Bull. Environ. Contam. Toxicol. (1987) 38:661–666

Bulletin of Environmental Contamination and Toxicology

Growth of Mixed Microbial Populations in Ground Water Containing Highly Chlorinated Organic Wastes

William Pelon and John A. Mayo

Department of Microbiology, Immunology, and Parasitology, Louisiana State University Medical Center, New Orleans, LA 70112

In Louisiana, there are about 300 chemical waste dump sites. Some have been abandoned, while others are still active. Most of the wastes deposited by the petro-chemical industries, are often highly chlorinated and considered not to be readily biodegradable. Biodegradation studies with polychlorinated biphenyls (PCB) have shown that the percent biodegradation inversely related to the percent by weight of chlorine present in the PCB molecule (Tucker et al 1975). Chlorinated insecticides and herbicides also have been found to persist in the environment (Safe Drinking Water Committee 1977).

The contamination of ground water by hazardous organic chemicals deposited at these locations has become a matter of concern, because of the risk of drinking water sources becoming polluted. In many areas, geological strata are continuous over great distances, and protect aquifers from contaminated ground water. However, in south Louisiana, strata are discontinuous, providing ready access to aquifers.

There have been many reports presented regarding the microbial biodegradation of oil pollutants and other recalcitrant compounds by communities of microorganisms (see Bull and Slater 1982 for review). Based upon such findings, we suspected that natural mechanisms that would detoxify hazardous organic wastes, would exist at waste sites. We here report the isolation of microbial populations that would grow in organic-saturated ground water, with no added carbon sources.

MATERIALS AND METHODS

Ground water was obtained from the Petro Processor Inc. waste dump site in North Baton Rouge, Louisiana. Highly chlorinated hazardous organic by-products,

Send reprint requests to William Pelon at the above address.

primarily associated with petrochemical industry had been deposited there in bulk (an estimated 225,500 cubic yards). The compound in greatest abundance is hexachlorobutadiene, followed by 1,1,2-trichloroethane, tetrachloroethene, 1,1,2,2-tetrachloroethane, hexachlorobenzene, and 1,2-dichloroethane (NPC Services Inc. 1985). All are carcinogenic in animals (Lewis and Tatken 1982). Thirty eight additional volatile and semivolatile compounds were also detected, although in lesser quantities. Of these, 10 are carcinogenic in animals. The qualitative and quantitative nature of the nonvolatile organic compounds that may also be present is unknown.

The ground water sample, obtained from a monitoring well at a depth of 30 feet, overlaid an accumulation of organic wastes that had soaked into the soil. Samples of soil were also collected in the immediate vicinity of the monitoring wells. The ground water sample was brought to the laboratory in New Orleans, where it was distributed into 500 ml amber glass bottles that were sealed with Teflon-lined caps. the bottles of water sample were stored at 4°C in the dark. Subsequent tests showed that the water was microbiologically sterile, even though it was not collected aseptically. Soil samples were also sealed immediately upon collection and stored in the dark at 4°C.

Initial studies utilized a simple continuous culture apparatus consisting of a supply reservoir, a reaction vessel, and a collection reservoir. The ground water was used at a concentration of 90 percent, with the remaining 10 percent consisting of a 10X concentrated inorganic salts solution without trace metals (Evans et al.) prepared in activated carbon-filtered, deionized, distilled, autoclaved water. The pH of the ground water-salts solution was 6.4. The solution was introduced into the reaction vessel at a flow rate of 6.0 ml per hour. To maintain a constant volume (125 ml), the contents of the reaction vessel were removed at the same rate. Cultural conditions were aerobic, with rapid mixing, and an incubation temperature of 25°C. The reaction vessel was inoculated with two ml of a lightly centrifuged 20 percent suspension of the soil sample. Fifteen hundred ml of ground water-salts flowed through the reaction vessel in 6 to 7 days. The first phase of the experiment was terminated when the supply reservoir was exhausted. The contents of the reaction vessel were collected and stored at 4°C for additional study.

