Journal of the Korean Chemical Society
2007, *Vol. 51, No. 2*
Printed in the Republic of Korea

고정화된 *Bacillus Brevis*에 의한 퀴놀린 분해의 증가

Balasubramaniyan. S and Swaminathan. M.*
Department of Chemistry, Annamalai University, Annamalai Nagar – 608002, India
(2006. 11. 9 접수)

Enhanced Degradation of Quinoline by Immobilized *Bacillus Brevis*

Balasubramaniyan. S and Swaminathan. M.*
Department of Chemistry, Annamalai University, Annamalai Nagar – 608002, India
(Received November 9, 2006)

요 약. 유리 및 고정화된 *Bacillus brevis*에 의한 퀴놀린의 분해를 조사하였다. 코코넛 껍질 탄소에 고정화된 *Bacillus brevis*에 의한 퀴놀린 분해 속도는 폼조각에 고정화되었거나 유리된 미생물에 의한 속도보다 빠르다. 시료에 존재하는 퀴놀린 100 ppm을 완전히 제거하기 위해서는 코코넛 껍질 탄소에 고정화된 *Bacillus brevis*로 만든 생촉매를 물속에서 20시간 유지시키면 되었다. 이 생촉매는 꽤 긴 보존기간과 적절한 재생력을 가지고 있었다.

주제어: 고정화, *Bacillus brevis*, 생분해, 퀴놀린, 코코넛 껍질 탄소

ABSTRACT. Biodegradation of Quinoline by free and immobilized *Bacillus brevis* has been investigated. The rate of quinoline degradation by immobilized *Bacillus brevis* on coconut shell carbon is faster than the rate by the microorganism immobilized on foam pieces and free cells. A complete removal of 100 ppm of Quinoline in the sample was achieved at a hydraulic retention time of 20 hours with the biocatalyst prepared by immobilizing *Bacillus brevis* onto coconut shell carbon. The biocatalyst had a reasonable shelf life and desirable recycle capacity.

Keywords: Immobilization, *Bacillus brevis*, Biodegradation, Quinoline, coconut Shell Carbon

INTRODUCTION

In recent years there has been increasing concern over the public health threat presented by the introduction of N-heterocyclic compounds into the environment. Little is known about the environmental fate of Quinoline, a heterocyclic compound found in coal tar, mineral oil and bone oil. In the Chemical industry it serves as a solvent and is the starting material for the synthesis of quinoline dyes and pharmaceuticals. Quinoline and some of its derivatives were reported to be toxic, carcinogenic and mutagenic.[1-4] The widespread use of quinoline and its derivatives entails that these compounds are distributed in the environment thus polluting soil and water.

Conventional biological processes (activated sludge tricking filters) can destroy a large fraction of biodegradable organic compounds found in wastewater and can remove additional materials by adsorption. But most of the hazardous compounds pass through conventional wastewater treatment facilities unaltered. In addition, they also have adverse impact in the composition and activities of microorganism in activated sludge flocs, thus reducing the overall performance of these facilities. The removal of these compounds is a real challenge for waste treatment engineers and scientists. There have been several reports on the bacterial degradation of quinoline.[5-9]

Investigation on the taxonomy of the quinoline degrading bacteria showed that in most cases they

were members of the genus *pseudomonas*. *Pseudomonas* are characterized by their ability to use a wide rage of organic compounds as sole sources of carbon and energy. Mostly these compounds are aromatic and heterocyclic substances.However, the ability to degrade quinoline does not appear to be confined to *pseudomonas*. A Gram-negative, aerobic *moraxella* bacterium and a *Nocardia* species were described showing these degrading properties.[5,8,10]

Immobilization of microbial cells have received increasing interest in recent years.[11,16] It offers a promising potential for the improvement of efficiency of bioprocesses. Compared with free cells, immobilized cells have several advantages. The main advantages in the use of immobilized cells are their higher operational stability, their ease of use in a continuous reactor and their ability for scale-up.

Activated carbons are a group of amorphous forms of carbon with a highly developed pore structure. Due to high adsorption capacity, activated carbon finds wider applications in many gas phase and liquid phase adsorption systems. It is used as an industrial catalyst. Activated carbon is also used as a carrier for cells or enzymes in biochemical reactions.[17]

Immobilized cells on carbon were widely used for treatment of numerous toxic compounds such as pentachlorophenol.[13,18-20] benzene derivatives and dichlorobenzoates,[21] 2, 4-dichlorphenoxy acetic acid[22] phthalic acid ester[12] and 2,4-dichlorophenol.[23,24]

In this paper we report the efficiency of free and immobilized bacterium *Bacillus brevis* on the degradation of Quinoline and the storage capacity and reusability of the immobilized biocatalyst.

