Effect of Emulsan on Biodegradation of Crude Oil by Pure and Mixed Bacterial Cultures

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Received 13 July 1988/Accepted 11 October 1988

Crude oil was treated with purified emulsan, the heteropolysaccharide bioemulsifier produced by *Acinetobacter calcoaceticus* RAG-1. A mixed bacterial population as well as nine different pure cultures isolated from various sources was tested for biodegradation of emulsan-treated and untreated crude oil. Biodegradation was measured both quantitatively and qualitatively. Recovery of ¹⁴C from mineralized ¹⁴C-labeled substrates yielded quantitative data on degradation of specific compounds, and capillary gas chromatography of residual unlabeled oil yielded qualitative data on a broad spectrum of crude oil components. Biodegradation of linear alkanes and other saturated hydrocarbons, both by pure cultures and by the mixed population, was reduced some 50 to 90% after emulsan pretreatment. In addition, degradation of aromatic compounds by the mixed population was reduced some 90% in emulsan-treated oil. In sharp contrast, aromatic biodegradation by pure cultures was either unaffected or slightly stimulated by emulsification of the oil.

Emulsan is the extracellular form of a polyanionic, cell-associated heteropolysaccharide produced by the oil-degrading bacterium *Acinetobacter calcoaceticus* RAG-1 (18, 31). The biopolymer stabilizes emulsions of hydrocarbons in water and has optimal activity when a mixture of aromatic and aliphatic components is present, such as in crude oil (17). The activity of the amphipathic emulsifier is due primarily to its high affinity for the oil-water interface (29) and its ability to orient itself at the interface to form a hydrophilic film around the oil droplets (21, 29, 30).

Studies with bacteriophages, antibodies, and emulsan-deficient mutants have demonstrated the following. (i) Emulsan accumulates on the cell surface of RAG-1 before its release into the medium as the cells approach stationary phase (6). (ii) Cell-bound emulsan serves as a specific receptor for a RAG-1 phage, ap3 (10). Cell-free emulsan is no longer recognized by the phage, but phage receptor activity of emulsan can be reconstituted in vitro at the oil-water interface stabilized by emulsan (11). (iii) Mutants of RAG-1 defective in emulsan production no longer grow well on crude oil either in the presence of exogenously added emulsan or in the presence of the wild-type emulsan-producing cell (13). This indicates that the cell-bound form of emulsan is required for growth on crude oil. (iv) Cells of mutants defective in emulsan actually adhere more avidly to hydrocarbons than the wild-type cells, indicating that emulsan on the cell surface may mask a hydrophobic component(s) and thus modulate adherence (12). In this regard, emulsan has been shown to inhibit bacterial adhesion to hydrocarbons (16) and to desorb adherent bacterial cells from hydrophobic surfaces (15).

Emulsan has potential applications in the petroleum industry, including formation of heavy oil-water emulsions for viscosity reduction during pipeline transport and production of fuel oil-water emulsions for direct combustion with dewatering (7; M. E. Hayes, K. R. Hrebenar, P. L. Murphy, L. E. Futch, Jr., and J. F. Deal, U.S. patent 4,618,348, October 1986). The affinity of emulsan for the oil-water interface suggests that it might affect microbial degradation of emul-

sified oils. This has implications both for the stability of the oil emulsions during storage and transport and for their biodegradability should the emulsions accidentally be spilled in the environment.

This report describes the degradation of emulsan-coated oil by both pure and mixed oil-degrading cultures. Two techniques were used: (i) ¹⁴C-labeled aromatic and saturated hydrocarbons were incorporated in emulsan-treated oil to monitor mineralization (production of ¹⁴CO₂), and (ii) capillary gas chromatography (CGC) was used to analyze residual, unlabeled oil. The ¹⁴C mineralization technique quantitatively measures the degradation of specific substrates, and the CGC procedure qualitatively assesses the alteration of a spectrum of substrates.

