Influence of Rhamnolipids and Triton X-100 on the Biodegradation of Three Pesticides in Aqueous Phase and Soil Slurries

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The effect of surfactants on the biodegradation of trifluralin and atrazine (by *Streptomyces* PS1/5) and coumaphos (by degrading consortia from a contaminated cattle dip) in liquid cultures and soil slurries was tested at different concentrations of a rhamnolipid mixture (Rh-mix) and Triton X-100 (TX-100). The extent of trifluralin biodegradation in liquid culture was improved at high concentrations of both surfactants. The extent of atrazine degradation dropped in the presence of either surfactant. Coumaphos biodegradation improved slightly at Rh-mix dosages >3000 μM; however, it was readily inhibited by TX-100 at amounts above the critical micelle concentration. In soil slurries, the extent of both trifluralin and atrazine biodegradation was higher in Hagerstown A (HTA) soil than in Hagerstown B (HTB) soil and was not significantly affected by the presence of either surfactant. The onset of trifluralin biodegradation was retarded at higher concentrations of surfactants. In the absence of surfactant, up to 98% of coumaphos in both soil slurries was transformed. At increasing dosages of Rh-mix, the onset of coumaphos biodegradation was retarded, but the removal efficiency of the pesticide increased. Rh-mix and TX-100 depletion was observed during *Streptomyces* PS1/5 growth in liquid cultures. Rh-mix concentration also decreased during coumaphos biodegradation, whereas TX-100 concentration was not affected. These results suggest that surfactants, added for the purpose of increasing the apparent water solubility of hydrophobic organic compounds, may have unintended effects on both the rate and extent of biodegradation of the target compounds if the surfactants can also be degraded by the microorganisms in the system.

Keywords: *Atrazine; trifluralin; coumaphos; biosurfactants; surfactants; biodegradation; Streptomyces; sorption; desorption; solubilization*

INTRODUCTION

It is widely accepted that the bioavailability of pollutants in the subsurface is limited to that portion of the compound dissolved in the aqueous phase (*1*). Numerous studies have investigated the enhanced solubilization of hydrophobic organic compounds (HOCs) in the presence of surfactants at concentrations above their critical micelle concentration (cmc) (*2*−*7*). Pesticides generally are known for their low aqueous solubility, high hydrophobicity, and tendency to stay sorbed in soil. Surfactants, at concentrations above their cmc, have been shown to enhance solubilization of hydrophobic compounds in soil and have been successfully used in soil washing or soil flushing for remediation of contaminated sites (*8*). Although the reported effects of surfactants on biodegradation enhancement of HOCs have been positive in some cases (*9*−*12*), in others either no effect or strong inhibition of microbial activity against the target pollutants has been observed (*3*, *4*, *10*, *13*, *14*). Due to their molecular structure, surfactant micelles can inhibit biodegradation of contaminants partitioned in the micellar phase because either (1) the hydrophilic micellar surface may form a barrier that can block the interaction of microorganisms with the pollutant partitioned in the micellar core or (2) the surfactant may irreversibly damage the cellular envelope (*10*). Even biosurfactants, essentially biodegradable and nontoxic relative to synthetic surfactants, can have specific compatibilities with the cell envelope structures of the microorganisms that produce them; hence, they might produce inhibitory effects on cell structures of other microorganisms (*10*, *13*). Although the results of some studies suggest that the bioavailability of contaminants is limited to that portion associated with surfactant monomers outside the micellar phase (*15*, *16*), others postulate that direct bioavailability of HOCs from the micellar core to the cell is possible, can be quantified, and depends on the surfactant type, the surface characteristics of the biomass, the surfactant concentration, and the mixing conditions (*7*, *17*, *18*).

Parallel or sequential biodegradation of nontoxic surfactants during enhanced bioremediation of aqueous wastes and soils can lead to either beneficial or inhibitory effects. Zhang et al. (*19*) showed that the addition of mineral nutrients and acclimated microbial cultures enhanced the biodegradation of both chlorinated hydrocarbons and Witconol surfactant. After applying sophorose lipids on a mixture of alkanes and aromatics, Oberbremer et al. (*20*) not only observed a 2-fold increase in the extent of hydrocarbon biodegradation but also found that the biosurfactants were biodegraded

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during the experiment. Goudar et al. (*21*) investigated the effect of two anionic surfactants, sodium dodecyl sulfate (SDS) and sodium dodecyl benzene sulfonate (SDBS), and two nonionic surfactants, POE (20) sorbitan monooleate (T-maz-80) and octyl-phenolpoly(ethyleneoxy)ethanol (CA-620), on the biodegradation of a benzene−toluene−*p*-xylene mixture (BTpX) in the aqueous phase by acclimated microorganisms. The authors found that SDS was preferentially utilized, inhibiting the hydrocarbon biodegradation, whereas the other three surfactants had no impact on BTpX biodegradation. In this case none of the four surfactants were toxic to the microbial cultures. Tiehm et al. (*8*) studied polycyclic aromatic hydrocarbon (PAH) degradation in soil in the presence of the nonionic surfactants Arkopal N-300 and Sapogenat T-300. Although the surfactants enhanced desorption of the contaminants from the soil, they also were readily degraded, lowering the dissolved oxygen concentration in the system and inhibiting PAH degradation. This suggests that readily biodegradable surfactants can reduce the effectiveness of subsurface remediation technologies because of their potential inhibitory effects on biodegradation of HOCs.