EXPERIMENTAL

Microorganism

The microorganism used in this study was a pure strain, isolated from the soil suspension of carbonization plant effluent channel. One gram of the soil suspension was inoculated into sterile tubes with 9 ml of the medium containing nutrient broth and cetrimide agar. After two days of incubation, the contents were examined for turbidity and the turbid portion was streaked out on plates containing potassium thiocyanate as a sole source. Colonies that grow on the plates were selected for identification The bacterium was analysed by biochemical, morphological and physiological tests in Institute of Microbial Technology, Chandigarh. The bacterium is motile, mesophilic and salt tolerant (up to 9%). It grows up to a temperature of 37℃ and pH 9. There is a growth of the bacterium under aerobic and partial anaerobic conditions. The results of biochemical tests match with *Bacillus brevis* as per the Bergey's manual of systematic Bacteriology (Vol.1, 1989). Hence the organism was identified as *Bacillus brevis* and labeled as MTCC 3136.

Preparation of culture

The organism was grown in nutrient broth medium for 48 hrs at 35 ℃ under stationary condition. The culture was then harvested by centrifugation at 10,000 rpm for 20 minutes, washed twice with sterilized water and resuspended in sterile buffered water. The centrifuged biomass was used for the degradation.

Immobilization of organism

Foam pieces and coconut shell carbon were used as matrices for immobilization. The bacterial suspension used for immobilization contains 48 hrs-grown cells incubated at 35 ℃ in the nutrient broth. The immobilization was carried out by passing this suspension 5 times up and down through the column highly packed with either by sterilized foam pieces of uniform size (5×5 mm) or by coconut shell carbon spherical particles. The washings of the column after immobilization showed negative results in the biological analysis for the presence of cells. This reveals that cells are inside the pores of the matrices. This observation was also confirmed by the same efficiency of immobilized cells on to coconut shell carbon with the repeated use.

Batch and Column Study

In biodegradation, the experiments were performed either in batch reactor or continuous flow reactor (*Fig.* 1) using free or immobilized cells respectively. Erlenmeyer flasks plugged with foam stop-

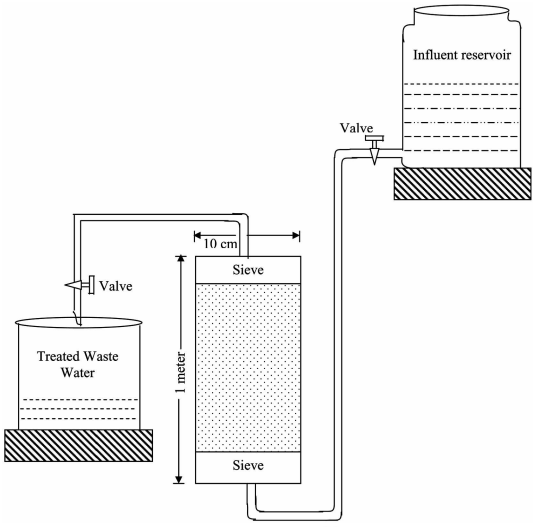

Fig. 1. Schematic flow diagram of continuous column reactor.

per were used as batch reactor. For the continuous flow reactor acrylic tube of 1m length and 10 cm diameter was used. The samples were withdrawn periodically and analysed for growth of the bacterium and quinoline degradation using Hitachi U-2001 spectrophotometer. The concentration of quinoline was determined by measuring the absorbance of the centrifugate at 313 nm. The growth of the cells in batch reactor was determined by measuring the absorbance of the sample at 540 nm.

RESULTS AND DISCUSSION

Quinoline degradation by *Bacillus brevis* free cells

The inoculums of *Bacillus brevis* was added to the solutions with solutions of different concentrations of quinoline. The samples were taken at regular intervals of time and the cell growth and quinoline concentration were determined. The growth of the bacterium at different initial concentrations of quinoline is shown in *Fig.* 2. The degradation of quinoline for various initial concentrations of quinoline is shown in *Fig.* 3. The growth of the bacterium decreases and the lag period increases with increase in the concentration of quinoline. The time required for complete degradation increases with the increase in initial concentration. The bacterium is capable of completely degrading the quinoline up to a concentration of 200 ppm in 50 hrs. A comparison of *Figs.* 2 and 3 reveals that the growth of the bacterium matches with the degradation. This indicates that the bacterium grows only by taking quinoline as carbon, nitrogen and energy sources.

