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Effect of a *Pseudomonas* Rhamnolipid Biosurfactant on Cell Hydrophobicity and Biodegradation of Octadecane

YIMIN ZHANG AND RAINA M. MILLER*

Department of Soil and Water Science, University of Arizona, Tucson, Arizona 85721

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In this study, the effect of a purified rhamnolipid biosurfactant on the hydrophobicity of octadecane-degrading cells was investigated to determine whether differences in rates of octadecane biodegradation resulting from the addition of rhamnolipid to four strains of *Pseudomonas aeruginosa* could be related to measured differences in hydrophobicity. Cell hydrophobicity was determined by a modified bacterial adherence to hydrocarbon (BATH) assay. Bacterial adherence to hydrocarbon quantitates the preference of cell surfaces for the aqueous phase or the aqueous-hexadecane interface in a two-phase system of water and hexadecane. On the basis of octadecane biodegradation in the absence of rhamnolipid, the four bacterial strains were divided into two groups: the fast degraders (ATCC 15442 and ATCC 27853), which had high cell hydrophobicities (74 and 55% adherence to hexadecane, respectively), and the slow degraders (ATCC 9027 and NRRL 3198), which had low cell hydrophobicities (27 and 40%, respectively). Although in all cases rhamnolipid increased the aqueous dispersion of octadecane at least 10^4-fold, at low rhamnolipid concentrations (0.6 mM), biodegradation by all four strains was initially inhibited for at least 100 h relative to controls. At high rhamnolipid concentrations (6 mM), biodegradation by the fast degraders was slightly inhibited relative to controls, but the biodegradation by the slow degraders was enhanced relative to controls. Measurement of cell hydrophobicity showed that rhamnolipids increased the cell hydrophobicity of the slow degraders but had no effect on the cell hydrophobicity of the fast degraders. The rate at which the cells became hydrophobic was found to depend on the rhamnolipid concentration and was directly related to the rate of octadecane biodegradation. These results suggest that the bioavailability of octadecane in the presence of rhamnolipid is controlled by both aqueous dispersion of octadecane and cell hydrophobicity.

Biodegradation of organic compounds with limited water solubility is slow because of the low availability of these compounds to microbial cells. As shown in previous work, the availability of slightly soluble organic compounds can be enhanced by microbially produced surfactants (biosurfactants), which can increase aqueous dispersion by many orders of magnitude (19). In many instances, biosurfactants also stimulate the biodegradation of organic compounds. For example, alkane degradation is stimulated by rhamnolipids (19), sophorose lipids (5, 10), and phospholipids (7). However, biosurfactants can also inhibit biodegradation. Inhibition can be genus specific or substrate specific. For example, sophorose lipids were found to stimulate hydrocarbon degradation by a *Torulopsis* sp. but to inhibit degradation by other yeast genera (5). Substrate-specific inhibition was demonstrated by Falatko and Novak (3), who showed that biosurfactants produced from growth on glucose or vegetable oil inhibited biodegradation of gasoline hydrocarbons, while biosurfactants produced from growth on gasoline did not inhibit degradation.

The reason for variable biosurfactant enhancement of biodegradation is not yet known. Clearly, though, biodegradation requires uptake of the substrate by the cell, which in turn requires contact between the substrate and the cell. Contact is determined by two factors: (i) available substrate surface area and (ii) affinity of microbial cells for the substrate. Biosurfactants increase dispersion or surface area for microbial attachment, which should increase biodegradation. However, there is evidence that biosurfactants may also interfere with the interaction between biosurfactant-dispersed substrates and microbial cells (4, 13).

The objective of this research was to investigate the effect of different rhamnolipid concentrations on cell hydrophobicity and the resultant impact on biodegradation of octadecane. It has been suggested previously that the hydrophobicity of the cell surface is an important factor in predicting adhesion to surfaces (17). Thus, cell hydrophobicity was used as a measure of potential cell affinity for hydrophobic substrates and was determined by the bacterial adherence to hydrocarbon (BATH) assay. In this study, four *Pseudomonas aeruginosa* strains which differed in octadecane biodegradation rates were used to determine the effect of rhamnolipid on cell hydrophobicity and octadecane biodegradation.

