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Environmental Contamination and Toxicology

Growth of Bacteria in an Oil Shale Retort Water by Indigenous Microorganisms

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Previous studies have shown that relatively high aerobic and anaerobic (or facultatively anaerobic) heterotrophic bacterial population densities occur as indicated by an increase in the turbidity of freshly filtered (0.4μm) Omega-9 retort water after a few days incubation at room temperature (Farrier et al., 1977). Growth of these microorganisms alters the nature and concentrations of dissolved organic (Felix et al., 1977; Pellizzari, 1978) and inorganic (Fox, 1980, Ph.D. dissertation, Univ. California, Berkeley, CA) constituents. Bacteria are the only microorganisms known to have demonstrated a capacity to grow in undiluted Omega-9 retort water (Gauger, 1981, Ph.D. dissertation, Univ. Wyoming, Laramie, WY). DeVore (1980, M.S. Thesis, Univ. Wyoming, Laramie, WY) studied the effects of the Omega-9 retort water on yeast and filamentous fungi. She found that four unidentified yeasts and one fungus, **Scopulariopsis candida**, were capable of growth on this retort water diluted 50%.

Bacterial growth experiments are performed for a variety of reasons. In some situations microorganisms are cultivated to yield a specific product (e.g., antibiotic), as a protein source, or because their growth in a particular medium removes certain undesired constituents (e.g., organic carbon in waste waters). Nutritional and physical parameters will often govern the rate at which growing microbial populations proliferate. It was considered important, therefore, to establish what rates of bacterial growth were occurring in the Omega-9 retort water by indigenous, mixed bacterial populations.

Since bacteria have demonstrated capability for growth in the undiluted process water, it may be possible to use bacteria to ameliorate undesirable water quality parameters as part of a treatment strategy or to mitigate, through detoxification of inimical components, environmental hazards as the result of an inadvertent spill. The study reported here was devised to assess bacterial growth characteristics in an example retort water. Information of this type may have implications in 1) the development of biological treatment systems, 2) establishing hazard as-

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sessment and abatement criteria, and 3) in assessing the stability of research samples.

MATERIALS AND METHODS

A large sample (47,300 l) of Omega-9 retort water was collected to provide material for a concerted environmental research effort by biologists, chemists, engineers and physicists (Farrier et al., 1977). It was mixed, filtered and stored in barrels of 114 liters each (3-5°C) to ensure homogeneity. Subsamples of this water were used to prepare liquid and solid culture media (Gauger, et al., 1980) and designated retort water broth (RWB) and retort water agar (RWA). Over the course of a nine month period, six bacterial growth experiments using an indigenous, mixed culture inoculum, were performed using sterile Omega-9 retort water obtained from two different barrels selected at random (Williams et al., 1979). The first three experiments used retort water obtained from one barrel while the latter three experiments were performed using Omega-9 retort water from a second barrel. A third barrel, also randomly selected, was used prior to these experiments for dissolved organic carbon studies. All barrels were stored under refrigerated conditions until sampled.

An Erlenmeyer flask containing 250 ml of membrane filter-sterilized Omega-9 retort water (1.2, 0.45 and 0.22 μm, Millipore filters and Swinnex filter holders) supplemented with 12 mmol NH_4NO_3 and 21 mmol Na_2HPO_4 was inoculated with 1 ml of untreated Omega-9 stock. This was incubated at room temperature (22-25°C) for 2-3 days until visually turbid. This culture was used as inoculum; the process being repeated each time a growth experiment was performed. For growth experiments, 1500 ml of filter-sterile RWB was inoculated with 1 ml of the turbid culture, placed on a rotary shaker (128 rpm) and incubated at room temperature (22-25°C). Aliquots of 1 ml each were withdrawn at hourly intervals for a period of 35-40 hours. A ten-fold dilution series was performed on these samples using filter-sterile retort water as diluent. Viable microbial populations were enumerated on nutrient supplemented RWA using the spread-plate technique (Koch, 1981). Plates were incubated at 20°C until colonies were large enough to be counted (5-7 days). Specific growth rate constants, μ ($hour^{-1}$) and generation times μ^{-1} were determined (Drew, 1981; Koch, 1981). Since bacteria divide by binary fission their growth is logarithmic in nature. The specific rate constant reflects this and is the slope of the curve exhibited by a bacterial population during the exponential phase of growth. Its numerical value is independent of the beginning viable cell concentration. Generation time is the period of time required for a bacterial population to double.