MATERIALS AND METHODS

Bacterial isolates. The pure bacterial isolates used in these experiments and their hydrocarbon-degradative capabilities are listed in Table 1. Two isolates have been identified: *A. calcoaceticus* RAG-1 (9) and *Pseudomonas* strain HL7b (5a); the others are environmental isolates of known degradative capability but undetermined generic affiliation.

The mixed population used for degradative studies is an oil-degrading bacterial culture which has been maintained for several years in the laboratory by transfer with crude oil as the sole carbon source. Its source and enrichment were described by Fedorak and Westlake (5). The inoculum for each experiment was pregrown for 24 to 28 days in BNP medium (see below) plus Prudhoe Bay crude oil and contained little residual oil.

Media. The mineral medium used for emulsification of oil (BNP) consisted of 0.5 g of K₂HPO₄, 1.0 g of NH₄Cl, 2.0 g of Na₂SO₄, 2.0 g of KNO₃, 0.2 g of MgSO₄ · 7H₂O, and trace FeSO₄ · 7H₂O per liter of twice-distilled water. BNP medium was supplemented with 1 g of yeast extract (Difco Laboratories, Detroit, Mich.) and 1 g of proteose peptone 3 (Difco) per liter to make BNP medium, used for biodegradation studies. The medium was supplemented after emulsan treatment because emulsification occurs more readily in a mineral salts medium than in an organic medium (D. L. Gutnick, unpublished studies).

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TABLE 1. Sources and oil degradation capabilities of pure bacterial cultures used in this study

Isolate	Gram stain reaction	Source	Oil degradation capability ^a for:	
			Saturates ^b	Aromatics ^c
1B-11	+	Marine sediment	—	+
VT4	—	Soil	—	+
HL7b	—	Fresh water	—	++
SE	+	Fresh water	++	—
ED	+	Fresh water	+	—
C1-Y ^d	+	Wastewater	++	—
SM	—	Fresh water	+	—
EA	—	Fresh water	+	—
RAG-1 ^e	—	Marine water	+	—

^a Degradative capability inferred from CGC analysis of residual oil after incubation with pure cultures (J. M. Foght and D. W. S. Westlake, unpublished results).

^b —, No degradation of saturates observed; +, rapid degradation of *n*-alkanes and slower degradation of isoprenoids; ++, rapid degradation of both *n*-alkanes and isoprenoids.

^c —, No degradation of aromatics observed; +, degradation of low-molecular-weight aromatic compounds including naphthalenes and biphenyls; ++, degradation of low- and higher-molecular-weight aromatics including phenanthrenes and anthracenes.

^d Isolate obtained from oil refinery wastewater provided by L. J. Huber, Federal Republic of Germany.

^e *A. calcoaceticus* RAG-1.

Prudhoe Bay crude oil was used for all experiments. A typical Prudhoe Bay crude oil has a specific gravity of 0.8883 g/ml, 23% (vol/vol) paraffins, and 25% (vol/vol) aromatics (1).

