MICROORGANISMS IN COASTAL WATERS

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Pathogenic viruses, bacteria, and fungi are present in the final effluent discharged through submarine wastewater outfalls, but the techniques for determining their presence in coastal waters are difficult and are rarely applied. Instead, other more common organisms, such as total coliform bacteria, are monitored as tracers and indicators of the possible presence of pathogenic forms in coastal waters, on beaches, and less frequently in shellfish.

During the past year, we have initiated a program to investigate the survival and distribution of common human and marine pathogens in the vicinity of outfalls and to examine the influence of waste discharges on native marine microbial populations. We have conducted several specific studies to date, including

- An investigation of the abundance and distribution of terrestrial, freshwater, and marine microorganisms around an outfall in Santa Monica Bay during and after discharge of chlorinated secondary effluent.

- A survey of marine bacteria in Santa Monica Bay.

- A laboratory study of the survival of pathogenic bacteria and viruses in seawater.

In each of the seven microbiology cruises conducted this year, samples of surface, middepth, and bottom waters, and cores of bottom sediments were screened aboard ship for the microbiological criteria and specific organisms listed on Table 1 (confirmative as well as presumptive tests were used). In several cruises, we also obtained samples of floatable materials, using a phytoplankton net or a 5 in. diameter floatables collector.

The cruises produced 144 samples, which were processed and analyzed in the Project's microbiology laboratory at California State College at Long Beach. Some of the results are presented here.

MARINE BACTERIA IN SANTA MONICA BAY

During fall 1973, we conducted two cruises to obtain a preliminary estimate of the abundance and distribution of marine bacteria populations near the Hyperion outfalls in Santa Monica Bay. Three stations were sampled one inshore, near the base of the outfalls, one at the end of the 5 ml outfall, where a combination of primary and secondary effluent is discharged, and one at the end of the 7 ml digested sludge outfall.

A general analysis of the samples showed that aerobic marine bacteria were most abundant in the sediments, we also found a rather high number of these organisms in the water column above the end of the 5 ml effluent outfall (Table 2). Anaerobic bacteria showed a similar trend.

We found that the water column samples from these cruises contained concentrations of aerobic, salt resistant bacteria ranging from 51 to 4,920 cells/ml. By comparison, coliform "MPN" (most probable number) counts for the same areas and times ranged from 0.3 to 2.3 per ml in surface waters to 3 to 240 at the 47 m depth (these values were furnished by Mr. J. Nagano, Hyperion Treatment Plant).

A number of samples from the cruises were positive for species of *Vibrio, Aeromonas, Pseudomonas, Streptococcus,* and coliforms. But we did not find pathogenic species of StaphyZococcus, *Salmonella,* and *Shigella.*

Overall, this survey indicated the presence of relatively abundant bacteria populations near the outfalls in Santa Monica Bay, especially in the sediments, and confirmed the occurrence of active, enhanced communities containing both aerobic and anaerobic marine bacteria.

GENERAL EFFECTS OF THE DISCHARGE OF CHLORINATED SECONDARY EFFLUENT

In 1974, we conducted four cruises to sample five sites in a 1 sq km grid around the unused 1 ml outfall in Santa Monica Bay (Figure 1). The purpose of these cruises was to observe microbiological populations during and after the weekly flushing of the outfall with chlorinated secondary effluent. On 31 January and 21 May, we sampled during the 4 to 6 hour flushing period, samples were also taken on 28 February and 9 May, 7 days after flushing.

As we expected, populations of fecal and freshwater bacteria were large during discharge, had declined after discharge, and did not survive in high numbers in the sediments. But the discharge had a surprising effect on the sediment and surface water concentrations of marine bacteria: These were present in much higher numbers 7 days after discharge than during the flushing the populations may have been enhanced by feeding on the organic matter released in the discharge.

The surface samples (Table 3) revealed another interesting result. During the flushing of the outfall with chlorinated secondary effluent, surface organisms appeared to be dominated by marine aerobic bacteria (about 74 percent of the populations measured) followed by freshwater aerobic bacteria (17.0 percent), freshwater anaerobes (8.5 percent), marine anaerobes (1.1 percent), and fecal streptococci (O.1 percent). Fecal coliform counts were low (less than 0.03 percent), as were counts for fungous yeasts and molds. These observations differ from those previously reported by R. Selleck of University of California, Berkeley, who found very high numbers of coliforms in surface films around Hyperion's 5 mile effluent outfall. There are several possible reasons for the discrepancy. First, as the sampler used in the Project's survey was small and not necessarily designed for surface sampling, small particulates and oil droplets may have been inefficiently sampled. Secondly, during our study, the discharge was chlorinated secondary effluent; the previous study was conducted around the 5 ml outfall, where a non-chlorinated mixture of primary and secondary effluent is discharged. This kind of surface sampling study should be repeated, using a device specifically designed for surface particulate sampling.

