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Screening of encapsulated microbial cells for the degradation of inorganic cyanides

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SUMMARY

Different encapsulation matrices were screened to encapsulate cells of *Pseudomonas putida* for degradation of inorganic cyanides. Degradation of NaCN by free cells and cells immobilized in agar, alginate or carrageenan matrices was studied. The rate of NaCN degradation was monitored for 120 h by measuring pH, bacterial growth, dissolved and gaseous NH_3 and gaseous CO_2. Alginate-immobilized cells degraded NaCN more efficiently than free cells or agar- or carrageenan-immobilized cells.

INTRODUCTION

Cyanide is a highly toxic metabolic inhibitor [20]. Considerable amounts of cyanides are found in the environment due to natural synthesis and industrial production [14,20]. Since higher concentrations of cyanides are too toxic for biodegradation, they are generally removed by expensive chemical means prior to biological treatment [5,12]. Microorganisms possess enzymes capable of converting cyanides into non-toxic compounds which may serve as carbon and nitrogen substrates [7,13,17]. Earlier studies have reported the use of acclimated microbial systems for the treatment of wastewaters contaminated with cyanide [5,11,12,14,22]. However, the application of immobilized cells in the treatment of wastewaters offers the possibility of degrading higher concentrations of toxic pollutants than can be achieved with free cells [3,10,21]. Under many conditions, immobilized cells have advantages over free cells or immobilized enzymes [1,2,4,6,16]. Immobilization allows a high cell density to be maintained in a bioreactor at any flow rate [9]. Hence, we evaluated the degradation of NaCN by free cells, and cells immobilized in agar, alginate and carrageenan matrices.

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MATERIALS AND METHODS

Chemicals

All chemicals (99% purity) including sodium alginate (Type VII), agar and carrageenan were purchased from Sigma Chemical Co., St. Louis, MO.

Isolation of the bacterium

The bacterial strain selected was isolated from soil samples collected from industrial waste sites. The soil sample was diluted (1:10) with sterile minimal medium and the suspension was incubated at room temperature for 1 h. One-milliliter portions of the suspension were inoculated into 9-ml portions of medium supplemented with different (50–400 ppm) concentrations of NaCN. The samples were incubated at 25 °C for 7 days and the tubes were then examined for turbidity. After five to seven serial transfers, turbid samples were streaked onto plates containing NaCN as the sole source of C and N. Successive streaking of the colonies onto NaCN agar allowed isolation of a pure culture that could utilize NaCN as its sole source of C and N. The bacterium is rod-shaped, motile, gram negative, fluorescent, catalase positive and oxidase positive. It has been identified and confirmed as a *Pseudomonas putida* based on the classification scheme described previously [15]. Tests were performed as mentioned in the manual [15] and as described by Smibert and Krieg [19].

Cell growth

P. putida was grown in a minimal medium (pH 7.0) containing the following (in g/l): K_2HPO_4, 4.3; KH_2PO_4, 3.4; $MgCl_2 \cdot H_2O$, 0.3. The medium was amended with 0.5 ml of trace element solution containing (in mg/l): $MnCl_2 \cdot 4H_2O$, 1.0; $FeSO_4 \cdot 7H_2O$, 0.6; $CaCl_2 \cdot H_2O$, 2.6; $NaMoO_4 \cdot 2H_2O$, 6.0. NaCN (4 mM/l) was added to the medium as sole source of C and N. Cell growth was determined by monitoring turbidity at 546 nm. When OD reached 1.0, the cells were centrifuged at $10000 \times g$ for 10 min at 5 °C and the cell paste (300–400 mg wet weight) was collected.

Immobilization in alginate

The cell paste was suspended in 100 ml of 0.85% sterile saline and mixed with 100 ml of 4% sterile sodium alginate. The alginate-cell mixture was extruded dropwise through a 25-gauge needle from a height of about 20 cm into cold 0.2 M $CaCl_2$. Each drop was hardened into a bead containing entrapped cells. These biobeads were further hardened by stirring them in $CaCl_2$ for 30 min, and they were then stored at 5 °C for 24 h.

Immobilization in agar or carrageenan

Equal volumes of cell slurry and 4% (w/v) agar or carrageenan were mixed and the mixture was added dropwise to cold 0.2 M KCl. Each drop with entrapped *P. putida* cells was hardened into a bead. The biobeads were further hardened by stirring in 0.2 M KCl for 30 min and then storing at 5 °C for 24 h.

The typical yield following immobilization of cells in alginate, agar or carrageenan was about 0.6 g of beads per ml of cell-alginate, agar or carrageenan suspension. Each biobead was 1–2 mm in diameter with wet and dry weights of 12–13 and 0.6–0.7 mg, respectively. At the time of immobilization, each biobead contained $1–2 \times 10^8$ viable cells as determined by pour plate technique.

Batch bioreactor experiment

The batch bioreactor experiment was performed in 250-ml Environmental Protection Agency (EPA) soil biometer flasks containing 1:1 biobead volume/0.85% NaCl solution supplemented with 4 mM NaCN. In the case of free cells, the minimal medium supplemented with 4 mM NaCN was used. The samples were incubated at 25 °C in a shaking water bath for 120 h. The rate of NaCN degradation by free cells and cells in beads was monitored by determining pH, bacterial growth (in the case of free cells), and dissolved and gaseous NH_3. Gaseous CO_2 was determined after 120 h of incubation. Gaseous NH_3 and CO_2 were trapped in 0.5 M boric acid and 0.5 M KOH, respectively. The samples (5 ml) were collected at regular intervals for analysis.