Preparation of emulsified oil medium for ¹⁴C-substrate mineralization studies. Purified emulsan was prepared as previously reported (20). The protein content was determined to be approximately 22% (wt/wt) (Bio-Rad Protein Assay; Bio-Rad Laboratories Chemical Division, Richmond, Calif.) with bovine gamma globulin (Bio-Rad) as a standard. Pure emulsan was suspended in a minimum volume of 95% ethanol, added to sterile boiling BNP medium to a final concentration of 50 µg/ml of medium, and boiled for 5 min. This treatment sterilized and dissolved the emulsan. The medium was cooled to room temperature before ¹⁴C-substrate-amended Prudhoe Bay crude oil was added to a final concentration of 1 µl of oil per ml of medium. The following ¹⁴C-substrates (Amersham Corp., Arlington Heights, Ill.) were used individually: [9-¹⁴C]phenanthrene, [9-¹⁴C]anthracene, [U-¹⁴C]carbazole, *n*-[1-¹⁴C]hexadecane, [1-¹⁴C]pristane, and [1-¹⁴C]hexadecanoic acid. The nanomolar amounts of ¹⁴C-substrate added (approximately 1,000 dpm/µl) did not significantly alter the crude oil composition. The labeled oil was emulsified by agitating the medium on a reciprocal shaker at 188 strokes per min for 3 h. After emulsification, a sterile stock solution of yeast extract and proteose peptone 3 was added to make BYP medium. For pure culture studies, 10 ml of the emulsified ¹⁴C-labeled oil medium was dispensed into 125-ml serum bottles, inoculated with 0.1 ml of culture pregrown in BYP medium, and sealed with a sterile serum stopper. Each replicate test bottle then consisted of 10 µl of ¹⁴C-substrate-amended oil plus 500 µg of emulsan in 10.1 ml of inoculated BYP medium. Unemulsified control bottles were prepared by dispensing 10 µl of ¹⁴C-substrate-amended oil into 10 ml of sterile BYP medium and inoculating the mixture with 0.1 ml of culture pregrown in BYP medium. Uninoculated control bottles were prepared, both with and without emulsan, to assess sterility of the media and to account for any volatility of the ¹⁴C-substrates. Sterility of

controls and purity of cultures were also assessed by streaking on BYP medium solidified with 1.5% agar (Difco). All bottles were incubated at 28°C with centrifugal shaking at 100 rpm.

For radiometric studies with the mixed population, media were prepared as outlined above. Ten-ml samples of medium were dispensed to 158-ml serum bottles, which were then inoculated with 1.0 ml of mixed population culture. Unemulsified and sterile controls were included, and all bottles were incubated at 28°C with centrifugal shaking at 100 rpm.

Measurement of ¹⁴C-substrate mineralization. At intervals, sealed replicate bottles were acidified to pH <2 with 4 N H₂SO₄ and flushed with nitrogen gas to recover ¹⁴CO₂ by using the trapping apparatus described by Fedorak et al. (2). Each vial contained 10 ml of ACS scintillation fluor (Amersham Corp.) plus 1 ml of Carbo-Sorb II (Packard Instrument Co., Inc., Downers Grove, Ill.) CO₂ trapping agent. The disintegrations per minute (dpm) recovered as ¹⁴CO₂ were adjusted for background dpm and calculated as a percentage of the added label.

Gas chromatography of residual oil. For qualitative CGC analysis of mixed-population oil degradation, media were prepared with and without emulsan as outlined above but omitting the ¹⁴C-substrates. Sterile emulsified oil medium (200 ml) was dispensed to 500-ml Erlenmeyer flasks and inoculated with 20 ml of mixed oil-degrading culture. Unemulsified controls inoculated with mixed population, plus uninoculated controls with and without emulsan, were included. Replicate flasks were fitted with foam plugs and incubated at 28°C with centrifugal shaking at 100 rpm for 3, 7, 14, or 28 days. Cultures for CGC analysis were acidified to pH <2 with 50% HCl, and the residual oil was collected by extraction with methylene chloride. The extracted, concentrated residual oil was separated into saturated and aromatic fractions by liquid column chromatography and analyzed by using the CGC columns and conditions described previously (5). In addition to saturated and aromatic compound detection by flame ionization, a sulfur-specific flame photometric detector allowed analysis of sulfur heterocycles in the aromatic fraction (4).

RESULTS

¹⁴C-substrate mineralization by pure cultures. All mineralization data have been corrected for background dpm. The dpm flushed from sterile controls were consistently less than 1% of the added label dpm, indicating that volatility of the labeled substrates did not contribute significantly to the recovered dpm; therefore, culture dpm have not been corrected for volatility. All cultures were found to be pure after incubation with the ¹⁴C-substrate-amended oil.

