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Environmental Aspects of PAH Biodegradation

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous pollutants, some of which are on the US Environmental Protection Agency priority pollutant list. Consequently, timely clean-up of contaminated sites is important. The lower-mol-wt PAHs are amenable to bioremediation; however, higher-mol-wt PAHs seem to be recalcitrant to microbial degradation. The rates of biodegradation of PAHs are highly variable and are dependent not only on PAH structure, but also on the physicochemical parameters of the site as well as the number and types of microorganisms present. PAHs sorb to organic matter in soils and sediments, and the rate of their desorption strongly influences the rate at which microorganisms can degrade the pollutants. Much of the current PAH research focuses on techniques to enhance the bioavailability and, therefore, the degradation rates of PAHs at polluted sites. Degradation products of PAHs are, however, not necessarily less toxic than the parent compounds. Therefore, toxicity assays need to be incorporated into the procedures used to monitor the effectiveness of PAH bioremediation. In addition, this article highlights areas of PAH research that require further investigation.

Index Entries: Polycyclic aromatic hydrocarbons; PAHs; biodegradation; bioavailability; water solubility; degradation kinetics; toxicity.

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INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) (Fig. 1) are widely distributed in the environment and may persist for extended periods of time. This has raised considerable concern because many of the high-mol-wt PAHs have been shown to be potentially genotoxic and carcinogenic (*1*). Human activity, including fossil fuel combustion and industrial processing, is the primary source of PAH contamination, although PAHs are also generated by natural phenomena, such as forest fires.

Microbial degradation of PAHs has been studied, and biochemical pathways and mechanisms of action for the lower-mol-wt PAHs have been elucidated (*1*). The majority of this work has emphasized metabolism in pure cultures of bacteria, and although this research has proven to be extremely valuable, research must continue in order to determine the capacity of microorganisms to degrade the more genotoxic higher-mol-wt PAHs, and to improve predictions of the fate and impact of PAHs in the environment. The objective of this article is to focus on the environmental aspects of PAH degradation. Special emphasis is given to incorporating points on research needs and gaps in our knowledge with the available information on PAH biodegradation.

PAH DEGRADATION RATES

PAHs in the environment are problematic not only because they are a human health risk, but also because they may alter native ecological communities. The general consensus is the predominant mechanism for PAH removal from soil and aqueous systems is biological (*1,2*), although physicochemical removal mechanisms can substantially reduce the concentrations of some lower-mol-wt PAHs (*3*). The current foci of PAH biodegradation research include (1) such factors as sorption and desorption, which may control natural biodegradation rates, and (2) enhancing degradation through various techniques, such as adding allochthonous microorganisms or high concentrations of laboratory-grown autochthonous organisms, adding inorganic nutrients, adding surfactants, and utilizing engineered systems to aid in mass transfer and to optimize growth conditions.

The rates at which PAHs are reportedly degraded vary widely and are contingent on factors that include:

1. The test culture system, i.e., one species vs a mixed culture;
2. The monitoring method, i.e., removal of parent compound vs production of CO_2,
3. The chemical structure(s) of the PAH(s) that are monitored; and
4. The optimization, or lack of optimization, for temperature, nutrients, water activity, and other laboratory-controlled parameters.