MATERIALS AND METHODS

Microorganisms. *P. aeruginosa* ATCC 9027, ATCC 15442, ATCC 27853, and NRRL 3198 were obtained from the American Type Culture Collection (Rockville, Md.) or from the University of Arizona Undergraduate Program in Microbiology Culture Collection. The cultures were stored at 4°C on nutrient agar plates or *Pseudomonas* agar P plates (Difco, Detroit, Mich.) and transferred monthly. All four strains produced rhamnolipids during growth in phosphate-limited proteose peptone-glucose-ammonium salts (PPGAS) medium (2). None of these strains produced rhamnolipid during growth in mineral salts medium (19) containing substrates used in this study, e.g., octadecane and glucose. Also, none of these strains can utilize rhamnolipid as a sole carbon source.

Rhamnolipid purification. *P. aeruginosa* ATCC 9027 rhamnolipid was produced and partially purified as described by Zhang and Miller (19). The partially purified yellow rhamno-

* Corresponding author. Phone: (602) 621-7231. Fax: (602) 621-1647.

lipid residue was then dissolved in 1 ml of chloroform and applied to a Silica Gel 60 (Aldrich, Milwaukee, Wis.) chromatography column (17 by 1.5 cm). The column was eluted with chloroform to remove the yellow pigment associated with the partially purified rhamnolipid and was then eluted with chloroform-methanol (10:1). The eluant fractions contained one anthrone-positive component, which was further analyzed by fast atom bombardment (FAB) mass spectrometry. Purified rhamnolipid was quantified by determinations of weights and amounts of rhamnose.

FAB mass spectra were obtained by using mass analyzer 1 of a custom-built four-sector instrument (AMD Intdectra, Harpstedt, Germany) of BEBE (B, magnetic; E, electric fields) configuration equipped with two KWS MC 68000 computer systems for instrument control and data acquisition. The ion source consisted of a 20-kV Cs-ion gun operating at a thermoionic emission current of 2 A at 12 kV. The samples were dissolved in high-performance liquid chromatography grade methanol, and an equal volume of thioglycerol was added. Four microliters of the resulting solution was placed on the target of the inlet probe held at 10°C with chilled methanol-water (1:1 [vol/vol]) at a source temperature of 34°C. The mass range 100 to 2,000 Da was scanned at 60-s/decade at a resolution of 2,000 (10% valley definition). The mass spectra are reported as the average of 10 accumulated scans.

[^{14}C]rhamnolipid. Some experiments in this study required the use of [^{14}C]rhamnolipid. This was synthesized by adding D-[U-^{14}C]glucose (specific activity, 251 mCi/mmol), which was obtained from Sigma (St. Louis, Mo.), into the PPGAS growth medium. The final concentration of glucose in the medium was 7.5 g/liter and was a mixture of unlabeled and ^{14}C-labeled glucose with a specific activity of 0.43 μCi/mmol. [^{14}C]rhamnolipid was purified and quantified as described above. The specific activity of the [^{14}C]rhamnolipid was 1.1 μCi/mmol, which indicated a 2.9% yield of [^{14}C]rhamnolipid from [^{14}C]glucose on the basis of recovery of radioactivity.

Biodegradation tests. Octadecane biodegradation was measured either by protein determination or by detection of $^{14}CO_2$ evolved during growth on [^{14}C]octadecane (19). For protein determination experiments, octadecane dissolved in chloroform was coated onto the bottom of a 250-ml flask. The solvent was then evaporated. Mineral salts medium (50 ml) containing rhamnolipid (0, 0.6, or 6 mM) was added, and the flask was placed briefly (30 s) into a 37°C water bath to melt the coated octadecane. The flask was then maintained at room temperature until the octadecane resolidified on the surface of the medium. The final octadecane concentration was 3.9 mM. Each flask was then inoculated with a 5% *P. aeruginosa* inoculum which had been grown in Kay's minimal medium (18) at 37°C for 24 h. The flasks were incubated at 23°C in a gyratory shaker (200 rpm). For protein measurements, 1-ml samples were removed periodically and heated for 1 min with 0.1 ml of 1 N NaOH (to lyse cells), and the protein content was determined (8).