The substrates *n*-[1-¹⁴C]hexadecane and [1-¹⁴C]pristane represent, respectively, the saturated straight-chain alkanes and branched-chain alkanes (isoprenoids) found in crude oil. The 1-¹⁴C label position on these substrates requires that only the terminal carbon(s) be metabolized for ¹⁴CO₂ to be produced.

Mineralization of both *n*-[1-¹⁴C]hexadecane and [1-¹⁴C]pristane in crude oil by the gram-positive isolate SE was significantly reduced by pretreatment of the oil with emulsan (Fig. 1). Hexadecane mineralization was reduced approximately 85% and pristane mineralization was reduced approximately 70% after 140 h of incubation. Extended incubation of emulsan-treated cultures to 14 days resulted in no change in pattern of ¹⁴CO₂ release. Similarly, mineralization of

FIG. 1. Mineralization of n -[1- ^{14}C]hexadecane and [1- ^{14}C]pristane in crude oil by isolate SE without (controls) and with emulsan pretreatment. Each point is the mean of three replicates; error bars represent 1 standard deviation.

n -[1- ^{14}C]hexadecane in emulsan-treated oil by the gram-negative isolate EA was reduced approximately 50% (Fig. 2). This isolate does not degrade pristane sufficiently for emulsan to have a significant effect on mineralization.

Mineralization of n -[1- ^{14}C]hexadecane and [1- ^{14}C]pristane by four other saturate-degrading isolates was determined by analyzing replicate bottles after 7 and 14 days of incubation. Results after 14 days of incubation (which were only slightly higher than the 7-day results) are presented in Table 2. Pretreatment of the labeled oil with emulsan resulted in inhibition of hexadecane and pristane mineralization. This inhibition was observed for all six saturate-degrading pure cultures tested, regardless of their origin, their overall saturate degradative capability, and their Gram stain reaction.

The effect of emulsan on mineralization of aromatic and nitrogen heterocyclic compounds was studied by using phenanthrene and carbazole, respectively. Production of $^{14}\text{CO}_2$ from [U- ^{14}C]carbazole requires only side-ring metabolism,

FIG. 2. Mineralization of n -[1- ^{14}C]hexadecane in crude oil by isolate EA without (control) and with emulsan pretreatment. Each point is the mean of three replicates; error bars represent 1 standard deviation.

TABLE 2. Mineralization of n -[1- ^{14}C]hexadecane and [1- ^{14}C]pristane in crude oil after 14 days of incubation, without (controls) or with emulsan pretreatment^a

Isolate	% Label recovered as $^{14}\text{CO}_2$ ^b			
	n -[1- ^{14}C]hexadecane		[1- ^{14}C]pristane	
	Control	+ Emulsan	Control	+ Emulsan
ED	35.2 $\pm$ 1.4	7.8 $\pm$ 0.29	46.5 $\pm$ 3.7	7.5 $\pm$ 0.53
C1-Y	38.5 $\pm$ 1.9	12.9 $\pm$ 2.2	60.1 ^c	16.4 ^c
SM	37.3 $\pm$ 1.8	7.9 $\pm$ 0.66	3.2 $\pm$ 0.55	0.49 $\pm$ 0.07
RAG-1 ^d	36.8 $\pm$ 1.7	9.1 $\pm$ 0.3	2.3 $\pm$ 0.49	0.2 $\pm$ 0.05

^a Emulsan (in this experiment only) was present at a concentration of 20 μg /ml of medium.

^b Means $\pm$ 1 standard deviation; $n = 3$.

^c Single sample assayed.