Relatively few soil-applied herbicides have been shown to be susceptible to mineralization by pure cultures of microorganisms. This is probably due to the fact that most herbicides contain a variety of structural groups requiring different catabolic enzyme systems not often found in a single organism. One way of getting complete degradation of pesticides in soils may be an initial enzymatic attack by nonspecific oxidases (such as the peroxidases produced by fungi and some actinomycetes) followed by further metabolism by hydrolases or ring cleavage enzymes, eventually resulting in products that are mineralized via catabolic pathways (*22*). Some actinomycete bacteria of the genus *Streptomyces* produce extracellular lignin peroxidases that can depolymerize/solubilize the lignin component of lignocellulose (*23*). This, plus several other characteristics such as an invasive mycelial growth habit (can penetrate soils, minimizing the need for mixing) and the ability to grow rapidly on environmentally benign substrates, makes *Streptomyces* an attractive organism for bioremediation of soils contaminated with pesticides. *Streptomyces* is a natural soil inhabitant that can survive and proliferate under unique conditions found in soil. Several studies have assessed the ability of *Streptomyces* strains to oxidize, dealkylate, and (or) dechlorinate pesticides and organochlorine compounds concomitant with growth on other carbon sources (*22*, *24*−*28*).

Coumaphos-degrading consortia have been isolated by Karns et al. (*29*) and Shelton and Somich (*30*) from coumaphos-contaminated soils and from cattle dipping vats in areas of the United States where coumaphos has been used intensively to prevent the reintroduction of ticks from cattle imported from Mexico. The authors proved that these organisms are capable of mineralizing coumaphos in dip suspensions or inoculated soil slurries, with the addition of adequate nutrients and maintenance of proper growing conditions.

The purpose of this research was to assess the effects of rhamnolipid and Triton X-100 surfactants on the degradation of trifluralin and atrazine by *Streptomyces* and on the biodegradation of coumaphos by a bacterial consortium.

Table 1. Composition and Characteristics of Selected Soils

	Hagerstown B (HTB)	Hagerstown A (HTA)	Beltsville A (BVA)
clay[a] (%)	59.5	27.0	16.0
sand[a] (%)	6.9	12.2	28.4
silt[a] (%)	33.6	60.8	55.6
f_{oc}[b]	0.0035	0.0311	0.0413
pH[b] (s)	5.39	6.58	3.78

[a] USDA-BARC. [b] This work.

MATERIALS AND METHODS

Microorganisms. *Streptomyces* strain PS1/5 was obtained from the Center for Agricultural Biotechnology at the University of Maryland. The microorganisms were cultivated at 30 °C with shaking in broth containing 5% w/w tryptone and 2% w/w dextrin. After 2 days, cells were plated on agar containing 5% tryptone and 2% dextrose. After 3 days, cells from agar plates were used to inoculate an aqueous solution containing dextrose (50 mM) and the minimal medium of Hylemon and Phibbs (*31*) as modified by Tomasek and Karns (*32*). After incubation for 2 days, the culture was mixed in a blender and used to inoculate liquid and soil slurries for biodegradation experiments with trifluralin and atrazine.

The source of coumaphos-degrading consortia was a contaminated cattle dip from vat 9 at El Salineño, TX. A volume of 50 mL of cattle dip was incubated at 30 °C for 8 days. The pH was monitored and adjusted periodically to a value of 7.5 until no further decrease was observed. The cattle dip culture was used to inoculate liquid and soil slurries for biodegradation experiments on coumaphos.

Materials. Rhamnolipid mixture (Rh-mix) produced by *Pseudomonas aeruginosa* UG2 has been previously identified as a mixture of $Rh_2C_{10}C_{10}$ dirhamnolipid (60% w/w) and $RhC_{10}C_{10}$ monorhamnolipid (21%), with the remaining 19% composed of a mixture of $Rh2C_{10}C_{12}$ dirhamnolipid and $Rh_2C_{10}C_{12}H_2$ "dehydro-dirhamnolipid". An average molecular weight of 624 and a cmc of 54 mg/L (86.5 μM) were obtained experimentally (*33*, *34*).

Triton X-100 (TX-100), a heterogeneous nonionic octylphenol ethoxylate surfactant, was obtained from Pierce Chemical Co., Rockford, IL (100 g/L solution, MW = 625). A cmc value of 125 mg/L (200 μM) was obtained experimentally (*35*). Trifluralin (98.7%) was obtained from Eli Lilly and Co. Indianapolis, IN, atrazine (94% w/w) from Ciba Geigy Corp., Greensboro, NC, and coumaphos (97.1%) from Bayvet Division of Cutter Laboratories Inc., Shawnee, KS. All of the chemicals were used as supplied.

Beltsville A soil (BVA) is a fine-loamy, mixed, semiactive, mesic Typic Fragiudults. Hagerstown A (HTA) and Hagerstown B (HTB) soils are fine, mixed, semiactive, mesic Typic Hapludalfs. All soils were obtained from the soil collection at the U.S. Department of Agriculture Beltsville Agricultural Research Center (USDA-BARC), Beltsville, MD. Their characteristics are presented in Table 1.

The fraction of organic carbon (f_{oc}) for each soil was verified by analyzing total organic carbon (TOC) in duplicate samples using a Shimadzu TOC-5000 analyzer. The detection limit for TOC was 0.05 ppm with a linear range up to 100 ppm.