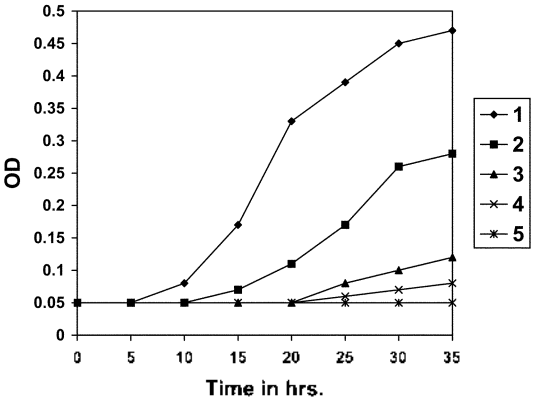

Fig. 2. Growth of *Bacillus brevis* with different concentrations of quinoline. 1. 100 ppm, 2. 200 ppm, 3. 300 ppm 4, 400 ppm, 5. 500 ppm.

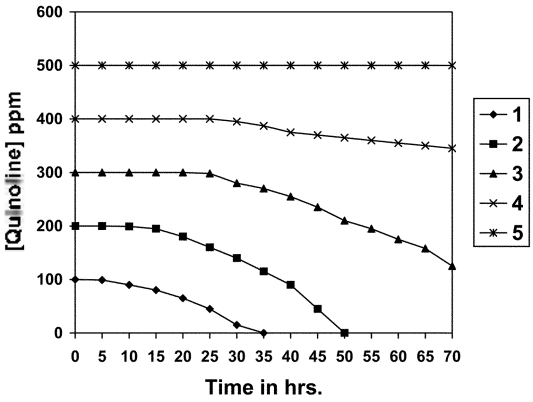

Fig. 3. Quinoline degradation by *Bacillus brevis* (free cells). 1. 100 ppm, 2. 200 ppm, 3. 300 ppm 4, 400 ppm, 5. 500 ppm.

The influence of pH on quinoline degradation had been carried out and the results are shown in *Fig.* 4. The degradation of quinoline increases with increase in pH from 6-9 and then decreases. The degradation is found to be efficient at pH 9 and it is taken as optimum pH.

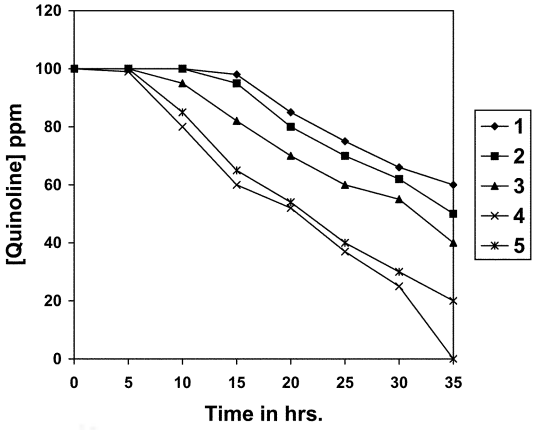

Fig. 4. Effect of pH on quinoline degradation by *Bacillus brevis* (free cells). 1. pH 6, 2. pH 7, 3. pH 8, 4. pH 9, 5. pH 10.

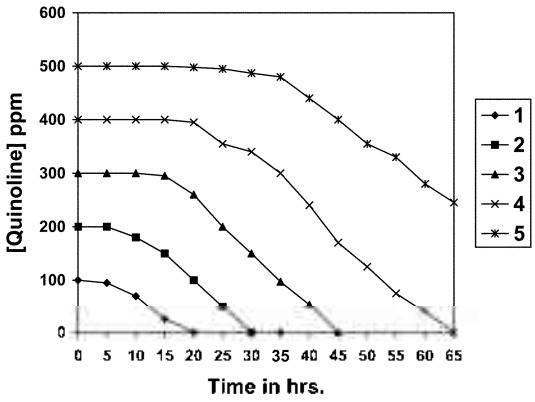

Fig. 6. Quinoline degradation by *Bacillus brevis* immobilized on coconut Shell carbon. 1. 100 ppm, 2. 200 ppm, 3. 300 ppm 4, 400 ppm, 5. 500 ppm.

