Journal of Inclusion Phenomena and Macrocyclic Chemistry **44:** 407–411, 2002.

Effects of Cyclodextrins on Dodecane Biodegradation

SILVIA STEFFAN[1], PATRIZIA TANTUCCI[1], LAURA BARDI[2] and MARIO MARZONA[1,*]
[1]*Dipartimento di Chimica Generale ed Organica Applicata, Corso Massimó D'Azeglio, 48 - 10125 Turin, Italy;* [2]*Istituto Sperimentale per la Nutrizione delle Piante. Via Pianezza, 115 - 10100 Turin, Italy (E-mail: laura.bardi@unito.it)*

(Received: 7 May 2002; in final form: 1 October 2002

Key words: bioavailability, bioremediation, cyclodextrins, hydrocarbons, soil

Abstract

Bioremediation of non-chlorinated hydrocarbon-polluted soils is mainly affected by low bioavailability, due to hydrophobicity of these xenobiotics. In fact, several microorganisms can use hydrocarbons as energy and carbon sources, but their degradative activity takes place into the aqueous phase of the soil, where just traces of hydrocarbons are found because of their low water solubility. So, natural attenuation usually occurs in hydrocarbon-polluted soils, but this process is very slow. It has already been demonstrated that cyclodextrins increase hydrocarbon solubility and bioavailability and accelerate their biodegradation. In this work it was investigated if their efficacy on biodegradation of a model hydrocarbon (dodecane) is affected by the kind (α, β-, γ- and hydroxypropyl-β-cyclodextrin) and the concentration of cyclodextrin and by environmental factors such as temperature and composition of the microbial indigenous population. The results obtained show that all the tested factors influence the biodegradation kinetics. The best results were obtained with β-cyclodextrin at a concentration near to its water solubility limit; moreover, biodegradation rate increases with temperature and different microbial strains show different degradative activity and metabolic behaviour.

Introduction

In situ bioremediation of hydrocarbon-polluted soils is among the most suitable remediation techniques, as it is cheap, frequently more effective than chemical and physical treatments and its environmental impact is very low [7].

Non-chlorinated hydrocarbons can be easily used as energy and carbon sources by several microorganisms until a complete mineralisation, and natural attenuation generally occurs in hydrocarbon-polluted soils, but this process is very slow [7]. In fact, low hydrocarbon bioavailability affects the rate and extent of hydrocarbon biodegradation [4, 6, 8, 14, 16]. Hydrocarbons are hydrophobic compounds; they are easily adsorbed on clay or humus soil fractions, so they transfer very slowly to the aqueous phase where they are metabolized by microorganisms. Cyclodextrins enhance the hydrocarbon water solubility by the formation of inclusion complexes, and highly improve microorganism performances [1, 5, 9, 10, 13, 17]. It has already been demonstrated that cyclodextrins accelerate degradation kinetics of polyaromatic and aliphatic hydrocarbons both in aqueous [2] and solid phase [3, 15].

The aim of this work was to test the effect on dodecane biodegradation of different kind (α, β, γ, hydroxypropyl-β-cyclodextrin) and concentration of cyclodextrins and of environmental parameters such as temperature and composition of indigenous microbial population. Biodegradation assays were performed in a liquid mineral medium, containing nutrilites required for microbial growth and dodecane as the sole carbon and energy source. Cells of strains selected from the indigenous microbial population of a hydrocarbon-polluted soil were used as biocatalyst. Cyclodextrins were added to the medium before microbial inoculation. Dodecane biodegradation kinetics were analysed monitoring hydrocarbon residual concentration by gas-chromatographic analysis.