Soil Preparation. Soils were ground and passed through a 1.0 mm stainless steel sieve to obtain a regular particle size. For the amendment of soils with pesticides, atrazine and trifluralin (1000 mg/L solutions in acetonitrile) and coumaphos (1000 mg/L solution in methanol) were prepared. Amounts of 500 g of each soil were spiked with 100 or 200 mL of pesticide solution. The mixtures were stirred vigorously to promote homogeneous distribution of the pesticide in the soil. The methanol and acetonitrile were evaporated by leaving the samples resting for 7 days at room temperature (~25 °C) inside a hood. The soils were rehydrated by the addition of deionized water (soil/water = 5:1 w/w) and allowed to dry at room temperature for 1 week. The resulting freshly amended soils with final concentrations of approximately 200 and 400 ppm

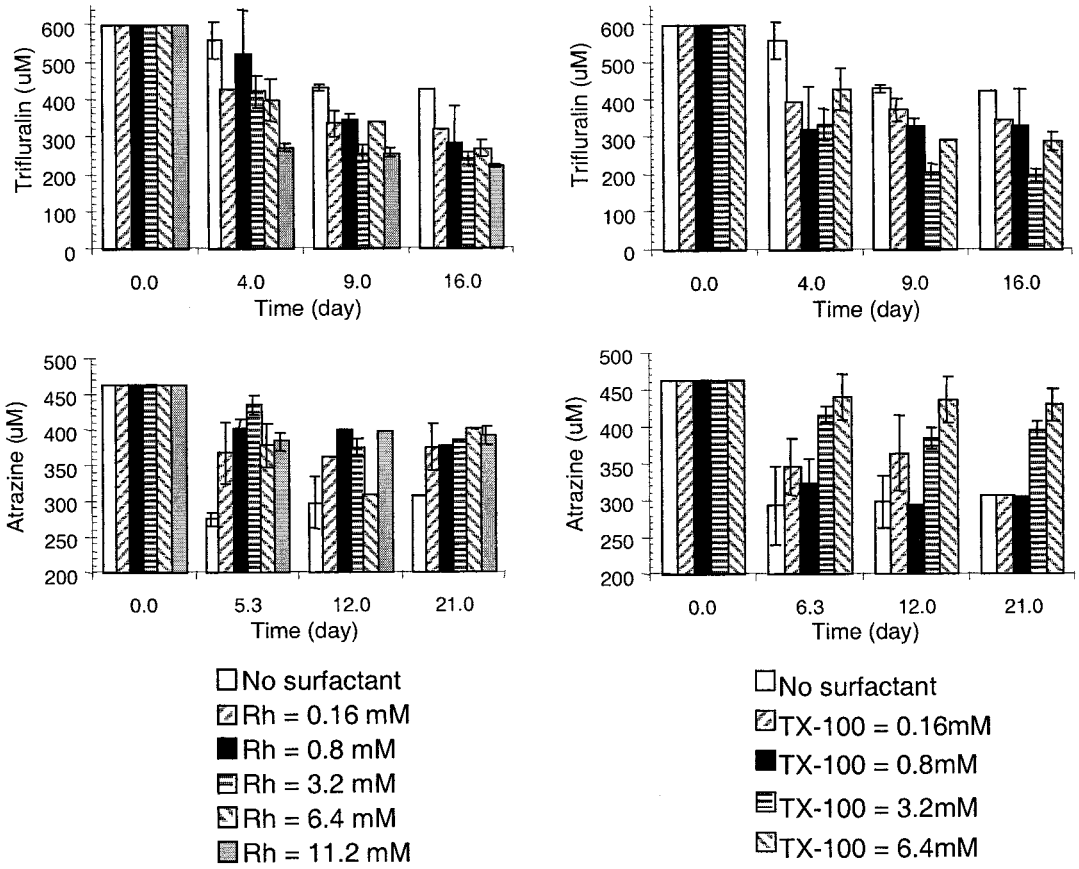

Figure 1. Effect of Rh-mix and TX-100 dosage on the biodegradation of trifluralin and atrazine (by *Streptomyces* PS1/5) in liquid media. Error bars represent standard deviation from triplicates.

of pesticide (0.6 and 1.2 mmol/kg soil for trifluralin, 0.93 and 1.9 mmol/kg for atrazine, and 0.55 and 1.1 mmol/kg soil for coumaphos) were used in desorption experiments.

Pesticide soil aging was simulated using soil drying and rewetting cycles as suggested by Shelton et al. (*36*). Soils, freshly amended with pesticide, were wetted with deionized water (soil/water = 5:1 w/w) and allowed to dry at room temperature for 1 week. The procedure was repeated five times before using the "aged" soils in desorption experiments.

Aqueous Incubations. Aqueous solutions containing minimal medium were amended with Rh-mix at concentrations of 0, 0.16, 0.8, 3.2, 6.4, and 11.2 mM or TX-100 at concentrations of 0.16, 0.8, 3.2, and 6.4 mM. The solutions were buffered with $NaHCO_3$ (15 mM) at pH 8. Dextrose at a concentration of 50 mM was added as carbon source to *Streptomyces* PS1/5 cultures used for trifluralin and atrazine biodegradation experiments. All solutions were autoclaved at 122 °C for 35 min.