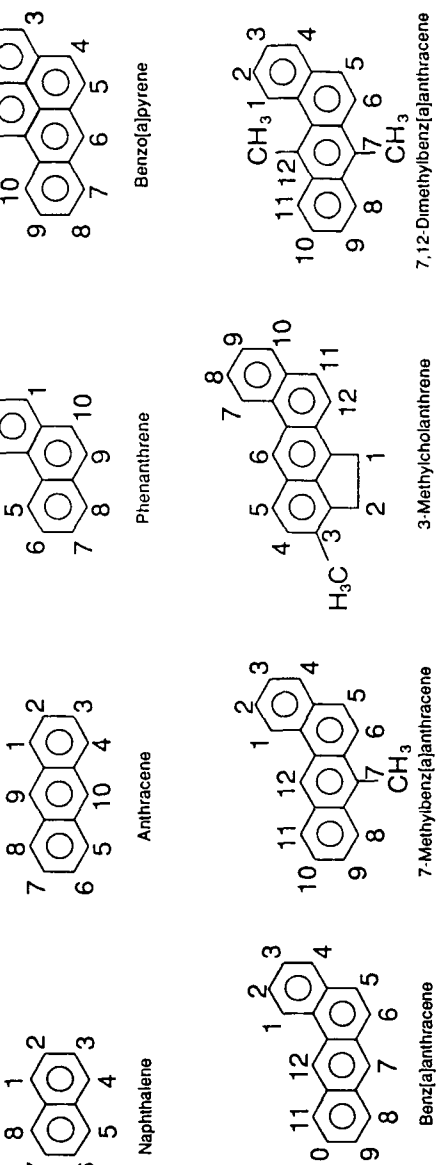

Fig. 1. Representative structures of PAHs described in this article.

Representative examples of half-lives of PAHs in various systems are given in Table 1. It is well known that the lower-mol-wt PAHs are more easily degraded than the higher-mol-wt PAHs (*1,2*); but the degradation rates for any given PAH are highly dependent on the test system. For example, reported half-lives for pyrene vary from a low of 19.4 d to a high of 90 wk (Table 1). In many cases, however, half-lives alone may not provide sufficient information to determine the potential effectiveness of bioremediation of PAHs, because degradation rates are frequently not linear. Initial degradation rates, after any lag phase, are usually faster than later rates. Degradation rates will decrease with time if any nutrient, such as oxygen, becomes severely limiting. If inorganic nutrients are not limiting, however, then it is likely that degradation rates will decrease as the most bioavailable PAH substrates are utilized, leaving the recalcitrant fraction behind. It is crucial to recognize that PAH biodegradability may be a function of soil history. In many cases, experiments are conducted with soils or sediments that are not from actual polluted sites; rather, PAH amendments are mixed into the soil just prior to starting degradation experiments. Unless a spill has recently occurred at the site targeted for bioremediation, such laboratory experiments will probably overestimate degradation potential in the field.

The length of contact time between PAHs and the soil can have a significant effect on availability of PAHs (*9*). Apparently, obtaining a true equilibrium between PAHs and soil matrices can be a slow process. What is not yet fully understood is the mechanism(s) that causes PAHs to become more recalcitrant with time. It is known that sorption of PAHs onto soil organic matter is a critical factor in determining bioavailability, and recently, predictive biodegradation models have been developed to account for the diffusion and sorption of organic molecules in complex matrices (*10–12*). One theory that may be applicable in a limited number of situations suggests that PAHs may be incorporated into soil humic material via enzymatic polymerization (*13*).

Since PAHs have limited water solubility and can be strongly sorbed onto soil organic matter, PAH degradation rates will often be controlled by mass transfer, dissolution rates, and solubility, and, in conjunction, the reported growth rates of bacteria cultivated on PAHs as sole carbon and energy sources are uniformly low (Table 2). One example of mass-transfer limitation is provided by Volkering et al. (*14*). They found that growth of a *Pseudomonas* sp. on naphthalene was a function of the size of naphthalene crystals added to the medium, with substantially faster growth rates as crystal size decreased. Similarly, Keuth and Rehm (*19*) showed that the growth rate of *Arthrobacter polychromogenes* increased as the phenanthrene concentration increased (Table 2). This is again probably a result of mass transfer. Alternatively, it is possible, although unlikely, that their observed degradation rates were determined by the microbial half-satura-

Table 1
Half-lives (in Days) for PAHs in Various Systems

PAH	Soil amended with PAH-containing sludge[a]	Soil mixed with oily waste, nutrients, and microbes[b]	PAHs added to two sandy loam soils[c]		PAHs added to two loam soils[d]		PAHs as sole C source in a fermentor[e]	PAHs added to lake sediments[f]		
			a	b	a	b		a	b	c
Acenaphthalene	—[g]	19.7	—	—	—	—	—	—	—	—
Naphthalene	28 ± 16	—	2.1	2.2	—	—	0.92	30.8	22.4	16.8
Phenanthrene	124 ± 48	24.4	16	35	—	—	1.54	126	56	28
Anthracene	141 ± 78	18.9	134	50	33	26	3.71	—	—	—
Fluoranthene	137 ± 35	—	377	268	—	—	—	—	—	—
Fluorene	—	—	—	—	24	21	—	—	—	—
Pyrene	225 ± 92	19.4	260	199	35	34	—	n.d.[g]	630	238
Benzo[k]fluoranthene	301 ± 105	—	—	—	—	—	—	—	—	—
Benzo[a]pyrene	211 ± 69	—	309	229	—	—	—	n.d.	>2100	>1400
Chrysene	—	—	371	387	129	407	—	—	—	—