* Author for correspondence. E-mail: mario.marzona@unito.it

Experimental

Microorganism selection and growth conditions

Dodecane degrading microorganisms were selected from 1 g of hydrocarbon-polluted soil suspended in 0.9% NaCl, by spreading onto selective solid mineral medium (K_2HPO_4 0.8 g/l, KH_2PO_4 0.2 g/l, $CaSO_4 \cdot 2H_2O$ 0.05 g/l, $MgSO_4 \cdot 7H_2O$ 0.5 g/l, $FeSO_4 \cdot 7H_2O$ 0.09 g/l, $(NH_2)_2SO_4$ 1 g/l, 2‰ w/v dodecane). After 15 days of incubation at 28 °C, two colonies, morphologically ascribed to a fungal and a bacterial strain respectively, were isolated. Pure or mixed cultures of these strains were used as inoculum for the biodegradation kinetics. 1 ml starter culture, obtained in selective liquid mineral medium after incubation at 28 °C for 5 days, was used for bacterial cell inoculation. A plug 0.5 cm diameter, obtained from selective solid mineral medium, with fungal cells grown at surface for 15 days at 28 °C, was used as inoculum for fungal strain.

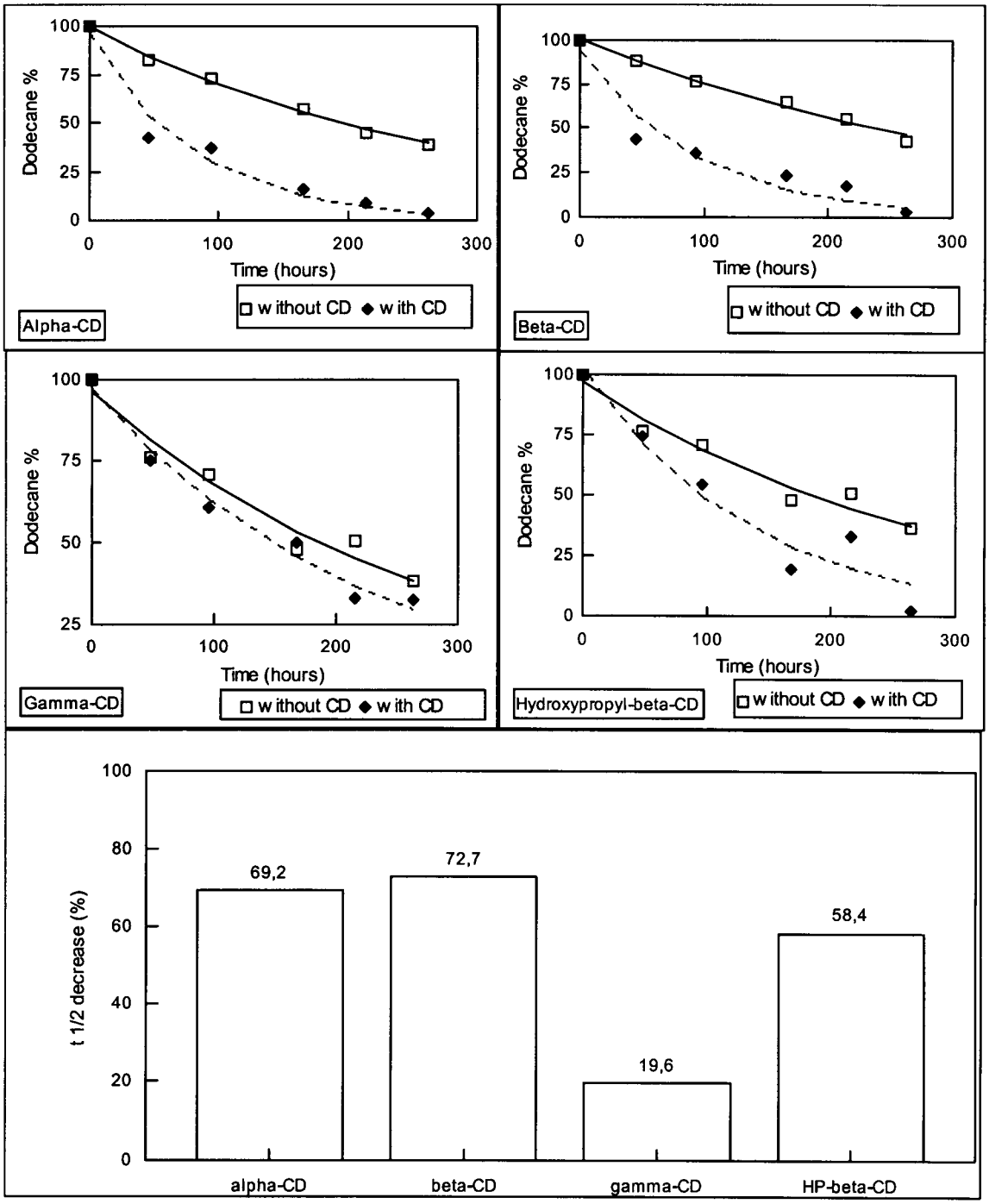

Figure 1. Effect of different cyclodextrins on biodegradation kinetic and on percent decrease of half degradation time ($t_{1/2}$).

