Short Communication

Biodegradation of quinoline by freely suspended and immobilized cells of *Comamonas* sp. strain Q10

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Quinoline and its derivatives have received attention because of their presence in the environment and potential as a health threat. Quinoline is often found at locations associated with oil shale, coal processing and at wood treatment sites (Fetzner, 1998). Quinoline is thought to be potentially carcinogenic and quite mobile due to its high solubility in water (Thomsen et al., 2000). Thus, quinoline resulting from industrial activities should be eliminated before it enters the environment.

Several reports are available on the degradation of quinoline by various microorganisms (Fetzner, 1998; Kaiser et al., 1996). Most quinoline-degrading microorganisms have been identified as *Pseudomonas* species (Brockman et al., 1989; Kilbane et al., 2000; Schwartz et al., 1989; Shukla, 1986). Quinoline-degrading strains of *Moraxella* (Grant and Al-Najjar, 1976), *Comamonas* (Miethling et al., 1993), *Rhodococcus* (O'Loughlin et al., 1996), *Nocardia* (Shukla, 1987), *Burkholderia pickettii* (Han et al., 2000) and *Desulfobacterium indolicum* (Johanson et al., 1997) have also been reported. An attractive method for

eliminating quinoline is biodegradation to convert it to essentially harmless compounds such as CO₂, H₂O and NH₄⁺.

When immobilized cells are used, the efficacy of biodegradation is often improved. Under many conditions, immobilized cells have the following advantages over freely suspended cells (O'Reilly and Crawford, 1989). First, a high cell concentration can be achieved. Second, increased flow rate, which is independent of the growth rate of the cells employed, is possible with minimal washout of immobilized cells. Third, catalytic stability as well as tolerance against toxic compounds can be improved. Common methods of whole cell immobilization include adsorption and entrapment. Entrapment of microbial cells in alginate has been well studied since it is easy to perform, inexpensive, and nontoxic for organisms. Degradation of xenobiotic compounds like phenols (Bettman and Rhem, 1984), pentachlorophenols (Lin and Wang, 1991), 3-chloroanilins (Ferschl et al., 1991), 2-chlorophenol (Lu et al., 1996), PCB (Mukerjee-Dhar et al., 1998), naphthalene (Manohar and Karegoudar, 1998), pyridine (Lee et al., 1994) and a mixture of monochlorobenzoates and 2,4-dichlorophenoxyacetic acid (Sahasrabudhe et al., 1991) by alginate-entrapped microbial cells have been reported.

Quinoline degradation by PVA-H₃BO₃-gauze immo-

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bilized cells has been attempted by Han et al. (2000); however, their study on the quinoline-degrading ability was conducted at a lower concentration (<3.88 mM) and the longevity of quinoline-degrading activity of immobilized cells was not investigated. In the present study, biodegradation of quinoline using immobilized pure strain Q10 in calcium alginate beads was investigated, and immobilized cells were compared with freely suspended cells with regard to quinoline-degrading capacity at various initial quinoline concentrations, and the longevity of quinoline-degrading activity of immobilized cells was also examined.

Strain Q10 was isolated from activated sludge by repeated transfers on a minimal salt medium with quinoline as carbon source and NH_4^+ as nitrogen source. The strain was characterized as a Gram-negative rod. The partial 16S rRNA sequences were determined for the strain. Genomic DNA of strain Q10 was purified by the method described by Jizhong et al. (1996). The DNA was amplified by the polymerase chain reaction, using specific primers corresponding to bases F27 (5'-AGAGTTTGATCCTGGCTCAG-3') and R1522 (5'-AAGGAGGTGATCCAGCCGCA-3') (Edwards et al., 1989). The sequence of the amplified 16S ribosomal DNA were determined by the direct sequencing method by TaKaRa Biotechnology (Dalian) Co., Ltd. and compared with other available 16S rRNA sequences by using the BLAST search option of the GenBank database to determine the closest phylogenetic neighbors. The 16S rRNA sequence of strain Q10 has been deposited in the GenBank nucleotide sequence database under accession number AF519533. Sequencing of the 16S rRNA gene and comparison with previously published 16S rRNA gene sequences resulted in classification of strain Q10 as a member of the genus *Comamonas* sharing 99% 16S rRNA sequence homology with *Comamonas testosteroni* strain JCM 10170. This culture will be maintained at Guangdong Key Laboratory of Microbial Culture Collection and Breeding, Guangdong Institute of Microbiology, China.