[a] Averages and SD for four types of soils amended with PAH-containing sludge. Moisture was 25–60%, and temperature was 20–30°C. Values include abiotic losses. Reference (4).

[b] Oil reclamation waste was mixed with uncontaminated soil, nutrients, and an enrichment of autochthonous microbes. Soil was land-farmed and data includes abiotic losses. Reference (5).

[c] Data are for two sandy loam soils. PAHs were added to these soils, which had no prior PAH exposure. Incubation was at −33 J/kg water potential and 20°C. Values are corrected for abiotic removal. Reference (3).

[d] PAHs were added to unpolluted soils, a light loam, and a loamy sand. Incubation was at 25°C, and values include abiotic losses. Reference (6).

[e] Data obtained from a cyclone fermentor with the PAH as sole carbon and energy source. A mixed enrichment culture was used. Reference (7).

[f] Data obtained from PAHs added to lake sediments in microcosms at 22°C. Sediments were from (a) lake with no known direct PAH exposure, (b) lake exposed to agricultural run-off, and (c) bay with PAH content of approx 2 ppm. Reference (8).

[g] Abbreviations: not determined (—); not detected (n.d.).

Table 2
Kinetic Rates for Growth of Bacteria on PAHs[a]

PAH	Concentration	Microorganism(s)	Growth rate	Reference
Naphthalene	Not given	Mixed culture	0.3 h^{-1}	(*14*)
Fluorene	1000 ppm	*Pseudomonas vesicularis*	0.043 h^{-1}[b]	(*15*)
Fluorene	100 ppm	*Arthrobacer* sp.	0.023 h^{-1}[b]	(*16*)
Phenanthrene	Not given	Mixed culture	0.03 h^{-1}	(*14*)
Phenanthrene	500 ppm	*Mycobacterium* sp.	0.069 h^{-1}	(*17*)
Phenanthrene	Not given	*Flavobacterium* sp.	0.19 h^{-1}	(*18*)
Phenanthrene	Not given	*Beijerinckia* sp.	0.23 h^{-1}	(*18*)
Phenanthrene	1000 ppm	*Pseudomonas paucimobilis*	0.058 h^{-1}[b]	(*15*)
Phenanthrene	75 ppm	*Arthrobacter polychromogenes*	0.079 h^{-1}	(*19*)
Phenanthrene	150 ppm	*Arthrobacter polychromogenes*	0.092 h^{-1}	(*19*)
Phenanthrene	450 ppm	*Arthrobacter polychromogenes*	0.117 h^{-1}	(*19*)
Fluoranthene	500 ppm	*Mycobacterium* sp.	0.040 h^{-1}	(*17*)
Fluoranthene	1000 ppm	*Alcaligenes denitrificans*	0.020 h^{-1}[b]	(*15*)
Pyrene	500 ppm	*Mycobacterium* sp.	0.056 h^{-1}	(*17*)
Pyrene	500 ppm	*Rhodococcus* sp.	0.023 h^{-1}[b]	(*20*)
Anthracene	Not given	Mixed culture	0.0003 h^{-1}	(*14*)

[a] All rates were determined in mineral salts media with the PAH as sole carbon and energy source, and all were incubated between 28 and 30°C.
[b] These values were calculated as the specific (instantaneous) growth rate constants, μ, based on the given generation times and $\mu = (\ln 2)/(t_{gen})$.