Degradation kinetics

Four sets of conical flasks containing 50 ml selective liquid mineral medium were prepared. In the first set, the kinetics of biodegradation were performed with 1.6% α-, β-, γ- or hydroxypropyl-β-cyclodextrin at 28 °C. In the second set, β-cyclodextrin was added at 0%, 0.4%, 1%, 1.6% or 1.8% concentration. In the third set, assays were performed with 1.6% β-cyclodextrin comparing incubation temperatures of 10 °C, 28 °C and 35 °C. In the fourth set, 1.6% β-cyclodextrin was added and different microbial strains were compared, using as inoculum the fungal strain, the bacterial strain or the associated fungal-bacterial strains. After inoculum, flasks were incubated on orbital shaker at 150 rpm and 28 °C (10 °C and 35 °C for temperature comparison assay). Samples were analysed at regular intervals of time by residual dodecane extraction from the whole volume of a single flask. Data obtained from each degradation kinetic were modelized using a Sigma plot program according to a first order reaction: $y = Ae^{-kt}$.

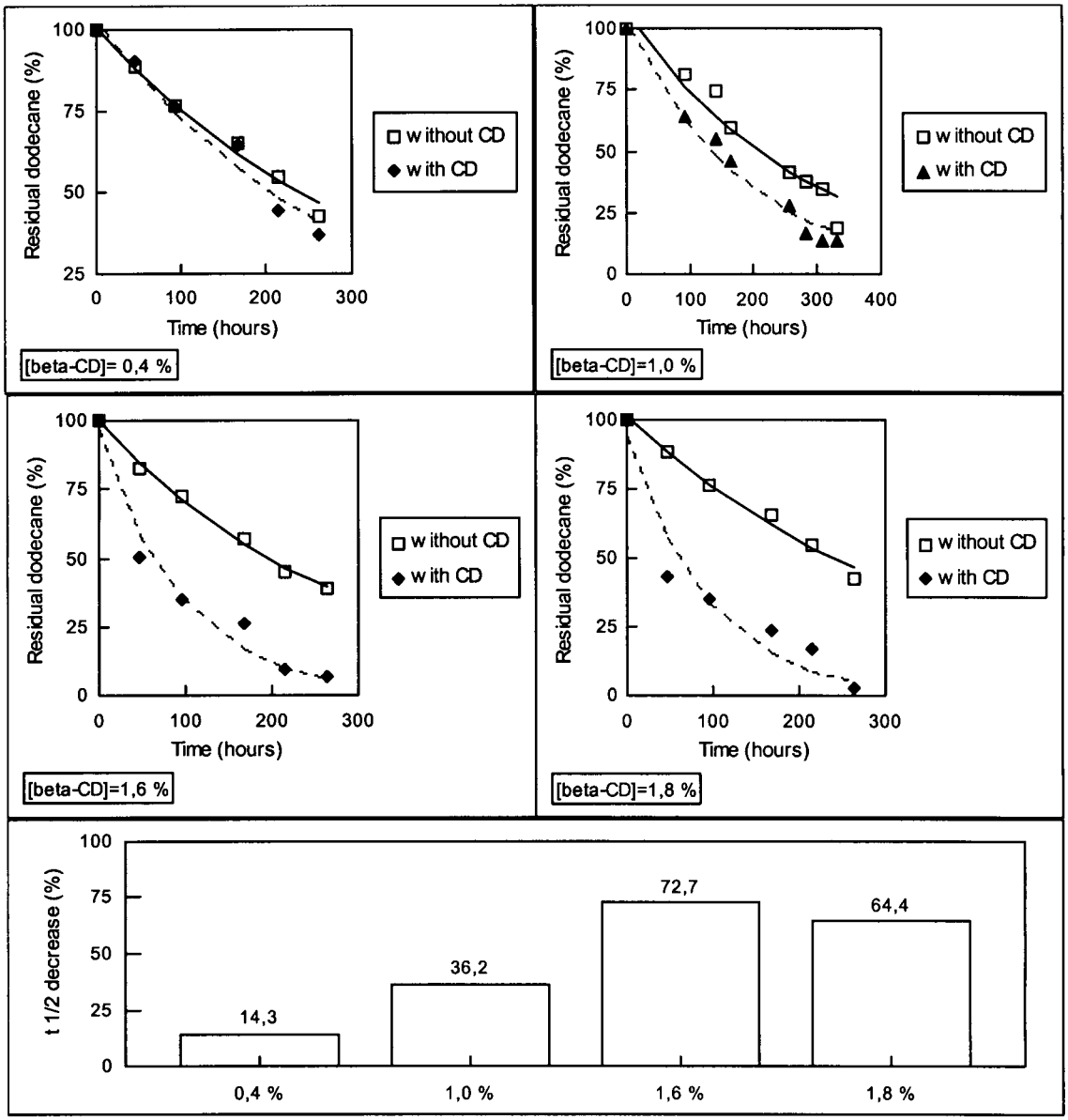

Figure 2. Effect of β-cyclodextrin concentration on biodegradation kinetic and on percent decrease of half degradation time ($t_{1/2}$).

Dodecane analysis

Dodecane was extracted from each sample in 3 × 10 ml toluene, then analysed by a gas chromatograph GC HP 5890-II equipped with FID detector on HP1 Cross-Linked Methyl Silicone capillary column, 15 m long and 0.32 mm i.d.; film thickness 1.0 mμ Operating conditions: oven temperature: 100-250 °C at 20 °C/min; injector temperature: 250 °C; detector temperature 280 °C; carrier gas: helium; flow: 5.2 ml/min; pressure: 13 psi; injection: 1 μl.

Results