Mineralization experiments were performed with [1-^{14}C] octadecane purchased from Sigma (specific activity, 3.6 mCi/mmol; 98% purity). A mixture of octadecane and [^{14}C]octadecane from Sigma was used to coat the bottoms of modified 125-ml micro-Fernbach flasks (Wheaton, Milville, N.J.), which were designed for the collection of $^{14}CO_2$ and ^{14}C-labeled volatile compounds. The solvent was evaporated, and 20 ml of mineral salts medium containing rhamnolipid (0, 1.2, or 7.6 mM) was added to each flask. The final concentration of octadecane was 1.6 mM, and the octadecane specific activity was 1.4 μCi/mmol. The octadecane was melted and the flasks were inoculated and incubated as described above. The micro-Fernbach flasks were periodically flushed through a series of six traps to collect $^{14}CO_2$ and ^{14}C-labeled volatile organic compounds. Radioactivity was determined with a Packard (Meriden, Conn.) Tri-Carb liquid scintillation counter (model 1600 TR).

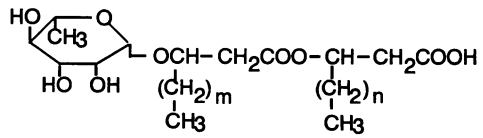

FIG. 1. *P. aeruginosa* ATCC 9027 monorhamnolipid structure. For C_{18} monorhamnolipid, m + n = 10; for C_{20}, m + n = 12; for C_{22}, m + n = 14; and for C_{24}, m + n = 16.

BATH assay. The relative hydrophobicities of bacterial cells were measured by a BATH assay (14). Bacterial cells to be assayed were prepared by the following procedure. The cells were first washed twice to remove any interfering solutes, particularly rhamnolipid that was added in some of the experiments. The cells were then resuspended in a buffer salts solution (pH 7.0) containing 16.9 g of K_2HPO_4, 7.3 g of KH_2PO_4, 1.8 g of urea, and 0.2 g of $MgSO_4 \cdot 7H_2O$ per liter to give an optical density (OD) at 400 nm of 1.0. Cells (4.0 ml) and hexadecane (1.0 ml) were mixed in a screw-top test tube (1.5 by 8 cm), and the test tube was vortexed for 60 s. After vortexing, the hexadecane and aqueous phases were allowed to separate for 30 min. The aqueous phase was then carefully removed with a Pasteur pipette, and the turbidity of the aqueous phase at 400 nm was measured. Hydrophobicity is expressed as the percentage of adherence to hexadecane, which is calculated as follows: 100 × (1 − OD of the aqueous phase/OD of the cell suspension).

The effect of rhamnolipid on the cell adherence to hexadecane was also determined. For these experiments, cells were harvested, washed twice, and resuspended in buffer salts solution as described above. Then, various concentrations of rhamnolipid (0 to 0.008 mM) were added to the cell solutions, and the BATH assay was performed.

Measurement of cell association with rhamnolipid. Bacterial cells grown in Kay's minimal medium at 37°C for 24 h were washed twice with mineral salts medium. Washed cells were added to 10 ml of mineral salts medium (OD at 400 nm of 2.0) containing [^{14}C]rhamnolipid (6 mM; specific activity, 0.75 nCi/mmol). This solution was incubated with and without octadecane (3.9 mM) in 125-ml flasks at 23°C with 200 rpm of gyratory shaking. Periodically, 1-ml samples were withdrawn and centrifuged. The cell pellet was washed twice, and radioactivity was determined.

RESULTS

Rhamnolipid purification and structure. The purified rhamnolipid was analyzed by thin-layer chromatography using a chloroform-methanol-water (65:25:4) solvent system. One anthrone-positive spot with an R_f value of 0.70, which corresponds to a monorhamnolipid, was identified (11). The sample was further analyzed by FAB mass spectroscopy. Mass spectra of the purified rhamnolipid were compared with known C_{18}-, C_{20}-, and C_{22}-purified monorhamnolipid samples generously provided by Graham W. Taylor (12). An intense signal molecular ion was found at *m/z* 543 (I_{rel} = 100.00%) which corresponds to $(M + K)^+$, where M is the C_{20} monorhamnolipid (Fig. 1). A series of signals at *m/z* 515 (I_{rel} = 4.18%), 543 (I_{rel} = 100.00%), 571 (I_{rel} = 31.88%), and 599 (I_{rel} = 2.87%)