Quinoline degradation by *bacillus brevis* immobilized on foam pieces and activated carbon

Quinoline degradation by *Bacillus brevis* immobilized on foam pieces and coconut shell carbon were carried out in a continuous flow reactor. *Figs.* 5 and 6 show the quinoline degradation by immobilized cells with different initial concentrations of quinoline. A complete degradation for 300 ppm in foam pieces and for 400 ppm in coconut shell carbon was observed in 65 hrs. The immobilized cells on coconut shell carbon are more efficient than those on foam pieces. *Fig.* 7 shows quinoline concentration – time – degradation diagram of *Bacillus brevis*

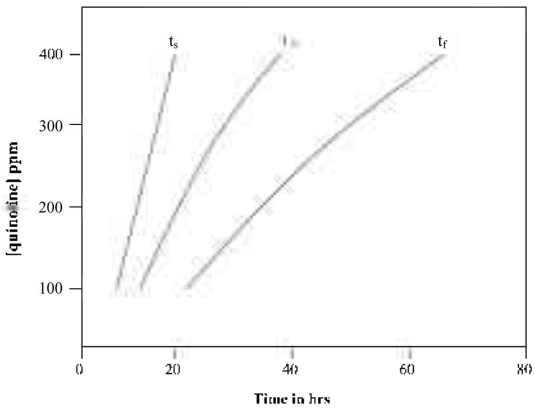

Fig. 7. Quinoline concentration – time of degradation diagram for *Bacillus brevis* immobilized on coconut shell carbon. t_s- time for the start of degradation, t_{50}-Time for 50% degradation, t_f- time for complete degradation.

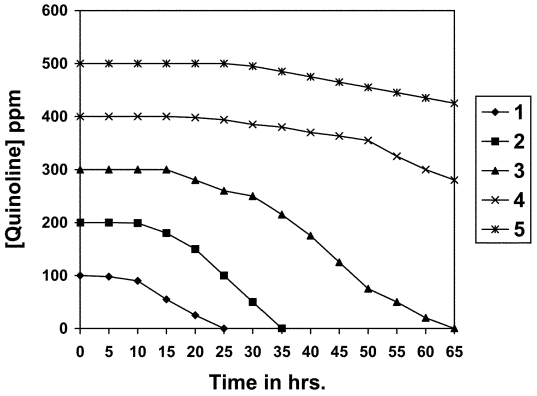

Fig. 5. Quinoline degradation by *Bacillus brevis* immobilized on foam pieces. 1. 100 ppm, 2. 200 ppm, 3. 300 ppm 4, 400 ppm. 5. 500 ppm.

immobilized on coconut shell carbon. The diagram is more advantageous as it gives the time required for the start of degradation (t_s), time required for 100% degradation (t_f) and time for 50% of quinoline degradation (t_{50}) at various quinoline concentrations. To the left of the t_s curve the quinoline degradation does not occur and to the right of the t_f curve quinoline in the medium is negligibly small. With the increase of quinoline concentration the increase in t_{50} and t_f are more when compared to the increase in t_s. This reveals that change in time for the start of degradation is not much but the time for 50% and

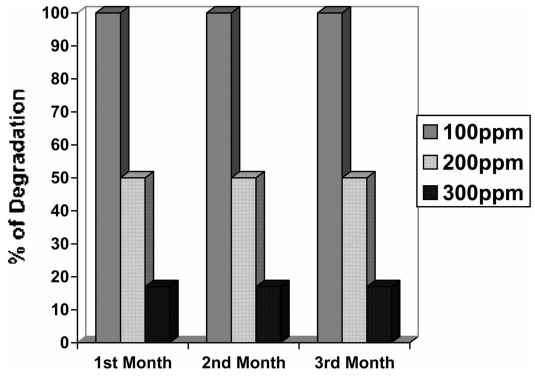

Fig. 8. Storage stability and repeated usability of the carbon immobilized cells on Quinoline degradation (20 Hours).

100% degradation increases sharply with the increase in quinoline concentration.

Storage stability and repeated usability of the cells immobilized on carbon

Since the efficiency of degradation is higher with the cells immobilized on coconut shell carbon, we had carried out an experiment to find its storage stability and repeated usability for three months. The concentrations 100,200 and 300 ppm of Quinoline were used for the degradation by cells immomobilized on coconut shell carbon. A complete degradation of 100 ppm of Quinoline is observed in 20 hours of hydraulic retention time and the percentage of removal decreases with increase in Quinoline concentration (*Fig.* 8). The biocatalyst after its use was washed with double distilled sterile water and kept in the column for a month. The experiment was repeated with this biocatalyst using the same concentrations of Quinoline two times and the results are displayed in *Fig.* 8. The figure shows that the efficiency of this biocatalyst remained same every month for three months.

The immobilized cells are advantageous in wastewater treatment due to easy transportation and storage. During storage and transportation the immobilized organism is likely to be affected due to environmental alteration and thus the efficiency deteriorates. If immobilization of cells were only on the surface of the carrier materials, the cells might be sloughed off by attrition during continuous treatment leading to a decrease in efficiency of the immobilized cells in degradation process. The experiment did show that the cells had maintained its efficiency in the removal of Quinoline. Hence the immobilized organism must be in the pores of the activated carbon. This fact is considered to be important while advocating the immobilized system for biodegradation or bioconversion of organics in wastewater.