Three different media were used in the present study. Medium I for inoculum preparation, used for both freely suspended and immobilized cells culture, contained (L^{-1}) KH_2PO_4 0.5 g, K_2HPO_4 1.5 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.2 g and trace element solution 10 ml. Glucose (5.0 mM) and quinoline (1.55 mM) were supplied as simultaneous carbon sources. Medium II for freely suspended cell culture contained (L^{-1}) KH_2PO_4

0.5 g, K_2HPO_4 1.5 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.2 g and trace element solution 10 ml. Quinoline at various concentrations (1.55–5.81 mM) was supplied as the carbon source and nitrogen source. To improve the strength of the calcium alginate beads, the salt composition and concentration in medium II were modified for immobilized cell culture. Medium III for immobilized cell culture contained (L^{-1}) KH_2PO_4 0.1 g, K_2HPO_4 0.1 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.2 g, CaCl_2 0.5 g and trace element solution 10 ml. Quinoline at various concentrations (1.55–5.81 mM) was supplied as the carbon source and nitrogen source. The trace element solution contained (L^{-1}) $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$ 12 g, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 2.0 g, CaCl_2 1.0 g, Na_2SO_4 10 g, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 0.4 g, MnSO_4 0.4 g, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 0.1 g, $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ 0.1 g, H_2SO_4 (98%) 5 ml. The pH of all media was adjusted to 7.0.

Cells were immobilized in gel beads of calcium alginate as follows. (1) Exponentially growing cells in an aerated fermentor containing 2 L of medium I were harvested into a paste by centrifugation at 10,000 rpm for 10 min. After discarding the supernatant, the cell paste was then resuspended in 1.5% sodium alginate. (2) A mixture of cells and sodium alginate was extruded dropwise from a needle (0.8 mm diameter) into a gently stirred solution of cold 1 M CaCl_2 . After immobilization, the calcium alginate beads were resuspended with gentle agitation in a fresh CaCl_2 solution for a hardening time for 2 h. Finally, these beads were washed with 0.85% NaCl solution and stored at 4°C for quinoline-degradation experimentation. The average diameter of the beads was about 2 mm.

For freely suspended cell culture, exponentially growing cells were inoculated to 250 ml conical flasks containing 100 ml medium II adjusted to different initial cell concentrations (1.1×10^6 cfu·ml $^{-1}$ and 5.5×10^6 cfu·ml $^{-1}$) and varying concentrations of quinoline (1.55–5.81 mM). The cultures were incubated in a shaker at 100 rpm and 30°C. For immobilized cells, 10 g wet weight of calcium alginate beads was inoculated into 250 ml conical flasks containing 100 ml medium III with various concentrations of quinoline (1.55–5.81 mM). The initial cell concentrations were 1.1×10^7 cfu·g $^{-1}$ beads and 5.5×10^7 cfu·g $^{-1}$ beads. Like the freely suspended cell culture, the immobilized cell culture was incubated in a shaker at 100 rpm and 30°C. The control experiments were carried out in sterile medium at the above-indicated quinoline loadings. For establishing the long-term stability of quino-

line degradation by immobilized cells, repeated batch fermentations were carried out. After every incubation period, the used medium was decanted and beads were washed with water and transferred into a fresh mineral salts medium containing quinoline. The fermentation process was carried out under identical fermentation conditions.