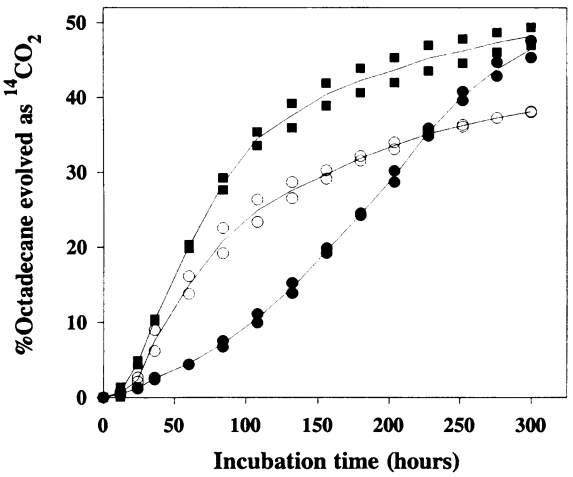

FIG. 2. Effect of rhamnolipid concentration on the mineralization of octadecane by *P. aeruginosa* ATCC 9027. A mixture of [^{14}C]octadecane (1.6 mM) and rhamnolipid was incubated in mineral salts medium with *P. aeruginosa* with gyratory shaking (200 rpm) at 23°C. Mineralization was measured as described in Materials and Methods. ○, no rhamnolipid; ●, 1.2 mM rhamnolipid; ■, 7.6 mM rhamnolipid. This experiment was done in duplicate, and each point represents a duplicate sample.

indicated a mixture of four monorhamnolipids, C_{18}, C_{20}, C_{22}, and C_{24}, although the signals for the C_{18} and C_{24} monorhamnolipids were very weak.

An average molecular weight of 504, which represents the C_{20} monorhamnolipid, was used in this study to calculate rhamnolipid concentrations when concentrations were determined by weight. We found that calculated rhamnolipid concentration values determined by weight were within 6% of the concentration values calculated by the rhamnose assay described in our previous work (19).

Effect of rhamnolipid concentration on biodegradation rates. As shown in Fig. 2, in the absence of rhamnolipid, cumulative CO_2 production showed a short lag phase and then rapid production of CO_2, which then tapered off after 100 h. In the presence of high rhamnolipid concentrations (7.6 mM), mineralization was enhanced relative to that of the control. Interestingly, low rhamnolipid concentrations initially inhibited the rate of mineralization relative to that of the control; however, the presence of either high or low rhamnolipid concentrations resulted in a higher cumulative amount of CO_2 produced after 300 h than the amount produced in the absence of rhamnolipid.

A comparison of cumulative protein productions by four different rhamnolipid-producing strains of *P. aeruginosa* during growth on octadecane is shown in Fig. 3. The rate of protein production (see slope of each line), a measure of octadecane biodegradation, varied among the four bacterial strains. In the absence of rhamnolipid, ATCC 15442 and ATCC 27853 were found to degrade octadecane rapidly (fast degraders), while ATCC 9027 and NRRL 3198 degraded octadecane more slowly (slow degraders). The addition of a high rhamnolipid concentration (6 mM) stimulated the rate of biodegradation for ATCC 9027 and NRRL 3198, the slow degraders. The same addition of rhamnolipid to ATCC 15442 and ATCC 27853, the fast degraders, did not seem to affect biodegradation. Interestingly, the addition of a lower concentration of rhamnolipid (0.6 mM) inhibited octadecane biodegradation by