The time required for 50% quinoline degradation by the free and immobilized cells are given in *Table* 1. The immobilized cells are found to be efficient than free cells. The cells immobilized on coconut shell carbon are more efficient than those on foam pieces.

CONCLUSIONS

The microbe *Bacillus brevis* grows by consuming quinoline as C, N and energy sources. The growth of the organism and quinoline degradation efficiencies are more at pH 9. The immobilized cells are efficient than free cells. Cells immobilized on activated coconut shell carbon are found to be more efficient than cells immobilized on foam pieces. The storage stability and repeated usability of the biocatalyst developed by immobilizing the bacterium on to coconut shell carbon will be very useful for the degradation of Quinoline in wastewater.

Table 1. Comparison of the efficiencies of free and immobilized cells

[Quinoline] ppm	Time required (in hrs) for 50% degradation		
	Free cells	Foam Immobilized cells	Carbon Immobilized cells
100	22	15	11
200	38	25	20
300	67	43	30
400	-	-	42
500	-	-	65

REFERENCES

1. Minako, N.; Takio, Y.; Yuko, S.; Takashi, S. *Mutant. Res.* **1977**, *42*, 335.
2. Sideropoulos, A. S.; Secht, S. M. *Mutant . Res.* **1996**, *11*, 59.
3. Jianlong, W.; Liping, H.; Hanchang, S.; Yi, Q. *Chemosphere.* **2001**, *44*, 1041.
4. Sugaya, K.; Nakayama, O.; Hinata, N.; Kamekura, K.; Ito, A.; Yamagiwa, K.; Ohkawa, A. *Chem.Technol and Biotechnol.* **2001**, *76*, 603.
5. Grant, D. J. W.; Al-Najjar, T. R. *Microbes.* **1976**, *15*, 177.
6. Dzumedzei, N. V.; Shevchuko, A. G.; Turovskii, A. A.; Staroviotov, I. I. *Microbiology.* **1982**, *52*, 157.
7. Bennet, J. L.; Updegraff, D. M.; Pereira, W. E.; Rostad, C. *Microbios Lett.* **1985**, *29*, 147.
8. Shukla, O. P. *Appl. Environ. Microbial.* **1986**, *51*, 1332.
9. Jianlong, W.; Xiangchun, Q.; Liping, H.; Yi, Q.; Hegemann, W. *Chemosphere.* **2001**, *44*, 1041.
10. Han, L.; Wang, J.; Shi, H.; Qian, Y. *J. Environ. Sci.* **2000**, *12*, 22.
11. Wang, J. D.; Hou, W.; Qian, Y. *Biotechnol. Techniques.* **1995**, *9*, 203.
12. Wang, J.; Liu, P.; Qian, Y. *Environ. Int.* **1997**, *23*, 775.
13. Wang, J.; Quan, Y. *Chemosphere.* **1999**, *38*, 3109.
14. Cassidy, M. B.; Lee, H.; Trevors, J. T. *J. Ind. Microbial.* **1996**, *16*, 79.
15. Gosmann, B.; Rehm, H. J. *Appl. Microbiol. Biotechnol.* **1986**, *23*, 163.
16. Trevors, J. T.; Elsas, J. D.; Lee, H.; Wolters, A. C. *Appl. Microbial. Biotechnol.* **1993**, *39*, 637.
17. Ehrhardt, H. M.; Rehm, H. J. *Appl. Microbiol. Biotechnol.* **1985**, *21*, 32.
18. Balfanz, J.; Rehm, H. J. *Appl. Microbiol. Biotechnol.* **1991**, *35*, 662.
19. OReilly, K. T.; Crawford, R. L. *Appl. Environ. Microbiol.* **1989**, *55*, 213.
20. Hu, Z. C.; Korus, R. A.; Levison, W. E.; Crawford, R. L. *Environ. Sci. Technol.* **1994**, *28*, 491.
21. Sahasrabudhe, S. R.; Modi, A. J.; Modi, V. V. *Biotechnol. Bioeng.* **1988**, *31*, 889.
22. Shreve, G. S.; Vogel, T. M. *Biotechnol. Bioeng.* **1993**, *41*, 370.
23. Quan, X.; Shi, H.; Wang, J.; Qian, Y. *Chemosphere.* **2003**, *50*, 1069.
24. Quan, X.; Shi, H.; Liu, H.; Lv, P.; Qian, Y. *Wat. Res.* **2004**, *38*, 245.