tion constant. If the inherent maximum degradation rate of a microorganism is much slower than the mass-transfer rate, then the inherent microbial kinetics, and not the dissolution or mass-transfer rates, will control degradation rates. This apparently was the case for another *Pseudomonas* sp. (*21*). Additionally, in natural soil systems where cell density and activity can be substantially lower than in laboratory-controlled cultures, solubilization of PAHs may play a less important role. In fact, Stucki and Alexander (*18*) reported that in the early phase of growth of a *Pseudomonas* culture, the dissolution rate of biphenyl was not limiting, but appeared to be limiting toward the end of the growth curve. More recently, Volkering et al. (*22*) did a definitive study using mathematical models in conjunction with experimental data to evaluate the interrelationships between microbial kinetics and dissolution rates of naphthalene, and they clearly demonstrated growth of a *Pseudomonas* sp. was not limited by mass transfer

when the cell numbers were very low, i.e., at the beginning of the growth curve. The growth of the culture quickly became mass-transfer-limited, however, as the cell number increased. Nonetheless, it has not been determined whether or not solubilization in the aqueous phase is always essential for biodegradation of PAHs, since the potential exists for attached organisms to degrade insoluble substrates (*23*). Provisional support for the importance of solid-phase degradation is provided by Aronstein et al. (*24*). They reported, in unpublished data, that some bacteria were capable of using sorbed hydrocarbons that were not detectable in the aqueous phase. Similarly, Wyndham and Costerton (*25*) also reported that bacteria colonized the surfaces of bitumen and suggested that this was the first stage in the degradation of this insoluble substrate. This area of research has not yet been adequately addressed and warrants further attention.

Another factor that is related to solubility and contributes to observed biodegradation rates is the inherent capacity of the microorganisms to transform very low concentrations of PAHs. Lewis and Gattie (*26*) suggest that for substrate levels substantially below the levels needed for cellular maintenance, degradation rates are likely to be very low, but also highly dependent on the species of microorganisms present. Few data are currently available in this area, although preliminary results in our laboratory suggest that mineralization of ppb concentrations of phenanthrene may be rapid. However, at polluted sites, multiple carbon sources are available, so even if one carbon source is below the concentration needed for maintenance level, the sum of carbon available probably will be above maintenance level. Furthermore, in low concentration, mixed-substrate situations, substrates are frequently used simultaneously (*27*). The extent to which mixed-substrate utilization may enable bacteria to remove very low concentrations of PAHs is not known, because most studies of bioremediation employ mixed cultures of organisms, and work is focused on utilization of either a single PAH or a mixture of PAHs. Comparative studies of the effects of multiple carbon sources vs one carbon source are rare; however, Bauer and Capone (*28*) did show that the degradation rate for anthracene in the presence of naphthalene in mixed-culture sediment slurries was the same as the rate for anthracene degradation in the absence of naphthalene. In contrast, Molina et al. (*29*) reported that pyrene mineralization could be inhibited by addition of other PAHs, although this inhibition could have been related to toxicity as well as substrate utilization patterns. In addition, pyrene metabolism by a *Mycobacterium* sp., which was inoculated into a microcosm containing other bacteria, was inhibited by the addition of 0.01% glucose plus 0.01% peptone, even though the *Mycobacterium* sp. can only cometabolize pyrene (*30*). Clearly, this is an area of research that could be explored further.

Various types of nutrients in addition to exogenous carbon sources have also been used in attempts to enhance microbial degradation of

PAHs. As would be expected, the results of these studies are diverse (*30,31*), since, for example, addition of phosphorus would be expected to enhance PAH degradation in a phosphorus-limited system, but not in a nitrogenor oxygen-limited system. Several studies on the addition of exogenous organisms to enhance PAH degradation have also been conducted. Mueller et al. (*32*) have had considerable success in removing a complex mixture of PAHs in a pilot-scale bioreactor that was repeatedly inoculated with specific microorganisms. Grosser et al. (*33*) were able to enhance pyrene degradation in a soil by reintroducing very high concentrations (2×10^9 CFU/g) of a native pyrene degrader, although concentrations as high as 10^8 CFU/g soil were ineffective in enhancing pyrene removal in this soil, which already had a very active indigenous degrader population.

