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DISTRIBUTION AND EFFICIENCY OF HYDROCARBON-OXIDIZING BACTERIA IN A FRESHWATER RESERVOIR

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ABSTRACT

Hydrocarbon-oxidizing bacteria were identified from three stations on DeGray Reservoir, Arkansas. The organisms were primarily gram-negative rods representing 9 taxa and 37 biotypes. *Pseudomonas* spp. were the most common isolates. The largest populations were found in areas most frequently used by boaters, although seasonal fluctuations were apparent during the spring and fall. The degradation of outboard motor oil by the five most rapidly growing isolates was studied. Each species had a different decomposition profile, and substrate oxidation rates were variable. *Acinetobacter calcoaceticus* var. *anitratus* was the most efficient decomposer.

INTRODUCTION

Much research has been directed toward hydrocarbon degradation by bacteria in aquatic environments (Atlas, 1981). The marine environment has attracted the most attention and there have been relatively few studies on freshwater habitats (Cooney and Summers, 1976; Horwitz and Atlas, 1977; Atlas, 1981). The purpose of this study was to measure both the quality and quantity of hydrocarbon-oxidizing bacteria in a relatively unpolluted freshwater reservoir, and to compare their efficiencies in decomposing certain molecular weight hydrocarbons.

MATERIALS AND METHODS

The study site was DeGray Reservoir, located on the Caddo River in southwestern Arkansas. It covers approximately 5500 hectares and has been filled about 10 years. Three major compartments of the reservoir were represented by sampling stations 4, 10 and 12 (Figure 1).

Figure 1. Location of sampling sites on DeGray Reservoir.

by standard methods of membrane filtration (APHA, 1976). Cultivation was on a mineral salts medium (Aaronson, 1970) containing 1% sterile Mercury Quicksilver outboard motor oil as a carbon source and 0.01% sterile triphenyltetrazolium chloride to facilitate counting. Samples were collected at 2 m intervals from the surface to 12 m, and at 5 m intervals thereafter, from November, 1979 through October, 1980. The samples were contained in Whirl-Pak bags (NASCO) and transported on ice to the laboratory. Aliquots of 100 ml were analyzed Sterilization was by autoclaving since such treatment does not alter composition (Walker and Colwell, 1975). Purified agar (Difco) was used as the solidifying agent. Controls containing no oil were used to check for the ability of the agar to support growth, but none was observed during the study. All samples were incubated at 24 °C for 4 weeks and colonies enumerated.

After incubation, isolates were streaked on McConkey's medium (Difco) to obtain pure cultures, and identified with the API 20E system (Analytab, Inc.).

Studies to determine the rate of hydrocarbon degradation were carried out on the five most rapidly growing isolates. The strains were cultured in a liquid mineral salts medium overlaid with 1% outboard motor oil (Austin et al., 1977). After incubation, the oil fraction was extracted from replicate sets of cultures at one week intervals for 4 weeks, and from controls containing no bacteria, with 10 ml pesticide grade hexane. Extracts were concentrated to 1 ml and analyzed by injecting 3 ul into a Tracor MT 222 gas chromatograph equipped with flame ionization detectors. The instrument incorporated a 6 ' x ¼ " glass column packed with 5% SE-30 on chromosorb, W,AW,DMS,HP,80/100 mesh. Nitrogen was used as carrier gas at a flow rate of 60 cc/min and the column oven was maintained at 210 °C.

Because n-alkanes are generally considered the most readily degraded components in a petroleum mixture (Atlas, 1981), straight chain hydrocarbons (ASD/Milton Roy Co.) ranging from $C_{18} - C_{24}$ were used as markers to aid in the characterization of the motor oil fractions being oxidized.

DATA AND RESULTS

Fifty-seven isolates representing 37 biotypes were identified as hydrocarbon oxidizers (Table 1). The most common bacteria were *Pseudomonas* spp. and the unnamed group CDC Group VE-1 which

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Table 1. Taxa of hydrocarbon-oxidizing bacteria isolated from all sampling stations.

Taxa	Isolates	Biotypes
Acinetobacter calcoaceticus var. anitratus	4	1
CDC Group 5E-1	4	1
CDC Group VE-1	12	9
Citrobacter freundii	2	2
Enterobacter cloacae	8	8
Enterobacter sakazakii	1	1
Klebsiella pneumoniae	1	1
Pseudomonas aeruginosa	1	1
Pseudomonas fluorescens	13	5
Pseudomonas maltophilia	4	1
Pseudomonas spp.	6	6
Serratia rubidaea	1	1

includes gram-negative fermenting rods with physiological characteristics similar to *Pseudomonas*. Approximately 95% of the total isolates were gram-negative rods.

The occurrence of larger populations in areas most frequently used by boaters was expected. In DeGray Reservoir, this area corresponds to the lower compartment (station 4). Data suggested this assumption to be generally true although heavy rainfall in March resulted in greater densities in the upstream compartments (Figure 2).