Total dissolved organic carbon levels (T-DOC) were measured at the beginning of each of the experiments. Direct injection of previously filtered (0.45 μm, silver membrane filters) 0.2 ml samples into a quartz combustion tube containing tungstic anhydride (WO_3) impregnated quartz wool (to remove salts which reduce

the life of the combustion tube), barium chromate (the combustion catalyst), and silver metal (removes most interfering gases), followed by heating to 960-980°C in a tube furnace. Oxidized T-DOC (as CO_2) was titrated coulometrically using a CO_2 Coulometer (Coulometrics, Inc., Wheatridge, CO) after removal of nitrogen oxides, NO_x, with a manganese dioxide scrubber (Huffman, 1977). The carrier gas used was CO_2-free oxygen. These measurements, carried out in duplicate for each experiment, were performed to determine if psychrotrophic bacterial growth in the stored retort water was reducing T-DOC levels via carbon assimilation.

RESULTS AND DISCUSSION

The most apparent observation (Table 1) is the chronological increase in the specific growth rate constants for each of the barrels. Generation times decreased from 2.6 h to 1.7 h in Barrel 1 and from 3.3 h to 1.4 h in Barrel 2. These data indicate that microbial growth was relatively rapid.

Table 1. Specific growth rate constants (μ) in two barrels of Omega-9 retort water for different experiments.

Barrel #	Time, Days	μ (Hours^{-1})
1	0	0.385
	61	0.511
	83	0.598
2	97	0.306
	161	0.518
	249	0.739

Psychrotrophic growth by the bacteria apparently occurred even though the retort water had been filtered and stored at cool temperatures to prevent this from happening (Fig. 1). Initially lower rates of growth may have been due to the absence of an essential nutrient (e.g., O_2, readily metabolized substrates) or due to a period required for induction of catabolic enzymes. Increase in specific growth rate constants within the respective barrels was significant (Barrel 1: Rate constant = 0.002*Days + 0.380, correlation coefficient, r = 0.987, probability level, p = 0.1; Barrel 2: Rate constant = 0.003*Days + 0.043, correlation coefficient, r = 0.997, probability level, p = 0.05; Fig. 1). These findings suggest that the indigenous bacteria adapted to the retort water milieu and that this capability was not a characteristic of an individual barrel, but was probably occurring simultaneously in all of the stored barrels.

Since one of the goals of cold storage was to ensure homogeneity and stability of the Omega-9 retort water, total dissolved carbon concentrations (T-DOC) were compared over the storage period.

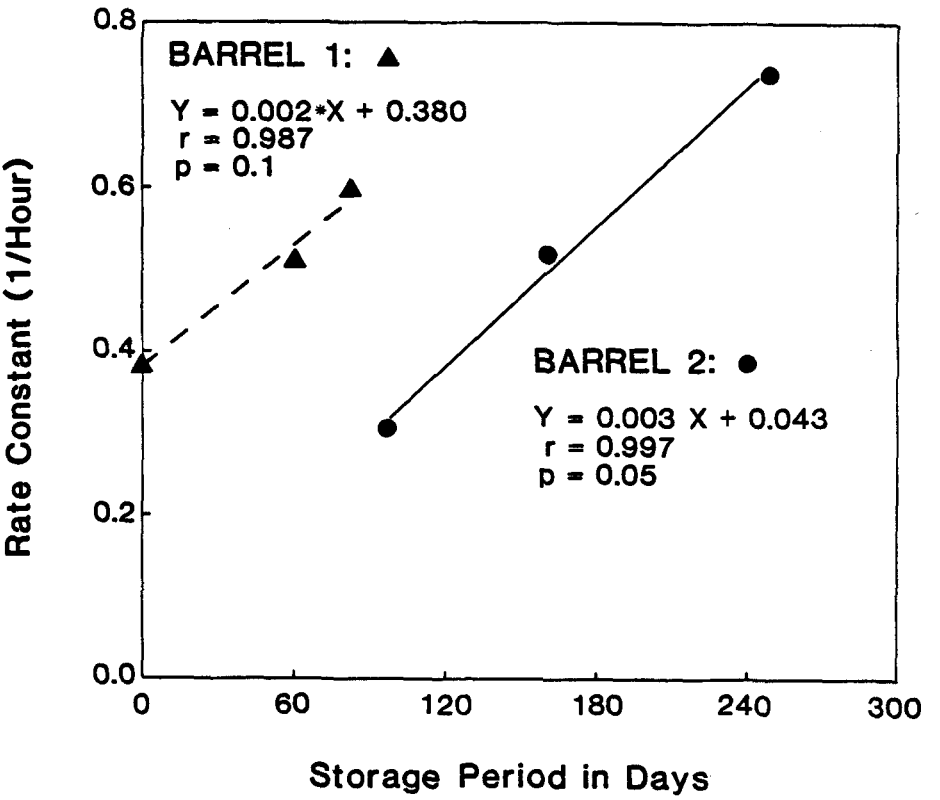

Figure 1. Changes in bacterial growth rates of stored Omega-9 retort water. Estimate denotes the best fit derived from linear regression. (Y = Specific growth rate constant, X = Days, r = correlation coefficient, p = probability level).

