function of incubation-time.

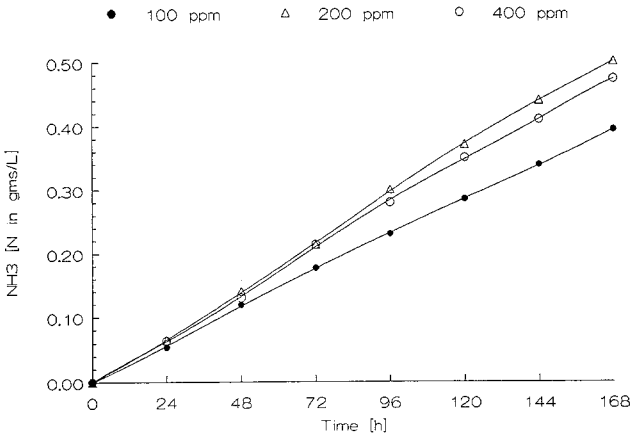

Fig. 4. Formation of gaseous NH_3 by *P. putida* in presence of NaCN as a function of incubation-time.

was observed at 100, 200 and 400 ppm of NaCN, respectively. The data in Fig. 3 indicate that CO_2 production in the presence of NaCN was increased in a concentration-dependent manner with increase in incubation time. The levels of gaseous (Fig. 4) and dissolved NH_3 (Fig. 5) were also increased with increase in incubation time. The NH_3 levels in general were higher at 200 ppm as compared to 100 or 400 ppm of NaCN.

Concentration of cyanide in excess of about 1 mM has been shown to be inhibitory and led to a decline in the rate of respiration [4]. The present data indicate that immobilized cells of *P. putida* cells were able to degrade higher concentration of NaCN into CO_2 and NH_3. Similar degradation of HCN by *Pseudomonas* sp. has been reported earlier [7]. The time-dependent increase in CO_2 and NH_3 formation indicates utilization of NaCN as carbon and nitrogen source by *P. putida*. An increase in pH of the medium could be attributed to NH_3 accumulation as a result of degradation of NaCN. The decline in pH after 96–120 h of incubation may be due to assimilation of NH_3 by bacteria. Evidence of this has been reported [4] where *Pseudomonas* sp. utilized NH_3 once a low medium cyanide concentration was attained. The levels of CO_2, gaseous and dissolved NH_3 were elevated with increase in concentration of NaCN from 100 to 200 ppm. The reduction in the formation of these products at 400 ppm indicates decreased degradation of NaCN by *P. putida*. This suggests that at higher concentrations, cyanide is toxic and may be inhibiting the enzymes responsible for its degradation into CO_2 and NH_3. The analysis of data on nitrogen and carbon content indicated that the total nitrogen content of both gaseous and dissolved NH_3 was found to be equivalent to nitrogen content of NaCN added to the medium; whereas the total carbon content in CO_2 represents only partial carbon content in NaCN added to the medium, suggesting incomplete oxidation of formate into CO_2. This confirms further that *P. putida* utilizes cyanide as a source of carbon and nitrogen. However, further studies on the enzymes involved in the conversion of NaCN to CO_2 and NH_3 may be needed to reconfirm the present data.

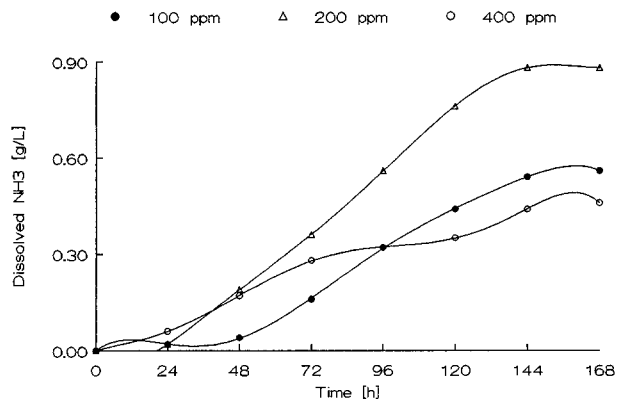

Fig. 5. Formation of dissolved NH_3 by *P. putida* in presence of NaCN as a function of incubation-time.

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