^d *A. calcoaceticus* RAG-1.

whereas extensive degradation is required to produce $^{14}\text{CO}_2$ from the internally labeled [9- ^{14}C]phenanthrene. Mineralization of [9- ^{14}C]phenanthrene (Fig. 3) by isolate HL7b was unaffected by emulsan treatment, whereas mineralization by isolate VT4 was slightly stimulated (a statistically significant difference at the 95% confidence level as determined by Duncan's multiple-range test). Extended incubation did not change the $^{14}\text{CO}_2$ release patterns of either HL7b or VT4. Similar stimulation was observed with the gram-positive isolate 1B-11, with which $4.6 \pm 0.38\%$ of added [9- ^{14}C]phenanthrene was recovered as $^{14}\text{CO}_2$ from unemulsified oil and $8.8 \pm 0.31\%$ was recovered from emulsan-treated oil after 14 days of incubation (mean $\pm$ 1 standard deviation; $n = 3$). A statistically significant stimulation of [U- ^{14}C]carbazole mineralization by isolates HL7b and VT4 was observed with emulsan pretreatment (Fig. 4).

^{14}C -substrate mineralization by mixed population. Mineralization of ^{14}C -substrates by the mixed oil-degrading population was determined by analyzing replicate bottles after 7 and 14 days of incubation. Results after 14 days of incubation, which once again were only slightly higher than those after 7 days, are shown in Table 3. Mineralization of all saturated and aromatic substrates was inhibited by $>90\%$ except hexadecanoic acid degradation, which was inhibited by 30%. These results contrast with the pure-culture data, which showed little or no effect of emulsan on aromatic degradation and much less inhibition of saturate mineralization.

A pure culture of isolate SE was used to test the observation that hexadecanoic acid degradation was not as severely inhibited by emulsan treatment as degradation of the corresponding n -alkane. After 14 days of incubation, an unemulsified control culture of SE released $50.0 \pm 1.7\%$ of [1- ^{14}C]hexadecanoic acid as $^{14}\text{CO}_2$, whereas the corresponding emulsan-treated culture released $39.2 \pm 0.36\%$ of the label as $^{14}\text{CO}_2$ (mean $\pm$ 1 standard deviation; $n = 3$). This represents an inhibition of 22%, while n -[1- ^{14}C]hexadecane degradation was inhibited by $>85\%$ (Fig. 1).

CGC analysis of oil incubated with mixed population. It is possible that results obtained from mineralization of specific ^{14}C -substrates are not representative of the degradation of whole crude oil. Therefore, gross changes in residual oil fractions were assessed by CGC, which is less sensitive and less specific than radiometric studies but spans a wider range of substrates.

Analyses of residual crude oil incubated with the mixed population showed that emulsan pretreatment had an inhibitory effect on degradation of a variety of saturated, aro-

FIG. 3. Mineralization of [9- ^{14}C]phenanthrene in crude oil by isolates HL7b and VT4 without (controls) and with emulsan pretreatment. Each point is the mean of three replicates; error bars represent 1 standard deviation.

matic, and sulfur heterocyclic components of oil (Fig. 5, and Table 4). Inhibition of aromatic and sulfur heterocycle degradation was overcome to a small degree by extending incubation to 28 days (Table 4), but saturate degradation inhibition was not relieved.

DISCUSSION

The results presented in this article indicate that pretreatment of oil with pure extracellular emulsan had both inhibitory and stimulatory effects on oil biodegradation. For example, the presence of emulsan at the oil-water interface (i) severely inhibited saturated alkane mineralization normally catalyzed both by pure cultures and by a mixed oil-degrading population, (ii) had little or even some stimulatory effect on aromatic (phenanthrene and carbazole) mineralization by pure cultures of aromatic degraders, and yet (iii) inhibited aromatic mineralization in crude oil by the mixed population.