Volumes of 150 mL of the sterilized solutions of medium containing surfactants were placed in 250 mL Erlenmeyer flasks. Trifluralin, atrazine, or coumaphos, previously ground into fine particles, was added to the flasks to obtain concentrations of 600, 465, and 1380 μM. Trifluralin and atrazine flasks were inoculated with 1 mL of *Streptomyces* PS1/5 blended broth, and coumaphos flasks were inoculated by adding 1 mL of cattle dip culture. Flasks containing each of the pesticides in liquid medium with 6.4 mM TX-100 or 11.2 mM Rh-mix were kept sterile as controls. Flasks were shaken at 30 °C, and triplicate samples were taken from time to time. Each sample was air-dried, redissolved in 1 mL of acetonitrile (trifluralin and atrazine samples) or methanol (coumaphos), centrifuged (3000*g*) for 10 min, and filtered (0.45 μm Millipore Millex HV, Millipore Products, Bedford, MA) prior to analysis.

Soil Slurry Incubations. Volumes of 60 mL of 0, 3.2, and 6.4 mM sterilized surfactant solutions prepared as described above were placed in 120 mL Erlenmeyer flasks. Amounts of 15 g of HTA soil freshly amended with pesticide were added to each flask. The operation was repeated for each pesticide. Similar soil slurries were prepared with HTB soils amended with the three pesticides and surfactant concentrations of 0, 6.4, and 11.2 mM. Soil slurries of aged pesticides in HTA soil were prepared identically. All of the flasks were inoculated with microbial cultures in the same way as the aqueous incubations. Triplicate samples were taken from time to time and placed in screw-cap tubes. The samples were air-dried, redissolved in 3 mL of acetontrile (trifluralin and atrazine) or methanol (coumaphos), and placed in a hot water bath at 85 °C for 1 h. Samples were vortexed for 1 min, centrifuged (3000*g*) for 10 min, and filtered (0.45 μm Millipore Millex HV) before analysis of pesticides or surfactants.

Analysis of Surfactants. Derivatization of Rh-mix and HPLC analysis of derivatives were performed using the method of Mata-Sandoval et al. (*33*). For the analysis of TX-100, a modification of the method for nonionic surfactant analysis as cobalt thiocyanate active substances (CTAS) was employed (*37*). Briefly, each sample was air-dried, followed by the addition of 1.5 mL of cobalt thiocyanate reagent [3 g of $Co(NO_3)_2$ (Aldrich Chemical Co., Inc., Milwaukee, WI) and 20 g of NH_4SCN (Aldrich Chemical Co.) in 100 mL of water] and 3 mL of CH_2Cl_2. The mixture was vortexed for 1 min and centrifuged (3000*g*) for 10 min. The organic phase containing the cobalt polyethoxylate complex was separated and its absorbance measured at 620 nm in a Beckman DU-7 spectrophotometer.

Analysis of Pesticides. Samples containing pesticides were analyzed in an HPLC instrument equipped with a Waters model 712 WISP autosampler, two Waters model 510 pumps, and a Waters model 996 photodiode array detector. For trifluralin analysis in rhamnolipid solutions an isocratic method with acetontrile/H_3PO_4 (0.01 N) (1.6:0.4 v/v) was employed at a flow rate of 2 mL/min through a Novapak C_{18} (8NVC184μ) column. Trifluralin analysis in TX-100 solutions required a 1.35:0.65 v/v acetontrile/H_3PO_4 mobile phase at the same flow rate through the same column. The absorbance of