To continue the experiment, the contents of the collection vessel were used as feed of a new reaction vessel. The reaction vessel was inoculated with a

second suspension of soil. Since some organics may become only partially biodegraded by the initial microbial population, there was the possibility that these would be utilized further by some microorganisms present in the second inoculum. This process was repeated again for a total of three reaction phases.

The development of the microbial populations in each phase was followed by daily microscopic examination of wet mounts and gram-stained preparations. Microbial counts were made at the end of each phase. Isolations of the microbial types that were present were made on brain heart infusion agar. Bacteria and fungi were identified by standard methods (Buchanan and Gibbons 1974; Krieg and Holt 1984; Barnett 1956).

RESULTS AND DISCUSSION

Table 1 summarizes the results of daily microscopic examinations of the fluids in the reaction vessel. These data indicate that microbial forms were present in the soil inocula that could grow in unsupplemented organic saturated ground water-salts solution, and were unaffected by the presumed toxicity of the water sample. There is also evidence that the microbial populations that emerged were dynamic, changing both quantitatively and qualitatively with time.

Although the red yeasts (Rhodotorula species) appeared to be numerous during the early phases of microbial growth when the ground water was viewed microscopically, relatively few colonies were observed when efforts at isolation were made. This suggested that the growth of the yeasts was inhibited by the predominating gram-negative bacterial species. When streptomycin was added to the agar medium to inhibit the growth of these bacteria, substantial numbers of not only the Rhodotorula species, but also the mold Aspergillis became evident. This is shown in Table 2.

Efforts were made to identify the microbial isolates to the genus level. Table 3 presents this data. The majority of the isolates were identified as gram-negative aerobes. Multiple isolates of a single genus may represent more than one species. Further evidence of the dynamic state of these populations is shown as seen by the different microbial species that were present during the different phases. The appearance of the fermentor type of organisms suggests cross-feeding from organisms arising earlier.

Table 4 shows the microbial population numbers that were present following each phase of incubation. The numbers were substantial considering the nature of the

Table 1. Daily microscopic examinations of reservoir fluids.

Day	Observations
	Phase I
0	Inoculation
1	Budding yeasts (+/- 1 for every 2 fields)
2	Yeasts (few); gram-neg. rods (few)
3	Yeasts (1-2/field); gram-neg. rods (very few)
4	Yeasts (+/- 10/field); gram-neg. rods & coccobacilli (40 - 50/field)
5	Yeasts (+/- 30/field; less gram-neg. rods & coccobacilli
6	Yeasts (1-2/field); emergence of gram-neg. rods & coccobacilli (20 - 30/field)
7	Wall growth - Many yeasts & coccobacilli
	Phase II*
0	Inoculation
1	Yeasts (1-5/field); few bipolar staining gram-neg. rods
2	Yeasts (few); bipolar staining gram-neg. rods (30-5-/field)
3	Numerous gram-neg. rods
4	Gram-neg. rods (30-50/field)
5	Gram-neg. rods (50-100/field)
	Phase III
0	Inoculation
1	Gram-neg. rods (50-100/field)
2	Gram-neg. rods (50-100/field); some bipolar, long & short forms
3	Gram-neg. rods (50-100/field); some bipolar, long & short
4	Gram-neg. rods; short forms predominating
5	Yeast-like bodies (no budding); long gram-neg. rods predominating

* Reservoir flask replaced.

substrate that is available to the organisms, and that growth had occurred under a continuous culture system where a part of the population was being removed continually.

Microbial population counts made with the soil inocula are shown in Table 5, and show that the populations present were less than seen in the reaction vessel (Table 4). This provides additional evidence that microbial growth was occurring, since the reaction vessel was inoculated with 2.0 ml of these suspensions. In summary, the data have shown that soil from the waste dump site contained microbial populations that were able to grow in a medium consisting of simple salts, and ground water saturated with highly

Table 2. Yeast and mold populations in reservoir fluid as revealed with streptomycin.*

Genus	Populations (per ml) when incubated at 20°C.	37°C.
Rhodotorula	3.8×10^5	8.3×10^5
Aspergillus	2.0×10^3	2.0×10^3

* 10,000 ug/ml.