Data from all three stations were combined to determine densities for the entire reservoir during each month (Figure 3). Hydrocarbon oxidizers gradually increased throughout spring after a low density during winter. A summer decline occurred, followed by a large increase during fall.

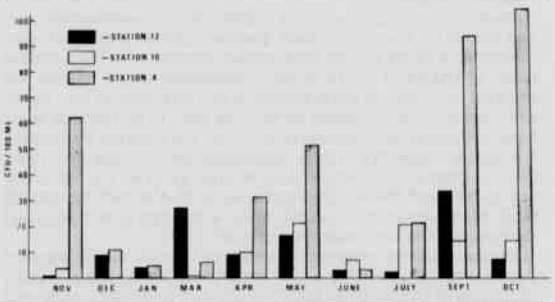

Figure 2. Comparison of populations in three compartments of DeGray Reservoir from November, 1979 through October, 1980.

Five taxa were studied to determine their abilities to degrade the major fractions of the outboard motor oil. CDC group VE-1 was found to be the least efficient (Figure 4a). It utilized only one fraction of the

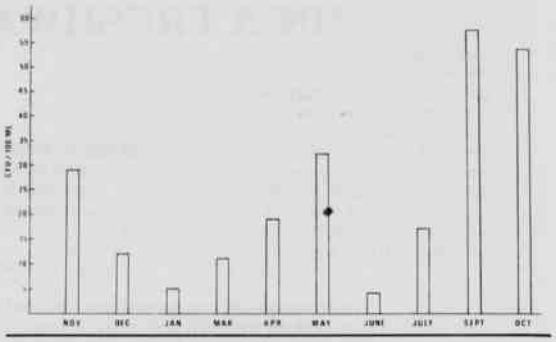

Figure 3. Mean populations for the entire reservoir from November, 1979 through October, 1980.

outboard motor oil in the $C_{22}-C_{24}$ range, reducing that fraction by approximately 72%.

Enterobacter cloacae utilized several fractions corresponding to the retention times of C_{18}, C_{18} and C_{20} standards (Figure 4b). The bacterium also decomposed the component nearest C_{22} as did CDC group VE-1.

Enterobacter sakazakii oxidized all but three of the major fractions (Figure 4c). There was a 12-100% concentration reduction in the $C_{18}-C_{24}$ range.

Most major components were decomposed by *Pseudomonas fluorescens* (Figure 4d). The organism degraded hydrocarbons in the $C_{20}-C_{24}$ range with efficiencies of 25-78%.

Acinetobacter calcoaceticus var. *anitratus* was most efficient in degrading the oil (Figure 4e). Data suggested that this bacterium was especially suited for oxidizing fractions from $C_{22}-C_{24}$. The two most fully oxidized components were diminished 81% (near C_{22}) and 93% (near C_{24}).

Relative oxidation rates for four species that could decompose the C_{22} (7.5 min retention time) fraction were determined (Table 2). CDC group VE-1 had an almost constant, but slow, rate during the 4 wks incubation. *E. sakazakii* and *E. cloacae* initially showed rapid rates of metabolism that began decreasing after 2 wks. *A. calcoaceticus* was most efficient, producing a 76% reduction of the oil component within 2 wks.

DISCUSSION

Many species of gram-negative bacilli are known to be capable of utilizing hydrocarbons. The species in this study have been recognized earlier in other investigations (Atlas, 1981; Austin et al., 1977; Jobson et al., 1972; Sedita, 1973). *Pseudomonas* spp. were the most comon group as reported previously (Atlas, 1980; Austin et al., 1977; Walker et al., 1975; Walker and Colwell, 1975).

Distribution of hydrocarbon oxidizers is affected by a number of variables including weathering (Atlas, 1980), nutrient composition (Austin et al., 1977; Horwitz and Atlas, 1977; Walker et al., 1975; Walker et al., 1976; Walker and Colwell, 1975) and water temperature (Atlas, 1980; Austin et al., 1977; Jobson et al., 1972). This study indicated heavy spring rains, and winter and summer temperature extremes influenced distribution. Some investigators found correlations between bacterial numbers and hydrocarbon concentrations, suggesting the possible use of these microbes as chronic pollution indicators (Austin et al., 1977; Horowitz and Atlas, 1977; Walker et al., 1975). Others have found no relationship between microbial density and hydrocarbons (Roubal and Atlas, 1977). Data presented here suggest larger populations were usually present in areas most frequently used by boaters, etc.