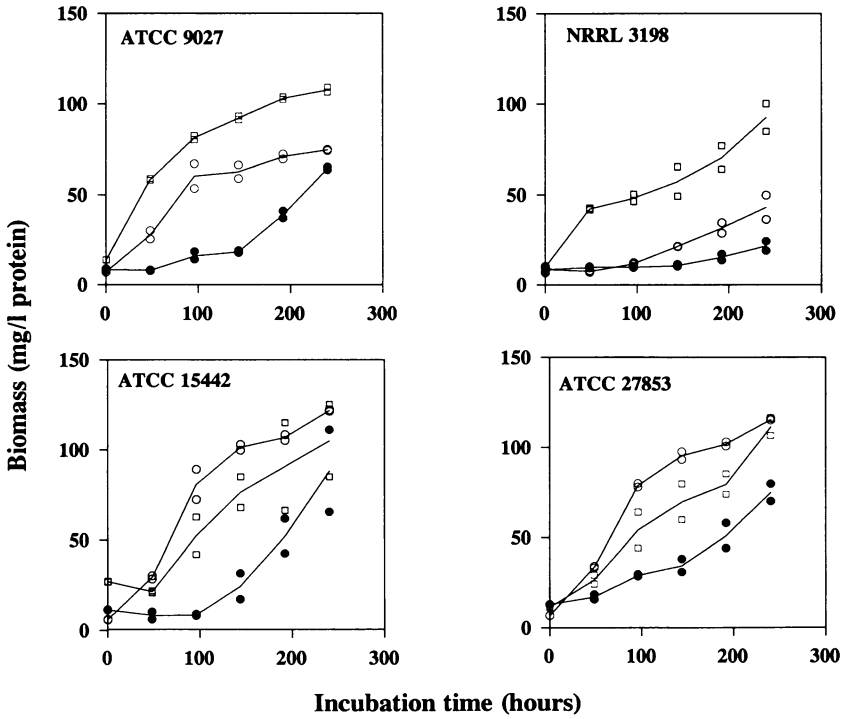

FIG. 3. Effect of rhamnolipid on octadecane biodegradation by four *P. aeruginosa* strains. A mixture of octadecane (3.9 mM) and rhamnolipid in mineral salts medium was inoculated with *P. aeruginosa* and the resulting mixture was incubated with gyratory shaking (200 rpm) at 23°C. Biomass was measured as described in Materials and Methods. ○, no rhamnolipid; ●, 0.6 mM rhamnolipid; □, 6 mM rhamnolipid.

TABLE 1. Hydrophobicity of *P. aeruginosa* cells (% adhered) grown on octadecane in the presence or absence of rhamnolipid

P. aeruginosa strain	% Adhered to hexadecane (mean ± SD)	
	Octadecane[a]	Octadecane + rhamnolipid[b]
ATCC 9027	27 ± 3	79 ± 6
NRRL 3198	40 ± 3	77 ± 6
ATCC 15442	74 ± 2	71 ± 5
ATCC 27853	55 ± 4	51 ± 4

[a] Bacteria were grown in mineral salts medium with octadecane at 23°C for 6 days, washed, and assayed.
[b] Bacteria were grown in mineral salts medium with octadecane and 6 mM rhamnolipid at 23°C for 6 days, washed to remove rhamnolipid, and assayed.

TABLE 2. Effect of carbon source on cell hydrophobicity of slow degraders

Carbon source	% Adhered to hexadecane (mean ± SD)			
	ATCC 9027		NRRL 3198	
	− Rhamnolipid	+ Rhamnolipid (6 mM)	− Rhamnolipid	+ Rhamnolipid (6 mM)
Hexadecane[a]	24 ± 3	83 ± 2	40 ± 7	53 ± 2
Octadecane[a]	27 ± 3	79 ± 6	40 ± 3	77 ± 6
Acetate[b]	36 ± 7	30 ± 9	8 ± 2	29 ± 1
Citrate[b]	19 ± 3	24 ± 6	11 ± 2	23 ± 8
Glucose[b]	20 ± 5	23 ± 4	61 ± 2	35 ± 10
Succinate[b]	22 ± 5	25 ± 7	49 ± 2	28 ± 1

[a] Bacteria were grown in mineral salts medium at 23°C for 6 days, washed, and assayed.
[b] Bacteria were grown in mineral salts medium at 23°C for 1 day, washed, and assayed.

all four strains for at least 100 h. After this initial inhibition, the biodegradation rates for ATCC 9027 (a slow degrader) and ATCC 15442 (a fast degrader) increased until they were comparable with controls.