Among the four tested cyclodextrins, best results were obtained using β-cyclodextrin, that produced a 72.7% reduction of the half biodegradation time (Figure 1). The effect of α-cyclodextrin was similar but lower (69.2%), while hydroxypropyl-β-cyclodextrin and γ-cyclodextrin were less effective (58.5% and 19.6% respectively) (Figure 1).

β-cyclodextrin concentration highly affected the biodegradation rate: biodegradation was accelerated when β-cyclodextrin concentration was increased. 1.6% β-cyclodextrin decreased the dodecane half biodegradation time from 231 to 63 h, while 0.4% induced a decrease from 231 to 198 h and 1% from 187 to 119 h. 1.8% gave results similar to 1.6% (Figure 2).

Temperature affected both rate and extent of biodegradation. At 10 °C, just 57.3% dodecane was degraded ($t_{1/2}$ calculated on degraded dodecane is 69.3 h), while at 35 °C the degradation was quicker and almost complete: $t_{1/2}$ = 39.5 h. Both kinetics fit to a first order reaction. The plot of 28 °C kinetic was more complex and seems to be the result of two subsequent phases (Figure 3). Anyway, the influence of temperature on the biodegradation process was significant.

In Figure 4 are shown the biodegradation kinetics of the two selected microbial strains. Both strains were effective, but their degradative activities were quite different. The strain morphologically ascribed to a bacterium gave a rapid attack, but when 50% dodecane was degraded the biodegradation rate decreased. The lag phase lasted about 100 h, then the process started again and reached the full mineralization. The trend of the kinetic obtained with microbial cells morphologically ascribed to a fungal strain was most regular and seems to be a first order kinetic $y = Ae^{-kt}$, with a reaction half time of 44.1 h. Bacterial-fungal association did not improve the performances of the pure fungal culture.