All species exhibited variations in decomposition rates for various fractions of the motor oil as has been reported previously (Walker et

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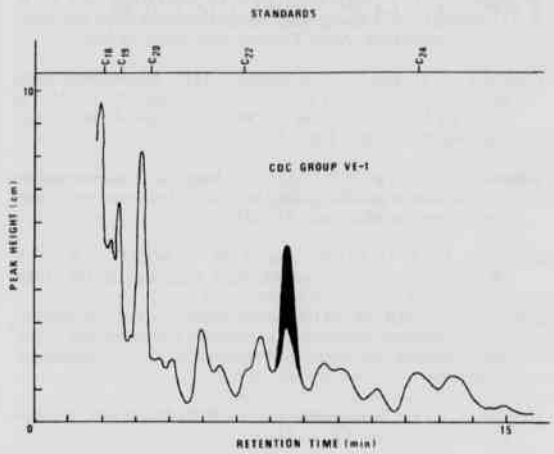

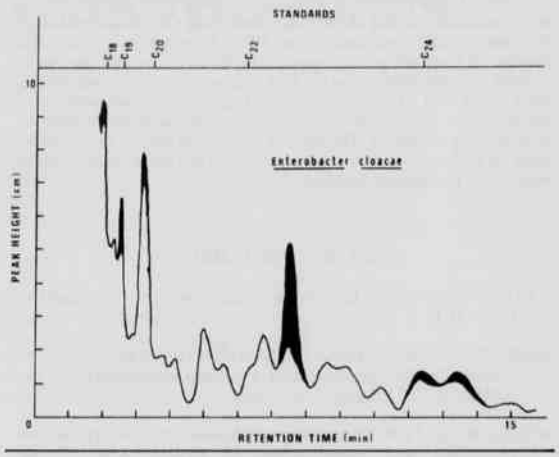

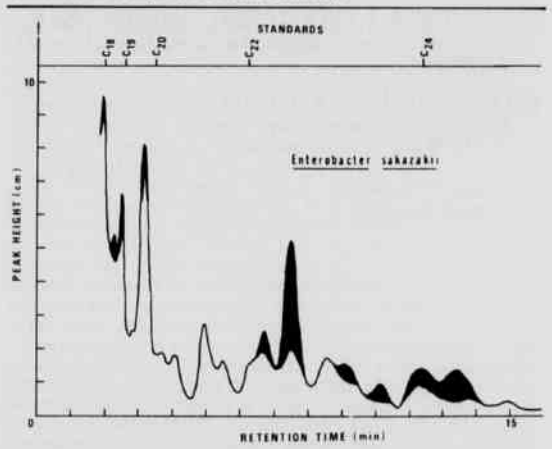

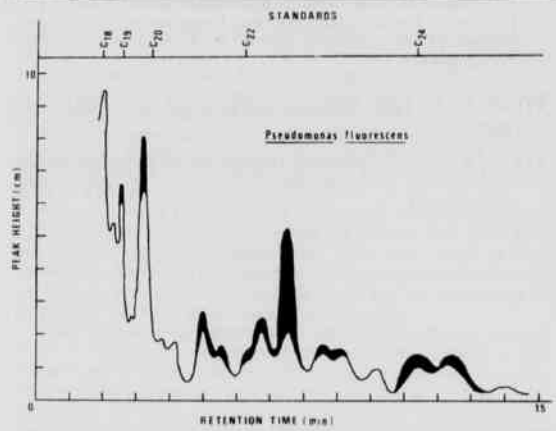

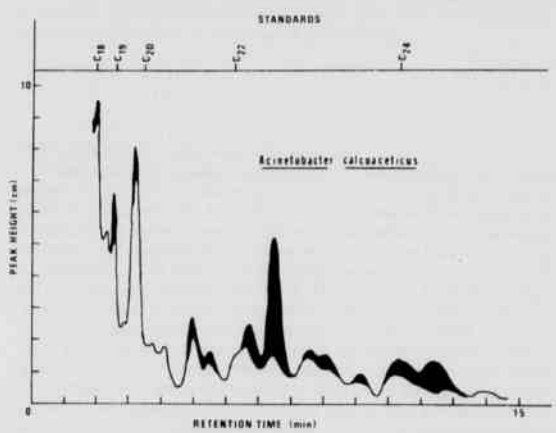

Figures 4A-E. Decomposition of the major fractions of outboard motor oil by five bacterial taxa (blackened areas represent the decrease in concentration as compared to sterile controls).

Table 2. Comparison of rates of decomposition by four species over a 4 week period, based on percent reduction of the major oil fraction having 7.5 minutes retention time.

Week	CDC	E. sakazakii	E. cloacae	A. calcoaceticus
1	0	0	3	0
2	29	47	57	76
3	43	82	67	79
4	72	89	74	81

al., 1976). Hydrocarbons in the $C_{22} - C_{24}$ range were metabolized by all 5 organisms studied. Others have shown that $C_{20} - C_{21}$ components were most readily oxidized (Atlas, 1979; Jobson et al., 1972). The number of fractions utilized varied considerably from one biotype to another. *A. calcoaceticus* was the least selective, apparently metabolizing most of the major fractions of the oil. In general, the microbial community of the reservoir can process hydrocarbon contaminants resulting from boating recreation. The bacteria responsible are not known to depend on hydrocarbons for growth, although increased concentrations may affect population densities.

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