BIOAVAILABILITY OF PAHs

Because bioavailability of PAHs is critical for effective bioremediation, a great deal of research has been devoted to increasing the availability of PAHs, particularly high-mol-wt PAHs that have a corresponding low water solubility. The most often used technique to enhance bioavailability has been addition of surfactants, but at this time, generalizations are not warranted. Laha and Luthy (*34*) found that mineralization of phenanthrene in a mixed-culture system was inhibited by nonionic surfactant concentrations greater than the critical micelle concentration (cmc), and was neither inhibited nor enhanced by concentrations below the cmc. Concentrations above the cmc did not, however, alter the degradation rates of glucose in their systems, so the inhibition was apparently not related to surfactant toxicity. Subsequent tests with more surfactants yielded similar results (*35*). In contrast, Aronstein et al. (*24*) reported that low concentrations of the nonionic surfactants, Alfonic 810-60 and Novel II 1412-56, did enhance mineralization of phenanthrene. Guerin and Jones (*36*) similarly claimed that phenanthrene degradation with *Mycobacterium* sp. was enhanced by the presence of various Tweens, but control values (i.e., no Tween) were not provided and therefore it is not clear whether or not the Tweens truly had a significant effect in their system.

Another method for enhancing bioavailability and thereby biodegradation rates involves the use of specially designed reactors, e.g., Liu et al. (*7*) used a cyclone fermentor to maintain high concentrations of PAHs in their system without resorting to addition of organic carrier solvents. Even benzo[a]pyrene has been efficiently degraded biologically in continuous stirred-tank reactors (*37*); unfortunately, the authors were only able to monitor parent compound removal, and therefore, the nature and quantity of metabolites are not known.

TOXICITY STUDIES

Increasingly, more researchers are incorporating toxicity assays into their biodegradation studies. This is an extremely valuable contribution, because the need for bioremediation is based on a need to decrease concentrations of toxic compounds at a site. Furthermore, bioavailability is a controlling factor not only in biodegradation, but also in toxicity. If PAHs are so tightly bound that they are not available for bioremediation, then the potential toxic impact of the PAHs may be minimized. This idea is supported by the work of Weissenfels et al. (*38*), who found that 62% of PAHs in a contaminated soil from a wood impregnation plant were removed, whereas there was no significant removal of PAHs from soil contaminated by a tar oil refinery. Nonetheless, in the Microtox assay, the tar oil soil was significantly less toxic than the wood impregnation plant soil. Although the wood impregnation plant soil had 1.8 times the PAH content of the tar oil soil (1815 and 1027 ppm, respectively), the former soil was at least 40 times more toxic than the later. Further experiments showed that this correlation was most likely attributable to stronger sorption of PAHs on the tar oil soil than on the impregnation plant soil. A comparable study by Erickson et al. (*39*) showed that PAH-contaminated (200–400 ppm) soils from a manufactured gas plant site were neither amenable to bioremediation nor toxic based on the Microtox test. Regardless of the recalcitrance of the PAHs at the site, the authors did find that exogenously added PAHs readily disappeared from that soil. However, the absence of abiotic controls meant that loss of exogenous PAHs may have been abiotic as well as biotic.

Toxicity studies are essential not only because intimate association of PAHs with the soil matrix may render the PAHs unavailable, but also because metabolites have the potential to be more toxic than the parent compound. In a set of carefully conducted studies, Mueller and his colleagues (*32,40*) have been using toxicity and teratogenicity assays to monitor the effectiveness of their bioremediation operations. They used a two-stage sequential bioreactor to remove PAHs from contaminated ground water; the effluent from reactor 1 was the feed into reactor two, which was necessary in order to achieve suitable removal efficiencies of the high-mol-wt PAHs. The final effluent (from reactor 2) was much less toxic than the feed as determined by the Microtox EC_{50} assay, two LC_{50} assays, and a teratogenicity assay. The final effluent was also less toxic than the effluent from the first stage reactor based on the mysid LC_{50} assays and the teratogenicity study, however, results from the Microtox assays and the *Ceriodaphnia* LC_{50} assay showed that the final effluent was slightly more toxic than the effluent from the first stage reactor. Thus, it is prudent to conduct several types of toxicity assays to determine if bioremediation has indeed achieved the desired goals, since different toxicity assays can yield different results. Ideally, the toxicity assay should reflect the potential for