Effect of rhamnolipid on cell hydrophobicity. Table 1 compares the effects of rhamnolipid on the cell hydrophobicities of the four *Pseudomonas* strains when they were grown on octadecane in the presence or absence of rhamnolipid. When they were grown on octadecane alone, the fast degraders, ATCC 15442 and 27853, had higher cell hydrophobicities (75 and 55%, respectively) than the slow degraders, ATCC 9027 and NRRL 3198 (27 and 40%, respectively). When grown on octadecane in the presence of rhamnolipid, the hydrophobicities of the slow degraders increased dramatically, while the hydrophobicities of the fast degraders remained unchanged. Additional experiments were carried out to investigate the effect of different carbon sources on the cell hydrophobicities of slow degraders. These carbon sources ranged from very water soluble (acetate, citrate, glucose, and succinate) to slightly water soluble (hexadecane [0.0063 mg/liter] and octadecane [0.006 mg/liter] [16]). As shown in Table 2, rhamnolipid increased the cell hydrophobicities of both ATCC 9027 and NRRL 3198 only when they were incubated in the presence of the slightly soluble carbon sources, e.g., hexadecane and octadecane.

Effect of cell hydrophobicity on biodegradation rates. Figure 4 compares the change in cell hydrophobicity (Fig. 4B) with cumulative protein production (Fig. 4A) during growth of *P. aeruginosa* ATCC 9027 on octadecane. As shown in Fig. 4B, the development of hydrophobicity in ATCC 9027 cells was dependent on rhamnolipid concentration. In the absence of rhamnolipid, there was a slight increase in cell hydrophobicity during growth to a final value of approximately 20% at 300 h. In contrast, the high rhamnolipid concentration (7.6 mM) stimulated an increase in hydrophobicity of ATCC 9027 cells to 80% in 100 h. At the lower rhamnolipid concentration (1.2 mM), there was a 100-h lag period before development of cell hydrophobicity started. After this lag period, the rate of increase in hydrophobicity was similar to the rate of increase at high rhamnolipid concentrations that was seen from 0 to 100 h. Development of high hydrophobicity appears to correlate with the rate of biodegradation (Fig. 4A). At the high rhamnolipid concentration, hydrophobicity increased immediately, and the initial rate of biodegradation was high. At the low rhamnolipid concentration, both development of hydrophobicity and octadecane biodegradation were inhibited for the first 100 h. After

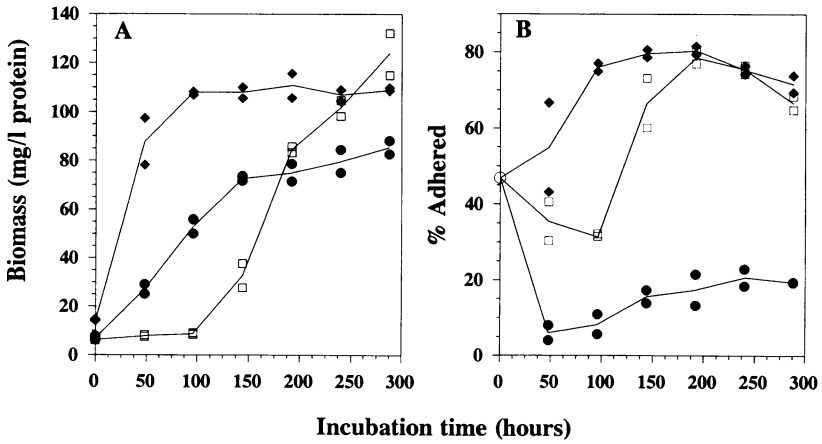

FIG. 4. Effect of rhamnolipid on the development of cell hydrophobicity and biodegradation of octadecane by *P. aeruginosa* ATCC 9027. A mixture of octadecane (3.9 mM) and rhamnolipid in mineral salts medium was inoculated with *P. aeruginosa* ATCC 9027, and the resulting mixture was incubated with gyratory shaking (200 rpm) at 23°C. (A) Amounts of protein were determined as a measure of biodegradation; (B) cells were assayed by the BATH test to determine hydrophobicity as described in Materials and Methods. ○, time point zero measured with cells precultured in Kay's minimal medium before dilution in the minimal salts medium used in the experiment; ●, no rhamnolipid; □, 1.2 mM rhamnolipid; ◆, 7.6 mM rhamnolipid.