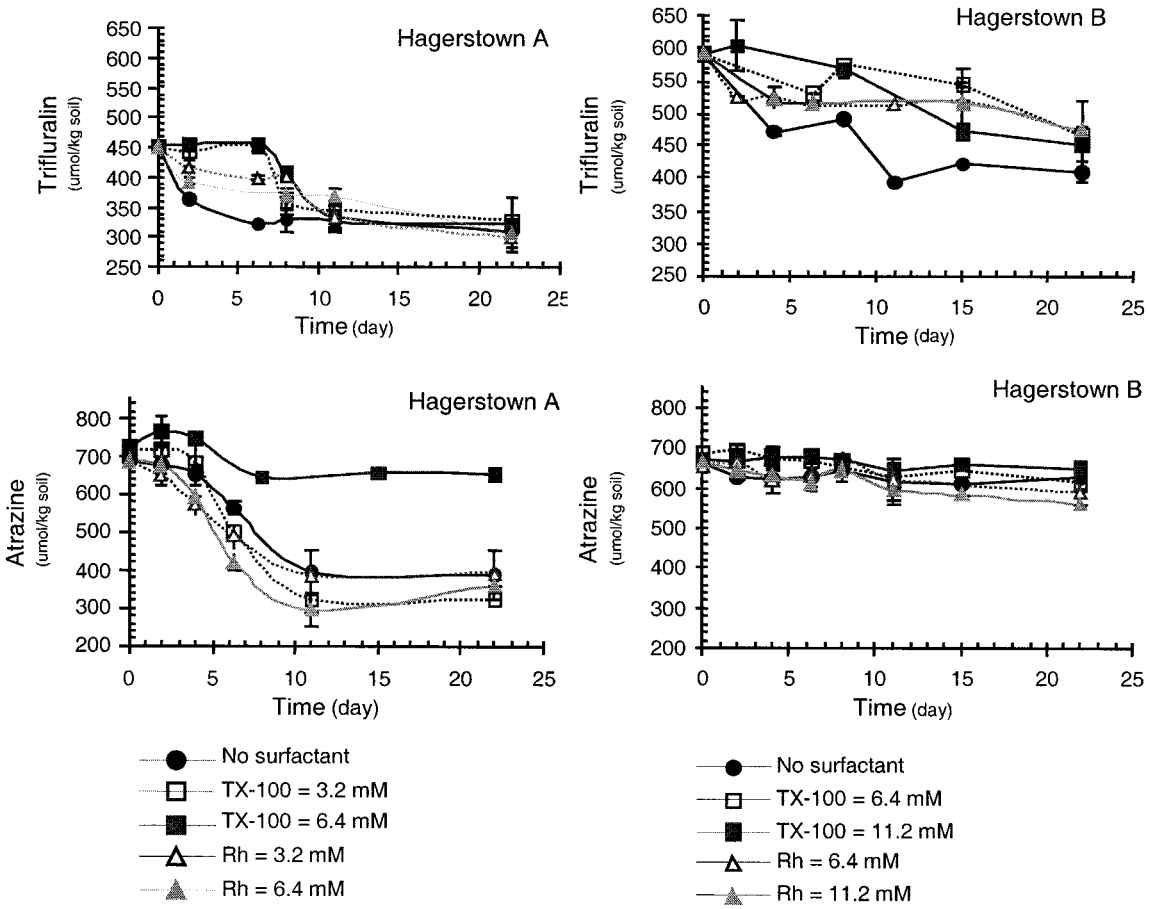

Figure 2. Effect of Rh-mix and TX-100 dosage on the biodegradation of trifluralin and atrazine (by *Streptomyces* PS1/5) in soil slurries. Error bars represent standard deviation from duplicates.

trifluralin was measured at 275 nm. Atrazine was quantified in both surfactant solutions with an isocratic method using acetonitrile/H_3PO_4 (0.01 N) (1:1 v/v). Atrazine's absorbance was measured at 228 nm. An isocratic method using methanol/H_3PO_4 (0.01 N) (1.5:0.5 v/v) was used for coumaphos analysis on rhamnolipid solutions, whereas the mobile phase was modified to 1.4:0.6 v/v methanol/H_3PO_4 (0.01 N) for coumaphos analysis in the presence of TX-100. Coumaphos absorbance was measured at 320 nm. The three pesticides were detected over a linear range at concentrations as low as 0.05 mg/L (equivalent to 0.15 μM for trifluralin, 0.23 μM for atrazine, and 0.138 μM for coumaphos).

Surface Tension. A CSC Scientific Co. model 70545 Du Nouy ring tensiometer was used to measure surface tension of aqueous incubations. The technique for measurements has been described previously (*33*).

RESULTS AND DISCUSSION

Both rhamnolipids and Triton X-100 had a positive effect on trifluralin degradation by *Streptomyces* PS1/5 in aqueous suspensions (Figure 1). Only 25% of total trifluralin was transformed in aqueous incubations in the absence of surfactants, whereas increasing concentrations of both surfactants improved the extent of pesticide biodegradation to 63−67%. In contrast, the surfactants generally had a negative effect on atrazine biodegradation, which was reduced from 34% in the absence of surfactants to only 5 and 13% at the maximum concentrations of 11.2 mM for Rh-mix and 6.4 mM for TX-100, respectively (Figure 1).

Results from Hagerstown A slurry incubations showed little difference between the amounts of trifluralin biodegraded in the absence of surfactant (30%) and in the presence of Rh-mix (33−38%) or TX-100 (26−28%) (Figure 2). However, a retardation in the onset of trifluralin biodegradation was observed in the presence of both surfactants during the first 6 days, being more noticeable for the cultures amended with TX-100 than those containing the biosurfactant. In Hagerstown B slurries, the amount of trifluralin biodegraded was reduced slightly from 30% in the absence of surfactant to ~24% in the presence of either surfactant. A slight retardation in the onset of trifluralin biodegradation was also observed in HTB slurries at high concentrations of TX-100. Up to 45% of the added atrazine was biodegraded in HTA slurries in the absence of surfactant. Rh-mix at low and high surfactant concentrations did not affect atrazine extent or onset of biodegradation in HTA slurries, whereas TX-100 at a dosage of 6.4 mM reduced the extent of degradation to 7%. In HTB slurries, only 7% of atrazine was transformed in the absence of surfactants. The presence of high-Rh-mix dosages improved atrazine degradation to 14%, whereas TX-100 at high dosages had no effect.

Coumaphos biodegradation in aqueous incubations was improved from 71% in the absence of surfactant to 81% at the maximum Rh-mix concentration of 11.2 mM (Figure 3A). In the presence of TX-100 at concentrations above its cmc (0.2 mM), the transformation of coumaphos was drastically reduced (Figure 3B). Simultaneously, a buildup of chlorferon, a byproduct resulting from the hydrolysis of coumaphos, was observed (Figure 4). No chlorferon was detected in samples to which Rh-mix had been added. In a separate experiment at TX-100 concentrations below its cmc (0.04 and 0.08 mM),

coumaphos biodegradation was neither enhanced nor inhibited (Figure 3C).

In the absence of surfactants, 98% of coumaphos was readily biodegraded in HTA and HTB soil slurries after the second day of incubation (coumaphos final concentration was ~15 μM), whereas at increasing dosages of Rh-mix the onset of its biodegradation was retarded, but eventually coumaphos levels dropped to final concentrations below the detection limit of 0.14 μM (Figure 5). TX-100 inhibited microbial action against the pesticide in both soil slurries.