PAHs to alter the actual ecosystem or community structure that would be expected to survive at the site, although virtually any measure of toxicity is better than solely monitoring disappearance of PAHs. However, even if toxicity is reduced, as determined by tests on aqueous extracts of the sample, any remaining PAHs could still enter other compartments of the ecosystem directly via ingestion of soil/sediment particles by the macrofauna or by uptake into plants. Apparently, there has not been much research in the area of direct uptake into biota, although investigations on the potential transfer of PAHs in this manner should be completed to determine whether or not previously contaminated sites are truly decontaminated.

CONCLUSIONS

Monitoring the effectiveness of bioremediation of PAH-contaminated sites can be problematic, because PAHs can be mineralized, transformed into more or less toxic daughter compounds that are not substantially different from the parent compound, volatilized, sorbed, or leached out from the system. Monitoring $^{14}CO_2$ production provides the best evidence for mineralization; however, it is unlikely that radiolabeled compounds will be in true equilibrium with PAHs in aged soils, and in most cases only a fraction of a compound is converted to CO_2 with the remainder, an unknown percentage, becoming incorporated into biomass. In many cases, radiolabeled PAHs are labeled only at one specific position and are, unfortunately, not commercially available as uniformly labeled compounds. This has the potential to skew the results of mineralization studies, since some carbon atoms in a given compound will be more or less likely to be converted to CO_2 than will other carbon atoms from the same molecule. Provided that high recoveries can be obtained, this problem can be overcome to a large extent by completing an accurate and detailed mass balance for the total radiolabel added. Monitoring disappearance of parent compound also does not give a completely accurate representation of biodegradation, since disappearance of parent material may be the result of a minor transformation, e.g., a hydroxylation, or abiotic changes. Additionally, many PAH bioremediation studies have been completed without any abiotic controls or without sufficient replication to allow for statistical analysis between control and experimental sites, making it more difficult to assess true biodegradation potential. Even under the best of circumstances, actually proving biodegradation in field situations can be difficult, and a comprehensive review on the subject is available (*41*). However, despite some inherent inaccuracies in monitoring bioremediation of PAHs, overall patterns of removal and reduction in toxicity have demonstrated that bioremediation can be a feasible treatment method for some sites. The most successful and reproducible methods appear to be managed systems, such as bioreactors.