Discussion

Among tested cyclodextrins, the best results were obtained with β-cyclodextrin; α-cyclodextrin gave similar results, while γ- and hydroxypropyl-β-cyclodextrin were less effective. According to Sanemasa *et al.* (1990), an aliphatic hydrocarbon molecule, which section diameter is 4.5 Å, can be contained into the toroidal cavity of α-cyclodextrin (5 Å) and β-cyclodextrin (6.9 Å). Similar results were obtained by Ono *et al.* (1979) for complexation of α- and β-cyclodextrin with alkyl-carboxylates. The internal cavity diameter of γ-cyclodextrin (8.5 Å) could be too large to be favorable to the formation of a complex with an alcane molecule [Sanemasa]. Finally, hydroxypropyl-β-cyclodextrin lateral chains could interfere with the dodecane-complex formation because of either polar or steric interactions.

Best performances obtained for β-cyclodextrin are an advantage from the economic point of view, as β-cyclodextrin is the cheapest commercial form of cyclodextrin.

The positive effect of β-cyclodextrin is proportional to its concentration until its water solubility limit; so, it is useless to increase the amount of coadiuvant beyond this level.

Temperature effect was analysed in the range of average variation observed at the Italian soil surfaces. The highest temperature (35 °C) shown the best results, with a drastic reduction of the time required for the full hydrocarbon degradation. At 10 °C it was impossible to reach a full hydrocarbon degradation, while at 28 °C a peculiar degradation trend was observed, ascribable to diauxinic cycles. These results could be explained by the existence of two different metabolic pathways, one of which is not active when temperature is less than 10 °C and is prevalent when temperature is 35 °C. This effect was not observed when the fungal strain or the fungal-bacterial consortium were used as biocatalyst. That means that metabolic pathways for degradation are different in the tested strains; the fungal strain was more effective for degradation and it probably can be competitive to other indigenous microorganisms, as its activity shown to be prevalent when grown in consortium.

In conclusion, cyclodextrins as coadiuvant considerably increase both extent and rate of dodecane biodegradation. Best performances were obtained at 35 °C, using 1.6 %l β-cyclodextrin and enrichment culture of microbial strains selected from the indigenous population of the polluted soil. In these conditions more than 98% of the hydrocarbon was

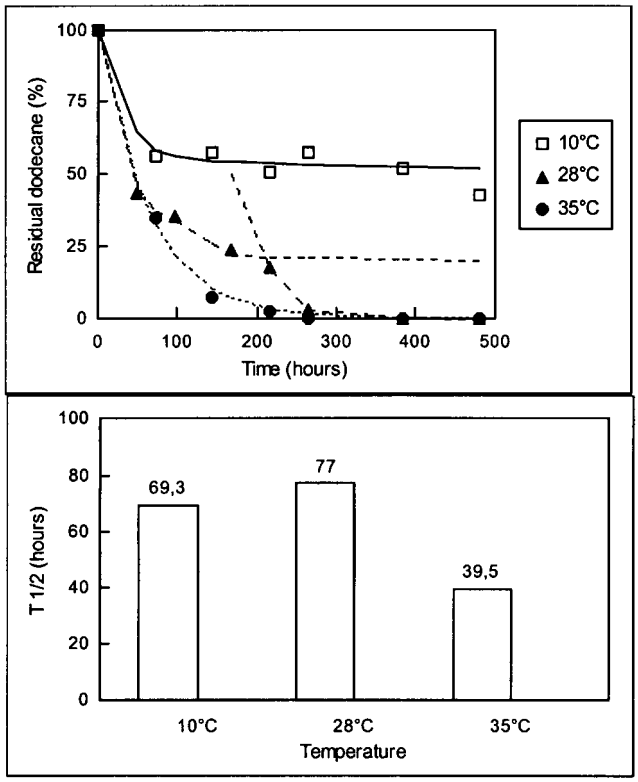

Figure 3. Effect of temperature on biodegradation kinetic and on half degradation time ($t_{1/2}$); $t_{1/2}$ was calculated on the basis of effectively degraded dodecane (57.3% at 10 °C, 100% at 28 °C and 35 °C).