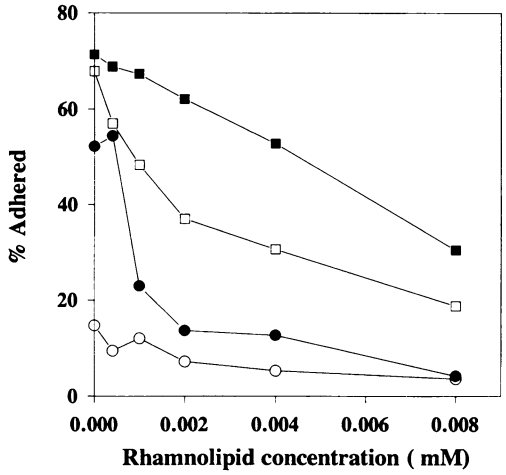

FIG. 5. Effect of rhamnolipid concentration on adherence of *P. aeruginosa* cells to hexadecane. *P. aeruginosa* cells were grown in Kay's minimal medium with gyratory shaking (200 rpm) at 37°C for 24 h. Cells were harvested and washed twice, and various concentrations of rhamnolipid were added. The BATH test was performed as described in Materials and Methods. ■, ATCC 15442; □, ATCC 27853; ●, ATCC 9027; ○, NRRL 3198.

development of hydrophobicity started at 100 h, the rate of octadecane biodegradation became comparable to the rate seen at high rhamnolipid concentrations from 0 to 100 h (Fig. 4A).

Rhamnolipid reduction of cell attachment to hydrocarbon. The results already presented have shown that biodegradation of octadecane by fast degraders was initially inhibited by rhamnolipid. Similarly, slow degraders were initially inhibited by the low rhamnolipid concentration (Fig. 3). To investigate the initial inhibition of octadecane degradation in the presence of rhamnolipid, cell adherence to hexadecane in the presence of rhamnolipid was determined for each of the test strains. For this experiment, cells were pelleted, washed, and then resuspended in buffer salts solution. The rhamnolipid was then added to the resuspended cells, and the BATH assay was performed. In all cases, the BATH test showed that extremely low rhamnolipid concentrations (0.008 mM) dramatically decreased the adhesion of cells to hexadecane. It should be emphasized that this BATH test did not measure true cell surface hydrophobicity but rather the apparent hydrophobicity in the presence of low concentrations of rhamnolipid. (Table 1 shows the actual hydrophobicity of each strain in the absence of rhamnolipid.) As shown in Fig. 5, the apparent hydrophobicity of each test strain was reduced with increasing concentrations of rhamnolipid of up to 0.008 mM. This experiment was also run at higher rhamnolipid concentrations (≥0.01 mM rhamnolipid); however, analysis of the BATH results was not possible because of the formation of rhamnolipid-hexadecane emulsions.

To investigate whether there was a strong interaction between the cells and the rhamnolipid, the cells were incubated with [^{14}C]rhamnolipid in the presence and absence of octadecane. After incubation periods of up to 48 h, no measurable [^{14}C]rhamnolipid was incorporated into the cells in either test.

DISCUSSION

Results of an earlier study from this laboratory showed that the aqueous dispersion of octadecane could be increased over 10^4-fold by the addition of rhamnolipid (19). The increase in aqueous dispersion was correlated with an increase in the rate of octadecane biodegradation. The results of the present study imply that the rate of octadecane biodegradation is dependent on both the aqueous dispersion of octadecane and on the surface properties of the degrading cells. As shown in Tables 1 and 2, cell hydrophobicity can be induced to change in the presence of a combination of both rhamnolipid and a slightly soluble substrate, e.g., octadecane. The importance of cell surface properties for the biodegradation of slightly soluble organic compounds has been indicated previously by Rosenberg and Rosenberg (15), who showed that the rate of hydrocarbon degradation by bacterial cells was dependent on cell affinity. Bacteria with high affinity for hydrocarbons utilized hexadecane more effectively than those with low affinity. Similarly, in this study, cells with high hydrophobicity, a measurement which can be used to indicate the affinity of cells for a nonpolar substrate, showed increased utilization of octadecane.