The inhibitory effects of emulsan on saturate mineralization, particularly in the case of the pure cultures, may be attributable to a requirement for direct physical interaction of the cells with the hydrophobic substrate in order to initiate

degradation (as has been found for *A. calcoaceticus* RAG-1 [19]). Previous observations have shown that emulsan prevents the adherence of various bacteria either to hydrocarbons or to hydrophobic biological surfaces such as buccal epithelial cells (15). It should be noted that different alkane-oxidizing strains may vary in their dependences on adherence to oil to effect degradation. In these cases hydrocarbon internalization is reported to proceed via "pseudosolubilization" through the action of specific microbial surfactants which presumably lower interfacial tension sufficiently to allow for microemulsion formation and hydrocarbon uptake (14). Previous coating of the oil with emulsan may prevent access of such surfactant(s), thus causing inhibition. Alternatively, emulsan may mask subcellular structures, such as the hydrophobic sites described by Pines and Gutnick (12), which could mediate adherence to hydrophobic substrates. The observation that degradation of hexadecanoic acid was less affected than degradation of hexadecane suggests the possibility that emulsan-mediated inhibition of degradation is reduced when the substrate has an amphipathic nature.

This inhibition does not appear to result from toxicity of emulsan-treated oil, because limited growth of pure cultures and the mixed population was observed in the presence of emulsan-treated oil. (Presumably this growth occurred at the expense of the low levels of yeast extract and proteose peptone present in BYP medium, since oil utilization was inhibited.) Moreover, the degree of mineralization by the same isolate differed for similar substrates. For example, there was a fourfold difference between mineralization of hexadecane and mineralization of hexadecanoic acid by

FIG. 4. Mineralization of [U- ^{14}C]carbazole in crude oil by isolates HL7b and VT4 without (controls) and with emulsan pretreatment. Each point is the mean of three replicates; error bars represent 1 standard deviation.

TABLE 3. Mineralization of ^{14}C -labeled substrates in crude oil without (control) or with emulsan pretreatment, after 14 days of incubation with mixed oil-degrading population

Substrate	% Label recovered as $^{14}\text{CO}_2^a$	
	Control	+ Emulsan
<i>n</i> -[1- ^{14}C]hexadecane	56.4 $\pm$ 1.6	5.4 $\pm$ 0.21
[1- ^{14}C]pristane	52.8	0.40
[1- ^{14}C]hexadecanoic acid	61.6	44.2
[9- ^{14}C]phenanthrene	72.3 $\pm$ 3.6	2.9 $\pm$ 2.1
[9- ^{14}C]anthracene	27.2	0.22
[U- ^{14}C]carbazole	23.6	0.35

^a Where more than one sample was assayed, the mean $\pm$ 1 standard deviation ($n = 3$) is given.

FIG. 5. Representative chromatograms of fractionated residual oil with or without emulsan treatment, after 14 days of incubation with mixed oil-degrading population. (A) Profiles of saturate fraction. nC_{16} , Hexadecane; pr, pristane; ph, phytane; chr, chrysene (marker). (B) Profiles of aromatic fraction. Peaks: 1, region containing C_2 -naphthalenes; 2, region containing C_3 -naphthalenes; 3, phenanthrene; 4, anthracene; chr, chrysene (marker). (C) Profiles of sulfur heterocycles. Peaks: A, C_2 -benzo[*b*]thiophene; B and C, C_3 -benzo[*b*]thiophenes; D, dibenzothiophene; E, region containing C_1 -dibenzothiophenes; F, region containing C_2 -dibenzothiophenes. (C_i denotes *i* alkyl carbons substituted on parent aromatic compound). For detailed peak identification, see references 3 and 4.

TABLE 4. Summary of CGCs of residual oil without (controls) or with emulsan pretreatment, after incubation with mixed population^a

Incubation time (days)	CGC result for:					
	Saturates ^b		Aromatics ^c		Sulfur heterocycles ^d	
	Control	+ Emulsan	Control	+ Emulsan	Control	+ Emulsan
3	+	—	±	—	±	—
7	+	—	+	—	++	ND
14	++	—	++	—	++	—
28	++	—	++	+	++	+

^a For representative chromatograms (14-day cultures), see Fig. 5.