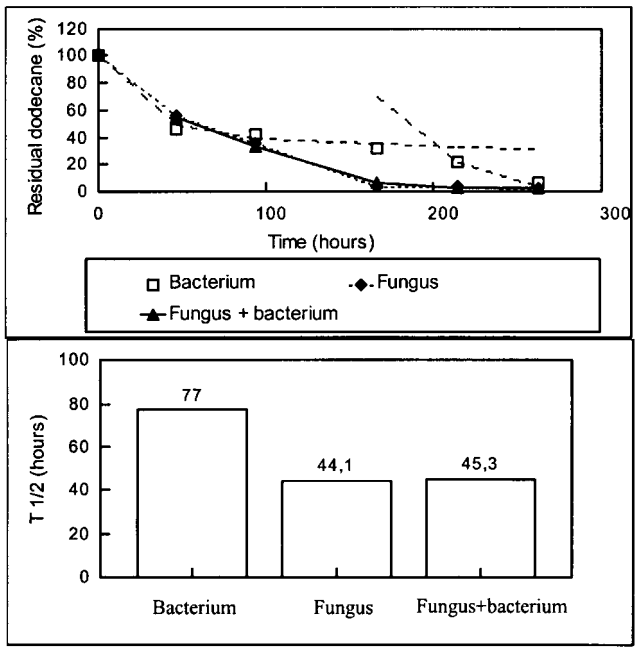

Figure 4. Microbial strain effect on biodegradation kinetic and on half degradation time ($t_{1/2}$).

removed in 230 h. These results confirm that cyclodextrin can greatly improve biodegradation increasing hydrocarbon bioavailability to natural, indigenous microorganisms of polluted soils; among the tested strains, fungal strains could be more active than bacterial strains.

References

1. B.K. Banky, K. Recseg, and D. Novak: *Magy. Kem. Lapja* **40**, 189 (1985).
2. L. Bardi, A. Mattei, S. Steffan, and M. Marzona: *Enz. Micr. Technol.* **27**, 709 (2000).
3. L. Bardi, R. Ricci, and M. Marzona: *Water Air Soil Pollut.*, Special Issue, in press (2002).
4. T. Barkay, S. Navon-Venezia, E.Z. Ron, and E. Rosenberg: *Appl. Environ. Microbiol.* **6**, 2697 (1999).
5. M. L. Brusseau: in R. Serra. C.I.P.A. S.r.l. (ed.), The Impact of Physical, Chemical and Biological Factors on Biodegradation: Implications for *in situ* Bioremediation (Biotechnology for Soil Remediation. Scientific Bases and Practical Applications), Milan, Italy (1998), pp. 81–98.
6. M.L. Brusseau, R.M. Miller, Y. Zhang, X. Wang and G.Y. Bai: *ACS Symposium Series* **594**, 82 (1995).
7. J.T. Cookson: in J.T. Cookson Jr. (ed.), *Optimizing Microbial Transformation of Hazardous Chemicals (Bioremediation -Engineering*, McGraw-Hill, Inc., USA (1995), pp. 95–164.
8. H. De Jonge, J.I. Freijer, J.M. Verstraten, J. Westerveld and F.W.M. Van der Wielen: *Environ. Sci. Technol.* **31**, 771 (1997).
9. K. Gruiz, E. Fenyvesi, E. Kriston, M. Molnar, and B. Horvath: *J. Incl. Phenom.* **25**, 233 (1996).
10. J. Olah, T. Cserhati, and J. Szejtli: *Water Res.* **22**, 1345 (1988).
11. K. Ono, M. Tokuda, and K. Muratami: *Polymer preprints*, *Japan* **28**(7), 1302 (1979).
12. I. Sanemasa, T. Osajima, and T. Deguchi: *Bull. Chem. Soc. Jpn.* **63**, 2814 (1990).
13. A. Schwartz and R. Bar: *Appl. Environ. Microbiol.* **61**, 2727 (1995).
14. A.M. Sekelsky and G.S. Shreve: *Biotechnol. Bioeng.* **63**(4), 401 (1999).
15. S. Steffan, L. Bardi, and M. Marzona: *Biol. J. Armenia*, **Special Issue 53**, 218 (2001).
16. H.H. Tabak and R. Govind: *Ann. N. Y. Acad. Sci.* **829**, 36 (1997).
17. J.M. Wang, E.M. Marlowe, R.M. Miller-Maier, and M.L. Brusseau: *Environ. Sci. Technol.* **32**, 1907 (1998).