The variations in TX-100 and Rh-mix concentrations during the biodegradation of atrazine and coumaphos in aqueous incubations are presented in Figure 6. Rapid depletion of Rh-mix to values below the detection limit (6 μM) was observed within the first 8 days of the experiments. TX-100 was rapidly transformed by *Steptomyces* PS1/5, but its concentration was not affected by the coumaphos-degrading consortia. Surface tension measurements were monitored throughout time for aqueous incubations of *Streptomyces* PS1/5 and coumaphos degraders in TX-100 (3.2 mM) and Rh-mix (6.4 mM). A stable surface tension of 33−34 mN/m was observed in all cases throughout the 21 days of the experiment (results not shown). This result suggests that Rh-mix and TX-100 were not completely mineralized but rather transformed into metabolites that still have surface-active properties.

Extents of biodegradation for aged and fresh pesticides in HTA soil were compared using Rh-mix and TX-100 at dosages of 6.4 and 3.2 mM, respectively. No significant difference (<4%) was observed in the extents of biodegradation of freshly added and aged pesticides at the surfactant dosages used here (results not shown). In previous desorption studies (*38*) similar water−soil partition coefficient values (K_{sol}*) were obtained for aged and fresh pesticides in HTA soil at different surfactant dosages. This indicated that the aging process did not affect the desorbing efficiency of surfactants, and the amount of solubilized pesticide available for microbial uptake is not being reduced by aging as done here. Thus, it is not surprising that the degrees of biodegradation were similar for aged and fresh pesticides.

Lignin peroxidases produced by *Streptomyces* are able to act only upon molecules that are dissolved in the aqueous phase. Results from previous studies (*35*) have shown that trifluralin's low aqueous solubility (8.98×10^{-4} mM) can be greatly enhanced through micellar solubilization in the presence of Rx-mix and TX-100. The improved degradation of trifluralin in aqueous incubations at increasing concentrations of surfactants suggests that by enhancing the solubilization of the pesticide, the access of peroxidases to trifluralin molecules partitioned into the micellar phase is favored, and this can improve the enzymatic reaction. Atrazine has a higher aqueous solubility of 0.176 mM, but the solubilization enhancement for this pesticide proved to be rather poor in the presence of either Rh-mix or TX-100 (*35*). Because *Streptomyces* enzymatic attack is relatively nonspecific, we assume that surfactant molecules present in the aqueous incubations are also susceptible to oxidation reactions catalyzed by peroxidases. They do not have a significant effect on the solubilization of atrazine, so surfactant molecules might act as scavengers of H_2O_2 and peroxidases and reduce the extent of pesticide transformation. This scenario agrees with our experimental results, because simultaneous inhibition

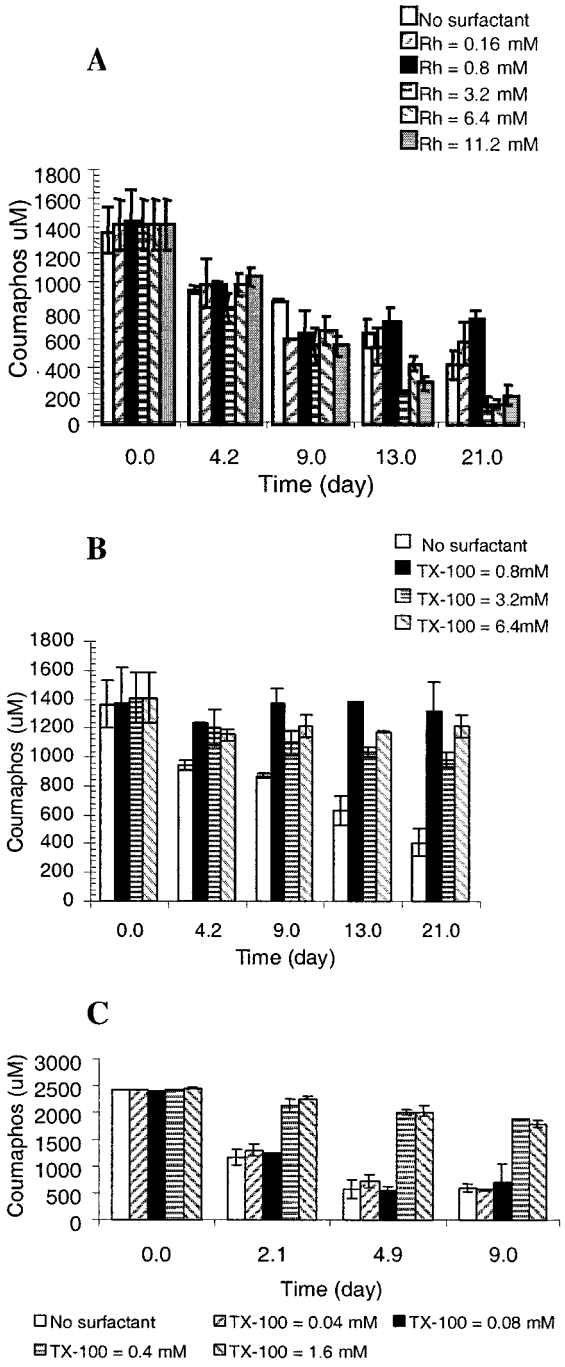

Figure 3. Effect of Rh-mix and TX-100 dosage on the biodegradation of coumaphos (by indigenous degrading consortia from cow dips) in two different liquid medium experiments (first experiment, A and B; second experiment, C). Error bars represent standard deviation from triplicates.

of atrazine degradation, together with the transformation of both surfactants, was observed during aqueous incubations.