Table 3. Genera of the microorganisms found to grow in the waste-contaminated ground water sample.

Phase I	Phase II	Phase III
Aeromonas (5)*	Aeromonas (2)	Azomonas (1)
Aspergillus (1)	Azotobacter (1)	Citrobacter (2)
Citrobacter (3)	Citrobacter (1)	Enterobacter (3)
Enterobacter (5)	Pseudomonas (4)	Pseudomonas (5)
Pseudomonas (7)	Rhodotorula (1)	Serratia (3)
Rhodotorula (1)	Fermentor A (2)	Fermentor B (1)
		Fermentor C (1)

* Isolates may represent the same or multiple species within the genus.

Table 4. Microbial population density in the reactor vessel at the end of each phase.

Phase	Incubation period (days)	Populations after incubation at 20°C.	37°C.
I	7	---	1.9*
II	6	1.7*	2.0
III	6	1.4	1.7

* $\times 10^6$

chlorinated organic compounds, with no supplemental carbon sources.

Such populations appeared dynamic, changing both qualititively and quantitatively with time. It is possible that some members of the population acted as commensals, using the products of earlier metabolism by other organisms.

Table 5. Microbial population densities in 20% soil suspensions used as inocula.

Soil Suspension	Populations (per ml) after incubation at 20°C.	37°C.
I	3.0×10^3	7.0×10^3
II	8.1×10^4	1.2×10^5

Microbial population counts made on successive days show that a steady state of growth was attained (Table 6). This is characteristic when successful microbial propagation occurs in a continuous culture system.

Table 6. Microbial population counts on successive days.

Exp. No.	Day	Populations after incubation at 20°C.	37°C.
I	4	2.5*	2.7*
	5	2.5	2.7
	6	1.7	2.0
II	4	2.3	2.1
	5	2.2	2.2
	6	1.4	1.7

* x 10^6

Acknowledgments. This investigation was supported by a grant from the U.S. Department of Interior, through the Lousisana Water Resources Research Institute, Baton Rouge, La. The cooperation of Mr. Harold F. Ethridge, Jr., La. State Dept. of Environmental Quality; and Dr. Lawrence Bohn, NPC Services Inc., is appreciated.

REFERENCES

Barnett, HL. Illustrated genera of imperfect fungi. Burgess Publishing Co. Minneapolis, Minn. 1956.

Buchanan RE, Gibbons NE (eds.) Bergey's manual of determinative bacteriology, 8th Ed., Cambridge University Press, Cambridge, Mass. 1974.

Bull AT, Slater JH (1982). Historical perspectives on mixed cultures and microbial communities. In: Bull AT, Slater JH (eds) Microbial Interactions and Communities, Vol 1. Academic Press, New York, p 1.

Evans CGT, Herbert D, Tempest DW (1970). The continuous cultivation of microorganisms. 2. Construction of a chemostat. In: Norris JR, Ribbons JW (eds.) Methods in Microbiology, Vol. 2, Academic Press, New York. p. 277-323.

Krieg NR, Holt JG (eds.) Bergey's Manual of Systematic Bacteriology, Vol. 1, Williams and Wilkins Co., Baltimore, Md. 1984.

Lewis RJ, Tatken RL (eds.) 1980 Registry of toxic effects of chemical substances. Vols. 1 & 2. DHHS (NOISH) Pub. No. 81-116. Feb. 1982.

NPC Services Inc. (1985). Remedial planning activities, petroleum processors Inc. (Report submitted to the Louisiana State Department of Environmental Quality and to the U.S. Environmental Protection Agency.)

Safe Drinking Water Committee, National Research Council (1977). Organic Solutes. In Drinking Water and Health. National Academy of Science. Washington D.C. pp. 486-856.

Tucker ES, Saeger VW, Hicks O (1976). Activated sludge primary biodegradation of polychlorinated biphenyls. Bull. Environm. Contam. Tox. 14:705-713.

Received August 14, 1986; accepted November 20, 1986.