In examining the interaction among rhamnolipid, substrate, and degrading cells, the effects of rhamnolipid can be categorized as positive or negative. Positive effects can be summarized as (i) the increased dispersion of substrate and (ii) the enhancement of cell hydrophobicity. The negative effect of rhamnolipid is an apparent interference in the interaction between the microbial cells and octadecane.

Although the effect of rhamnolipid on octadecane dispersion was concentration dependent, both the low and high rhamnolipid concentrations used in this study significantly increased octadecane dispersion ($>10^4$-fold). Therefore, rhamnolipid-induced enhancement of cell hydrophobicity, which is also an effect of rhamnolipid addition, seems to be an important factor in determining the biodegradation rate of slightly soluble organic compounds. Rhamnolipid was particularly effective for cells with low initial hydrophobicity (slow degraders). For these cells, high rhamnolipid concentrations induced an immediate rapid increase in cell hydrophobicity (and biodegradation), whereas in the presence of low rhamnolipid concentrations, there was a 100-h lag period before cell hydrophobicity (and biodegradation) began to increase. These results imply that there is a concentration-dependent interaction of rhamnolipid with the degrading cells which seems to stimulate the development of cell hydrophobicity. In contrast, octadecane biodegradation by fast degraders, which had a high initial cell hydrophobicity, was either unaffected by the addition of rhamnolipid (high rhamnolipid concentrations) or was inhibited (low rhamnolipid concentrations). In this case, rhamnolipid addition did not have any effect on the measured hydrophobicity of the degrading cells.

The negative effect of rhamnolipid addition is an apparent interference in the interaction between the microbial cells and octadecane. This may be due to rhamnolipid-induced interference in the contact between the degrading cells and the substrate (octadecane). This hypothesis is supported by the data in Fig. 5, which showed that extremely small amounts of rhamnolipid severely reduced the adhesion of cells to hexadecane in the BATH assay (Fig. 5). One possible explanation for this interference is that at pH 7.0, both the bacterial cell walls and the rhamnolipid-octadecane complexes carry a net negative charge. Therefore, electrostatic repulsion between the similarly charged cells and complexes may prevent their interaction. To begin investigation of the interaction among the rhamnolipid, the octadecane, and the degrading cells, we performed some initial experiments which showed that filtration of rhamnolipid-octadecane solutions through a 300,000-molecular-weight membrane to remove particulate octadecane

did not affect the octadecane biodegradation rate (data not shown). This suggests that cells can obtain octadecane directly from the rhamnolipid-octadecane micellar structures described by Champion et al. (1). However, after incubation in filtered rhamnolipid-octadecane solutions, rinsed cells showed no accumulation of [^{14}C]rhamnolipid, suggesting that rhamnolipid is not taken up by the cell. Thus, there is only a weak association between the cell surface and the rhamnolipid-octadecane structures in comparison with the stronger associations which have been suggested for other systems (e.g., phospholipids), such as fusion between biosurfactant structures and the cell membrane (6, 9).

In summary, the results of the study present show that the enhancement of biodegradation of slightly soluble organic compounds, e.g., octadecane, by biosurfactants is not simply a matter of increased dispersion of the organic compounds. The rhamnolipid used in this study also affected the surface properties of the degrading cells, in some cases leading to enhanced biodegradation (ATCC 9027 and NRRL 3198) and in other cases inhibiting biodegradation (ATCC 15442 and ATCC 27853). Thus, there is a complex interaction among the rhamnolipid, substrate, and the degrading cells which needs to be explored more carefully before the full potential of this biosurfactant can be realized in practical applications, e.g., bioremediation of petroleum-contaminated sites. This observation can be extended to include the many different biosurfactants produced in nature. There are a variety of chemical structures which are produced; however, presently very little is known about the relationship between the chemical structures of these biosurfactants and their ability to either increase dispersion of slightly soluble organic compounds or to change cell surface properties. Successful use of biosurfactants in remediation will require knowledge of such structure-function relationships.

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