The culture broth samples were filtered through a 0.22 μm filter and stored at 4°C. Quinoline was quantified using high-performance liquid chromatography (HP1100, Agilent Co., Ltd., Waldbronn, Germany). A reversed-phase column was used: Diamonsil™ C18, 5 μm , 150×4.6 mm. The mobile phase was composed of methanol and water (60:40) and the flow rate set at 1.0 ml·min⁻¹. The compounds were detected at 275 nm. Total organic carbon (TOC) was determined by UV-persulfate TOC analyzer phoenix 8000 (Dohrmann, Cincinnati, OH, USA). Ammonia was analyzed by standard methods (APHA, 1980). The content of the cells entrapped in the alginate beads was measured by dissolving the gel beads in 10 ml sodium pyrophosphate (1% w/v) followed by serial dilution and plating on nutrient agar plates using a bacteriological colony counter (Manohar and Karegoudar, 1998).

The results for the degradation of 1.55 mM of quinoline by freely suspended cells and immobilized cells are given in Fig. 1. It was shown that quinoline was completely degraded within 12 h with freely suspended cells concentration of $1.1 \times 10^6 \text{ cfu} \cdot \text{ml}^{-1}$. The degradation of quinoline was concurrent with the disappearance of TOC. After 18-h incubation time, approximately 27% of substrate nitrogen was fixed in ammonia, and 56% of TOC disappeared. The degradation of quinoline with immobilized cells was slightly lower than that with freely suspended cells; only about 89%

Fig. 1. Degradation of 1.55 mM of quinoline by freely suspended cells (■ quinoline, × TOC, ● ammonia) and immobilized cells (◆ quinoline, △ TOC, * ammonia).

quinoline was degraded within 12 h.

Figure 2, a, b and c show the quinoline-degrading profile at concentrations of 1.94 mM, 3.88 mM and 5.81 mM, respectively. It is evident that the 1.94 mM quinoline was completely degraded within 14 h of incubation with a freely suspended cell concentration of $1.1 \times 10^6 \text{ cfu} \cdot \text{ml}^{-1}$, whereas the same concentration of quinoline was completely degraded after only 10 h of incubation when the initial cell concentration was increased to $5.5 \times 10^6 \text{ cfu} \cdot \text{ml}^{-1}$. No significant difference in the degradation of 1.94 mM quinoline by immobilized

Fig. 2. Quinoline-degrading profile at concentrations of 1.94 mM (a), 3.88 mM (b), and 5.81 mM (c) by freely suspended cells (◆ $1.1 \times 10^6 \text{ cfu} \cdot \text{ml}^{-1}$, ■ $5.5 \times 10^6 \text{ cfu} \cdot \text{ml}^{-1}$) and immobilized cells (△ $1.1 \times 10^7 \text{ cfu} \cdot \text{g}^{-1}$ beads, × $5.5 \times 10^7 \text{ cfu} \cdot \text{g}^{-1}$ beads), * sterile control.

cells and freely suspended cells was observed. At 3.88 mM quinoline concentration, about 19% quinoline was degraded after 64-h incubation time at an initial freely suspended cell concentration of 1.1×10^6 cfu·ml⁻¹, whereas quinoline could be completely degraded within 56 h with equivalent immobilized cells. When the initial freely suspended cell concentration increased to 5.5×10^6 cfu·ml⁻¹, 3.88 mM quinoline was completely degraded within 40 h, and there is almost the same degradation rate of quinoline with equivalent immobilized cells. When the concentration of quinoline increased to 5.81 mM, only 8% quinoline was degraded after 64-h incubation time at an initial freely suspended cell concentration of 1.1×10^6 cfu·ml⁻¹, whereas nearly 33% quinoline was degraded after 64-h incubation with equivalent immobilized cells. Furthermore, approximately 46% quinoline was degraded within 64 h with the freely suspended cell concentration increased to 5.5×10^6 cfu·ml⁻¹, whereas quinoline could be completely degraded by equivalent immobilized cells. No significant disappearance of quinoline was observed in sterile controls from Fig. 2, a, b and c.