Adams et al. (*39*) reported the effects of H_2O_2/O_3 advanced oxidation processes (AOP) on the treatment of alkyl phenol ethoxylate surfactants (APE). Their results showed that oxidation of APE molecules followed two preferential pathways: (1) shortening of the ethoxylate hydrophilic chain and (2) hydroxylation and cleavage of the aromatic ring. Because the hydrophobic alkyl chain is facing the micellar core, it may be protected from the action of hydroxyl radicals, which in turn will preferentially attack the ethoxylate group located in the

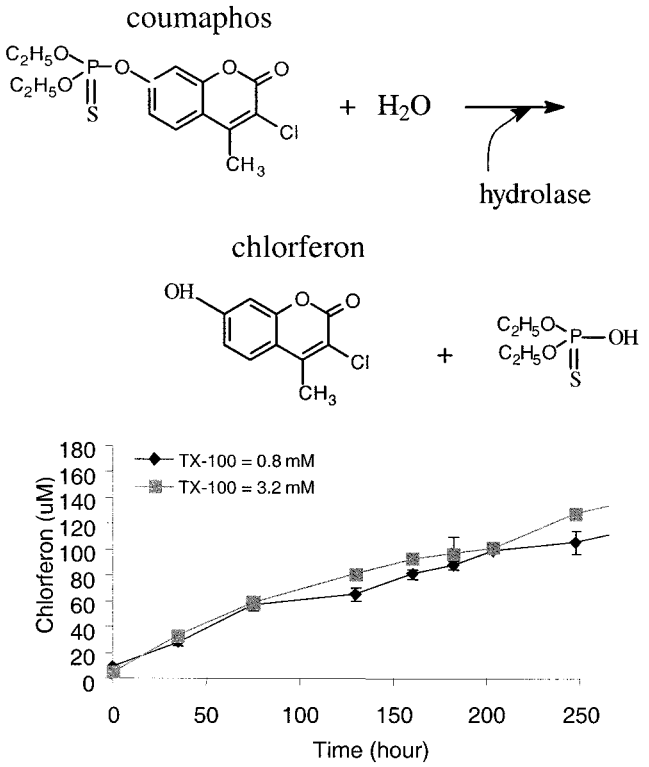

Figure 4. Chlorferon accumulation during degradation of coumaphos in aqueous incubations at different TX-100 dosages. Error bars represent standard deviation from triplicates.

micellar surface or the aromatic ring next to it. Although we did not identify any TX-100 metabolites produced during *Streptomyces* growth, we believe that this surfactant might go through transformations similar to those reported for other APEs during AOP.

Our experimental results showed that adding surfactants to HTA and HTB soil slurries did not improve significantly the extent of trifluralin and atrazine degradation, and in some cases even reduced it. Previous studies have shown that Rh-mix and TX-100 can sorb to soils, increasing their hydrophobicity, and enhance the sorption of pesticides. In this case solubilization of the pesticides may be promoted only at very high surfactant concentrations (*38*). This could cause a reduction in the amount of pesticide available in the aqueous phase for enzymatic attack by *Streptomyces*.

Solubilization studies have shown that the low aqueous solubility (5.78×10^{-4} mM) of coumaphos is modestly enhanced by Rh-mix and TX-100, due to its tendency to partition onto the surfactant micellar surface rather than in the core (*35*, *40*). The moderate enhancement in the biodegradation of coumaphos by Rh-mix in aqueous incubations suggests that besides mildly increasing the pesticide apparent solubility, the biosurfactant is nontoxic to the microorganisms and either permits their access to the pesticide partitioned in the micellar phase or facilitates the transport of coumaphos out of the micelles for microbial uptake in the bulk water. Moreover, a depletion of rhamnolipid species occurred simultaneously with the depletion of coumaphos, confirming that not only is Rh-mix microbially compatible but it is also readily metabolized by the consortia. Retardation in the onset of coumaphos degradation at increasing concentrations of Rh-mix in HTA and HTB soil slurries indicates that microorganisms might be preferentially metabolizing the biosurfactant molecules, possibly due to a combination of low levels of coumaphos desorbed from soil together with much higher concentrations of Rh-mix in the aqueous phase.