^b —, No degradation of saturates observed; +, rapid degradation of *n*-alkanes and slower degradation of isoprenoids; ++, rapid degradation of both *n*-alkanes and isoprenoids.

^c —, No degradation of aromatics observed; ±, incomplete degradation of low-molecular-weight aromatics including naphthalenes; +, degradation of low-molecular-weight aromatic compounds including naphthalenes and biphenyls; ++, degradation of low- and higher-molecular-weight aromatics including phenanthrenes and anthracenes.

^d —, No degradation of sulfur heterocycles observed; ±, incomplete degradation of low-molecular-weight sulfur heterocycles including benzothiophenes; +, degradation of low-molecular-weight sulfur heterocycles including methylbenzothiophenes and dibenzothiophenes; ++, degradation of low- and higher-molecular-weight sulfur heterocycles including methylidibenzothiophenes; ND, not determined.

isolate SE. If inhibition had been due solely to toxicity, we would have expected similar levels of inhibition. Therefore, although it cannot be completely dismissed based on the data presented here, simple toxicity is not a likely explanation for the observed effects of emulsan treatment.

In contrast to alkane degradation, oil emulsification by emulsan had little or no effect on aromatic mineralization by the pure cultures tested. This may be related to the difference in uptake mechanisms of aromatics from those of saturated alkanes. For example, if the aromatic-degrading strains used in this study internalize only the hydrocarbon substrate dissolved in water, prior adherence may not be required. Bacterial growth on pure aromatic compounds has been shown to be proportional to the water solubility of the substrate (28), and bacteria were shown to use only the water-dissolved fraction of biphenyl, naphthalene, and phenanthrene (26, 27). If emulsan treatment increased the surface area of the oil droplets, it might have enhanced the partitioning of phenanthrene and carbazole in water, which in turn might account for the increased mineralization. However, the reported solubilization studies (24, 26–28) were conducted with aromatic substrates presented as solids, where solution rates were found to be dependent on particle size (24). Therefore, they may have only limited relevance to our experiments in which the aromatics were dissolved in a liquid crude oil carrier.

An alternate explanation which appears unlikely is that the various aromatic degraders used in this study all produced an emulsan depolymerase (23) which could overcome the inhibitory effects of emulsan. However, this enzyme would have been expected to break the emulsion (22). In fact, no change in the physical form of the oil emulsion was observed throughout the course of the experiments. Moreover, it is unlikely that each of the different aromatic degraders produced an emulsan depolymerase.

The mixed population appeared to be much more sensitive to emulsan treatment than the pure cultures. This was the case even for the aromatic substrates, which were mineralized by the pure isolates. In this regard, Horowitz et al. (8)

reported differences in growth and hydrocarbon degradation between oil-degrading strains examined in pure culture and those examined in reconstituted mixtures. Among the effects observed were competition for common substrates, enhanced die-off in mixed culture, and stabilization of populations in stationary phase. In that study, different components of the oil were found to be utilized sequentially. Sequential utilization by mixed cultures of oil-degrading microorganisms has also been reported by Walker and Colwell (25). It is possible that emulsan pretreatment could have inhibited an essential stage in the mineralization sequence. Whatever the mechanism, simple extrapolation of pure culture data to a mixed population is not valid.

Results from the mixed oil-degrading population suggest that industrial emulsan-stabilized oil-water emulsions should be recalcitrant to biodegradation. This would be beneficial in terms of transport and storage of such emulsions but suggests that emulsified oil spilled in the environment might be degraded more slowly than the original oil. It would be interesting to compare mixed enrichment populations isolated on emulsan- or other emulsifier-treated oils with those isolated on untreated oils to see whether specialized populations developed.

ACKNOWLEDGMENTS

We are grateful to Cecilia Anders for fine technical assistance and to Rina Avigad in the laboratory of D.L.G. for preparing the emulsan.

This work was supported in part by a Natural Sciences and Engineering Research Council Canada operating grant to D.W.S.W.

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