Figure 3 shows quinoline-degradation profiles using immobilized cells (cell concentration used was 5.5×10^7 cfu·g⁻¹ beads) in repeated batch fermentations. When the quinoline concentration was 1.94 mM, the immobilized cells could be reused 20 times without losing their quinoline-degrading activity during the culture period tested. When the quinoline concentration increased to 3.88 mM, the immobilized cells could be reused 10 times without losing their quinoline-degrading activity. The content of the viable cells in the calcium alginate beads during repeated batch fermentation experiment was also measured (Table 1). It was shown that the content of the viable cells in the calcium alginate beads increased at first, and then remained constant on the whole.

Quinoline is highly water-soluble. With an N-atom incorporated in the cyclic π -system of a benzene ring, the water solubility increases 200 times compared to that of the analogue PAH compound naphthalene (Thomsen et al., 2000). A higher concentration of quinoline is often toxic to freely suspended cells and abolishes growth (Shukla, 1986). An improvement in degradation by immobilized cells compared to freely suspended cells was observed at 3.88 mM and 5.81 mM of quinoline. At 5.81 mM quinoline concentration, in particular, freely suspended cells were significantly inhibited, whereas immobilized cells can also

Fig. 3. Degradation of quinoline by repeated batch culture of immobilized cells.

◆ quinoline concentration used, 1.94 mM, ■ quinoline concentration used, 3.88 mM.

Table 1. Change of the viable cell content in calcium alginate beads during repeated batch degradation experiment.

Concentration of quinoline	Viable cell content in calcium alginate beads (cfu·g ⁻¹ beads)			
	Initial	1 day	5 days	10 days
1.94 mM	5.5×10^7	1.4×10^8	5.0×10^8	5.0×10^8
3.88 mM	5.5×10^7	2.5×10^8	5.4×10^8	6.0×10^8

completely degrade quinoline. This was a result of the protection against substrate inhibition afforded by immobilization. However, the fact that immobilized cells had lower rates than freely suspended cells at 1.55 mM of quinoline may be due to the slow diffusion of the compound into gel beads. Pore size of gels used for entrapment of the biomass can affect the diffusion of substrate and products and limit the reaction of the entrapped cells (Aksu and Bülbül, 2000). These results were similar to those of Dwyer et al. (1986), who found that the degradation of phenol by immobilized cells was higher than that of freely suspended cells at higher phenol concentrations, whereas, at lower phenol concentrations, immobilized cells had low degradation rates due to mass transport limitation of phenol. However, the degradation of quinoline by PVA-H₃BO₃-gauze immobilized cells was always lower than that by freely suspended cells at concentrations in the range of 0.388–3.88 mM (Han et al., 2000). We believe that improvement of degrading-activity of immobilized cells is dependent on matrices of immobilization.

From Figs. 1 and 2, we can see that biomass concentration in the reaction mixture strongly affected degradation of quinoline by immobilized and freely

suspended cells. Cultures with a high cell concentration degrade quinoline faster than those with a low cell concentration regardless of the concentration of quinoline used. Significant interdependence between the degradation of toxic organic compounds and the size of the inoculum used has been noted earlier (Mukerjee-Dhar et al., 1998) and it is postulated that the use of a high cell titer helps to lower the toxicity of the quinoline.

In some conditions, immobilized cells could not degrade toxic organic compounds on reuse because of the inhibition of degradation intermediates or the inactivation of one or more of the transformation enzymes (Mukerjee-Dhar et al., 1998; O'Reilly and Crawford, 1989), whereas, in our repeated batch fermentations, immobilized cells could be reused 20 and 10 times without losing their quinoline-degrading activity at a concentration of 1.94 mM and 3.88 mM. The results were similar to those of Lee et al. (1994), who found that the immobilized *Pimelobacter* sp. in calcium alginate beads can be reused in degrading pyridine. We also found that alginate beads containing living cells shrink in size, and there was some disintegration of beads upon repeated use in spite of the addition of CaCl_2 , whereas no loss of stability in control beads not containing living cells was observed. The depolymerization of the alginate molecule was probably due to enzymatic activity of the entrapped cells. These results indicated that the use of immobilized cells in alginate is quite efficient for degradation of quinoline; however it is necessary to improve the structural stability of the immobilized solid for its use in commercial reactors.

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