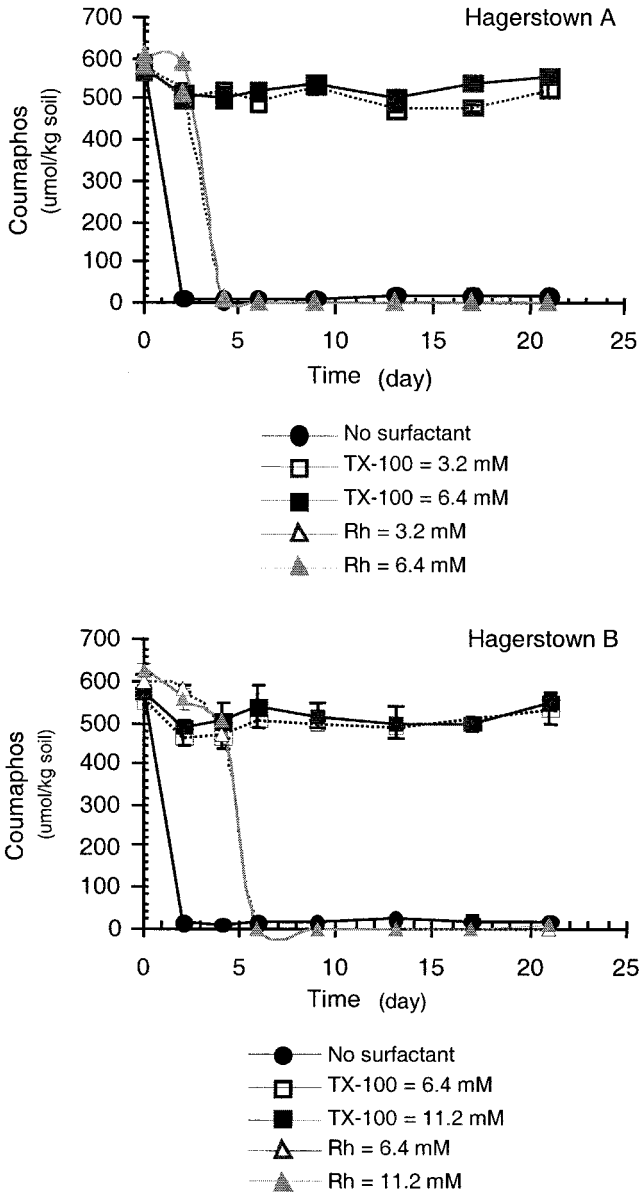

Figure 5. Effect of Rh-mix and TX-100 dosage on the biodegradation of coumaphos (by indigenous degrading consortia from cow dips) in soil slurries. Error bars represent standard deviation from duplicates.

The concentration of each rhamnolipid species was followed during the biotransformation of Rh-mix by *Streptomyces* PS1/5 and coumaphos degraders (results not shown). The results suggest that $C_{10}C_{10}$ species seemed to be preferentially degraded at the initial stages of the experiment, whereas the $C_{10}C_{12}$ species were more resistant. This behavior agrees with results observed after the production of Rh-mix by *P. aeruginosa* UG2 in aged cultures (*33*, *38*). The final composition of $C_{10}C_{12}$ species was higher in aged cultures than in normal cultures because once the carbon source was exhausted, the microorganisms seemed to preferentially degrade the $C_{10}C_{10}$ species.

The drastic inhibition of coumaphos mineralization in aqueous and slurry incubations at TX-100 dosages above the cmc of 0.2 mmol/L contrasts with results that

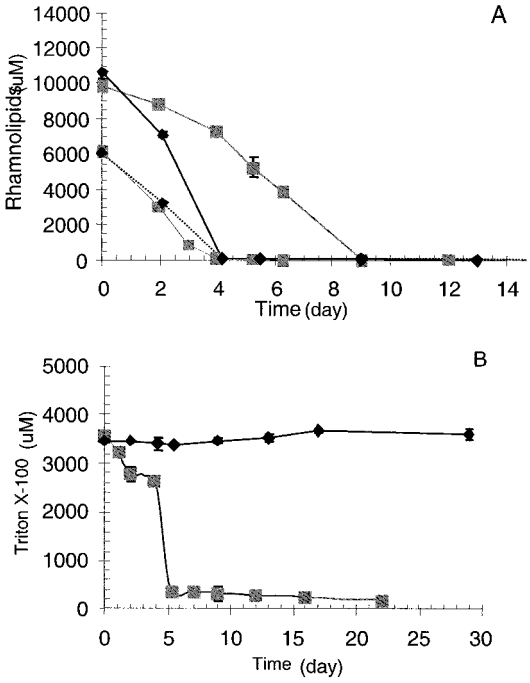

Figure 6. Accidental degradation of total rhamnolipids (A) and TX-100 (B) by *Streptomyces* PSI/5 (■) and coumaphos-degrading consortia (▲) in liquid media during the biodegradation experiments of atrazine and coumaphos. Error bars represent standard deviation from duplicates.

showed no inhibition at TX-100 dosages below the cmc. Preferential metabolism of TX-100 surfactant must be ruled out as an explanation for the inhibitory process, because experimental results showed that the consortium was unable to degrade it. Possible toxicity due to TX-100 levels is not a convincing argument because microorganisms passed drastically from activity to inhibition at a low surfactant concentration that corresponded to its cmc. We hypothesize that inhibition might be caused by interference of the micelles' structure with the transport of the substrate into the cell or to physical−chemical interference with the activity of enzymes involved in coumaphos degradation. Another explanation is that chlorferon accumulation in the system might have reached levels that are toxic for the microorganisms. Enzymatically catalyzed hydrolysis of coumaphos is one of the possible initial steps in the pathway of the pesticide degradation, and TX-100 micellar structure might be either improving this step or inhibiting the subsequent degradation of chlorferon, as no accumulation of this metabolite was observed either in incubations at TX-100 concentrations below the cmc or in those in which Rh-mix micelles were present.

CONCLUSIONS

This study demonstrates that the use of (bio)surfactants to increase solubilization of HOCs does not guarantee enhanced biodegradation. Biosurfactants such as the rhamnolipids produced by *P. aeruginosa* UG2 might increase the bioavailability of contaminants due to their microbial compatibility, yet depending on the applied concentrations, they might be used preferentially as substrate and inhibit the degradation of the target compound. On the other hand, synthetic surfactants such as TX-100 create micellar structures that might interfere with the activity of specific enzymes involved in the pathways of pollutant biodegradation. The attack of HOCs with nonspecific enzymes produced by actinomycetes such as *Streptomyces* PS1/5 might be improved by introducing surfactants to enhance the low solubility of the contaminants, but at high concentrations surfactants might act as enzyme scavengers and reverse the original effect.

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