T-DOC was selected as a test parameter to evaluate the validity of the refrigeration theory for several reasons. 1) If bacterial growth was occurring it should be reflected in a decrease in T-DOC levels as Omega-9 retort water DOC was being assimilated. 2) Any observable changes in T-DOC over the period of storage would suggest a lack of homogeneity or stability in the Omega-9 sample. 3) Since levels of T-DOC had been studied by other investigators (Fox et al., 1978) and found to be relatively high (1000 mg T-DOC/l or greater) it was reasoned that DOC measurement should be an adequate and appropriate measure of homogeneity and stability for the sample. It is evident (Fig. 2) that the levels of T-DOC dropped substantially (~300 mg T-DOC/l) over the storage period and that this decrease was statistically significant (T-DOC = -0.372*DAYS + 1017.03, correlation coefficient, r = 0.973, probability level, p = 0.005). These findings represent data collected from three different barrels, selected at random, of the stored Omega-9 sample. Other barrels had been used during the interim periods although they were not tested for T-DOC levels. These observations, coupled with the bacterial growth studies above, in-

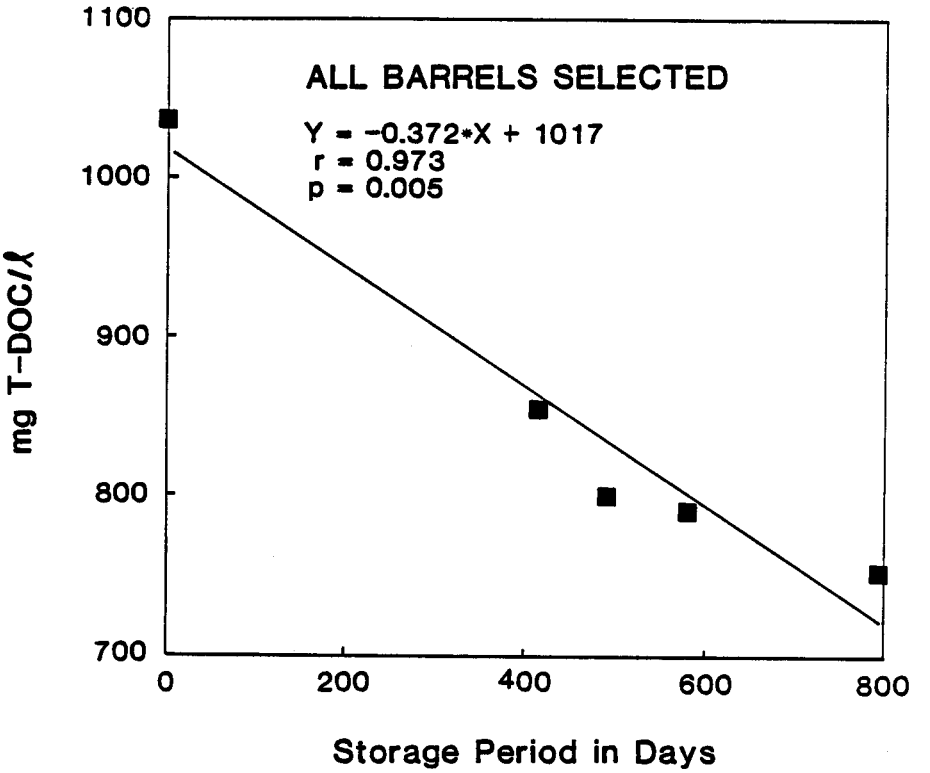

Figure 2. Decline in total dissolved organic carbon concentration over storage period for several randomly selected barrels of Omega-9 retort water. (Y = mg T-DOC/l, X = Days, r = correlation coefficient, p = probability level).

dicate that psychrotrophic bacterial growth was occurring in the stored Omega-9 barrels and that this growth effected nearly a 30% drop in T-DOC over that time period. The Omega-9 sample, was, therefore, neither stable nor homogeneous. Since the T-DOC drop is probably a reflection of assimilated carbon, it is conceivable that the microorganisms were probably also mediating transformations of the DOC constituents, thereby producing a modified process water as well.

Proliferation of indigenous bacteria was found to be fairly rapid in the Omega-9 retort water and the organisms were well adapted to this medium. In light of the stringent conditions imposed by physical and chemical characteristics of this waste water (Fox et al., 1978), such prolific growth was unexpected. It is evident that cold storage, as an attempt to maintain stability and homogeneity of the Omega-9 research stock, was not an effective deterrent to microbial growth. However, the potential for using these organisms in the development of waste treatment control technology should be of primary consideration in future research.

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