REFERENCES

1. Cerniglia, C. E. (1992), *Biodegradation* **3,** 351–368.
2. Cerniglia, C. E. (1993), *Curr. Opinion in Biotechnol.* **4,** 331–338.
3. Park, K. S., Sims, R. C., Dupont, R. R., Doucette, W. J., and Matthews, J. E. (1990), *Environ. Toxicol. Chem.* **9,** 187–195.
4. Wild, S. R. and Jones, K. C. (1993), *Environ. Toxicol. Chem.* **12,** 5–12.
5. Catallo, W. J. and Portier, R. J. (1992), *Water Sci. Technol.* **25,** 229–237.
6. Maliszewska-Kordybach, B. (1993), *Environ. Pollut.* **79,** 15–20.
7. Liu, D., Maguire, R. J., Pacepavicius, G. J., and Nagy, E. (1992), *Environ. Toxicol. & Water Quality: Int. J.* **7,** 355–372.
8. Heitkamp, M. A. and Cerniglia, C. E. (1987), *Environ. Toxicol. Chem.* **6,** 535–546.
9. Burford, M. D., Hawthorne, S. B., and Miller, D. J. (1993), *Anal. Chem.* **65,** 1497–1505.
10. Scow, K. M. and Alexander, M. (1992), *Soil Sci. Soc. Am. J.* **56,** 128–134.
11. Scow, K. M. and Hutson, J. (1992), *Soil Sci. Soc. Am. J.* **56,** 119–127.
12. Chung, G.-Y., McCoy, B. J., and Scow, K. M. (1993), *Biotechnol. Bioeng.* **41,** 625–632.
13. Qiu, X. and McFarland, M. J. (1991), *Haz. Waste Haz. Mater.* **8,** 115–126.
14. Volkering, F., Breure, A. M., Sterkenburg, A., and van Andel, J. G. (1992), *Appl. Microbiol. Biotechnol.* **36,** 548–552.
15. Weissenfels, W. D., Beyer, M., and Klein, J. (1990), *Appl. Microbiol. Biotechnol.* **32,** 479–484.
16. Grifoll, M., Casellas, M., Bayona, J. M., and Solanas, A. M. (1992), *Appl. Environ. Microbiol.* **58,** 2910–2917.
17. Boldrin, B., Tiehm, A., and Fritzsche, C. (1993), *Appl. Environ. Microbiol.* **59,** 1927–1930.
18. Stucki, G. and Alexander, M. (1987), *Appl. Environ. Microbiol.* **53,** 292–297.
19. Keuth, S. and Rehm, H.-J. (1991), *Appl. Microbiol. Biotechnol.* **34,** 804–808.
20. Walter, U., Beyer, M., Klein, J., and Rehm, H.-J. (1991), *Appl. Microbiol. Biotechnol.* **34,** 671–676.
21. Wodzinski, R. S. and Coyle, J. E. (1974), *Appl. Microbiol.* **27,** 1081–1084.
22. Volkering, F., Breure, A. M., and van Andel, J. G. (1993), *Appl. Microbiol. Biotechnol.* **40,** 535–540.
23. Costerton, J. W. (1992), *Int. Biodeter. Biodegrad.* **30,** 123–133.
24. Aronstein, B. N., Calvillo, Y. M., and Alexander, M. (1991), *Environ. Sci. Technol.* **25,** 1728–1731.
25. Wyndham, R. C. and Costerton, J. W. (1981), *Appl. Environ. Microbiol.* **41,** 791–800.
26. Lewis, D. L. and Gattie, D. K. (1991), *Ecol. Modelling* **55,** 27–46.
27. Harder, W. and Dijkhuizen, L. (1982), *Phil. Trans. R. Soc. Lond. B.* **297,** 459–480.
28. Bauer, J. E. and Capone, D. G. (1988), *Appl. Environ. Microbiol.* **54,** 1649–1655.
29. Molina, M., Nummer, B., and Araujo, R. (1993), Abstracts of Am. Soc. Microbiol. 93rd Gen. Meeting, May 16–20, Atlanta, GA.
30. Heitkamp, M. A. and Cerniglia, C. E. (1989), *Appl. Environ. Microbiol.* **55,** 1968–1973.
31. Manilal, V. B. and Alexander, M. (1991), *Appl. Microbiol. Biotechnol.* **35,** 401–405.

32. Mueller, J. G., Lantz, S. E., Ross, D., Colvin, R. J., Middaugh, D. P., and Pritchard, P. H. (1993), *Environ. Sci. Technol.* **27,** 691–698.
33. Grosser, R. J., Warshawsky, D., and Vestal, J. R. (1991), *Appl. Environ. Microbiol.* **57,** 3462–3469.
34. Laha, S. and Luthy, R. G. (1991), *Environ. Sci. Technol.* **25,** 1920–1930.
35. Laha, S. and Luthy, R. G. (1992), *Biotechnol. Bioeng.* **40,** 1367–1380.
36. Guerin, W. F. and Jones, G. E. (1988), *Appl. Environ. Microbiol.* **54,** 937–944.
37. Marks, R. E., Field, S. D., Wojtanowicz, A. K., and Britenbeck, G. A. (1992), *Water Sci. Technol.* **25,** 213–220.
38. Weissenfels, W. D., Klewer, H.-J., and Langhoff, J. (1992), *Appl. Microbiol. Biotechnol.* **36,** 689–696.
39. Erickson, D. C., Loehr, R. C., and Neuhauser, E. F. (1993), *Water Res.* **27,** 911–919.
40. Mueller, J. G., Middaugh, D. P., Lantz, S. E., and Chapman, P. J. (1991), *Appl. Environ. Microbiol.* **57,** 1277–1285.
41. Madsen, E. L. (1991), *Environ. Sci. Technol.* **25,** 1663–1673.