TABLE 1

Changes in the levels of OD, pH, CO_2 and NH_3 during degradation of NaCN (4 mM) by *Pseudomonas putida*

Time (h)	OD	pH	Gaseous NH_3 (g)	Dissolved NH_3 (g)	CO_2 (g)
Free cells					
0	0.100	6.7	–	–	–
24	0.121	7.2	0.013	0.013	–
48	0.138	7.5	0.028	0.026	–
72	0.169	7.8	0.042	0.044	–
96	0.272	7.9	0.057	0.058	–
120	0.446	7.9	0.073	0.064	0.190
Agar beads					
0	–	6.7	–	–	–
24	–	6.9	0.013	0.016	–
48	–	7.2	0.030	0.034	–
72	–	7.4	0.045	0.055	–
96	–	7.6	0.061	0.074	–
120	–	7.6	0.078	0.079	0.183
Alginate beads					
0	–	6.7	–	–	–
24	–	7.0	0.014	0.023	–
48	–	7.3	0.048	0.037	–
72	–	7.6	0.063	0.044	–
96	–	7.8	0.078	0.079	–
120	–	7.8	0.094	0.087	0.202
Carrageenan beads					
0	–	6.7	–	–	–
24	–	6.8	0.014	0.012	–
48	–	7.0	0.030	0.019	–
72	–	7.2	0.045	0.025	–
96	–	7.3	0.060	0.041	–
120	–	7.3	0.070	0.068	0.179

TABLE 2

Changes in carbon and nitrogen during degradation of NaCN (total NaCN at 0 h: 0.294 g; C: 0.072 g and N: 0.084 g) by free and immobilized cells fo *P. putida* after 120 h

Samples	Carbon (g)	% change over carbon at 0 h	Nitrogen (g)	% change over nitrogen at 0 h
Alginate	0.055	76.39	0.081	96.43
Agar	0.050	69.44	0.073	86.90
Carrageenan	0.049	68.06	0.063	75.00
Free cells	0.052	72.22	0.060	71.43

Carbon as total CO_2; nitrogen as total NH_3.

Analytical methods

Growth in liquid culture was determined by measuring the optical density at 546 nm. Dissolved NH_3 was determined colorimetrically by the procedure of Berthelot as described by Kaplan [8]. CO_2 and NH_3 were determined by back titration. Gaseous NH_3 in boric acid was determined by adding an equal volume of 0.5 M KOH and back titrated for free KOH using 0.5 M HCl. Gaseous CO_2 in 0.5 M KOH was assayed by titrating it against 0.5 M HCl.

The experiment was repeated thrice and the averages of the values obtained are presented.

RESULTS

Data on the degradation of NaCN by free cells and cells immobilized in agar, alginate or carrageenan matrices are presented in Tables 1 and 2. The initial pH of the growth medium increased from 6.7 to 7.9 during the 120-h incubation period (Table 1). The pH also increased in biobead preparations. Maximum increase in pH (7.9) was observed in the medium containing free cells followed by alginate (pH 7.8), agar (7.6) and carrageenan (7.3) biobeads. Dissolved and gaseous NH_3 also increased (Table 1). Bacterial growth occurred in samples containing free cells. The changes in the levels of NH_3 and CO_2 in general were more pronounced in the samples containing alginate-immobilized cells compared to other biobeads and free cells (Table 1).

The data in Table 2 indicate that the total carbon and nitrogen obtained from CO_2 and NH_3 during NaCN degradation by alginate was greater compared to other biobeads and free cells (Table 2). When the per cent change of C and N obtained from total CO_2 and NH_3 over C and N contents of NaCN added to the medium at 0 h was calculated, the following trends were observed: (i) carbon/alginate > free cells > agar > carrageenan and (ii) nitrogen/alginate > agar > carrageenan > free cells.

DISCUSSION

Although the microbial metabolism of NaCN has been well documented [13,14,18,22], the enzymatic pathways involved in the production of ammonia from cyanide are not clear [7,13]. The present study demonstrates that free cells and cells immobilized in alginate, agar or carrageenan can degrade NaCN into CO_2 and NH_3. Formation of NH_3 and CO_2 as a result of NaCN degradation indicates the utilization of NaCN as a C and N source by the encapsulated microbial cells. This suggests that the mechanism involved in the conversion of NaCN to NH_3 and CO_2 is identical in free and immobilized cells.

The increased formation of NH_3 and CO_2 from NaCN by alginate biobeads as compared to other biobeads may be due to the rapid exchange of substrates and products through the alginate matrix. It can also be inferred that alginate-immobilized cells have higher catalytic stability as demonstrated by the increased production of CO_2 and NH_3.

The data on mass balance indicate that complete remediation of NaCN can be achieved with cells immobilized in the alginate matrix. The N and C obtained in the samples containing alginate-immobilized cells indicate that the total C and N present in NaCN is nearly equal to that of C and N obtained in the form of CO_2 and NH_3. This confirms that immobilized cells of *P. putida* have utilized NaCN as a source of C and N, and suggests that immobilized cell technology can be employed in the environment for the remediation of inorganic cyanides.

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