Although pathogenic bacteria, such as *Salmonella* species, were present in the effluent before discharge on 21 May, they were not detected in samples from any of the four cruises. These organisms may either be well diluted or suffer significant mortality during discharge. Similarly, we did not find human pathogenic fungi, such as *Candida albicans* and *Cryptococcus neoformans*, in the samples, although we did isolate and identify hundreds of other species of fungi.

SURVIVAL OF PATHOGENS IN SEAWATER

The laboratory studies on the survival of various species of microorganisms in seawater showed that the physical, chemical, and biological conditions of the marine environment, the types of organisms tested, and the nature of the effluent play the major roles on their survival.

Figures 2 and 3 show the survival of several species of microorganisms in seawater. Under the test conditions, a significant percentage of surviving cells were observed even after 1 week of incubation at 4°C. When the temperature was increased to 25°C, their survival was slightly less than that of the lower temperature. When the seawater was filtered through a bacteriological membrane, survival rates were higher than those of populations placed in raw seawater, indicating that the filteration had removed most of the predators (marine bacteria and protozoans). When we enriched the seawater by adding some nutrient, survival was better, demonstrating the protective action of the nutrient for the cells. When the hydrostatic pressure was increased to the deep sea pressures (up to 1,OOO atmospheres), the death rates of the microorganisms were accelerated. But increased pressure failed to kill or inactivate all the cells even after 1 week of incubation. It is also interesting to note that viruses tested in this study (Bacteriophage 0X 174 (Figure 3) and Bacteriophage T4) showed remarkable stability even under the increased pressure. Since primary and secondary sewage treatment procedures, including chlorination, are generally known to be insufficient to inactivate the enteroviruses, which are most frequently found in wastewater, further investigations of the survival of viruses in the sea would be of a great importance.

We plan to continue these studies, as well as a program to study bacteria associated with fish diseases, in the next year.

FIGURES

Figure 1.
Changes in populations of marine bacteria during and after discharge of chlorinated secondary effluent in Santa Monica Bay, winter and spring 1974

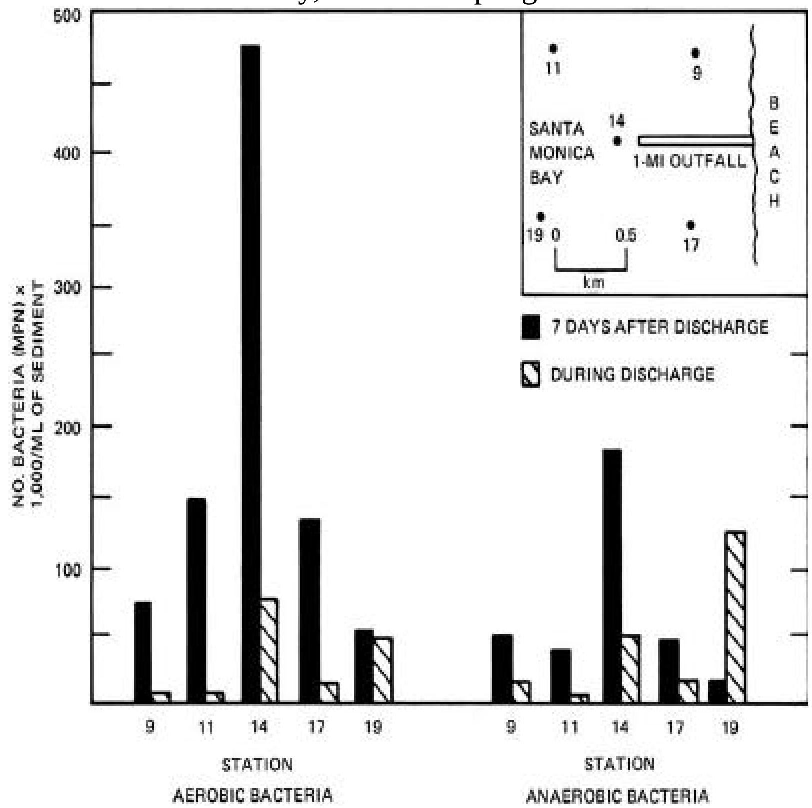


Figure 2.
. Survival of pathogenic bacteria in filtered seawater at 25°Cand pressures of 1 to 1,000 atmospheres

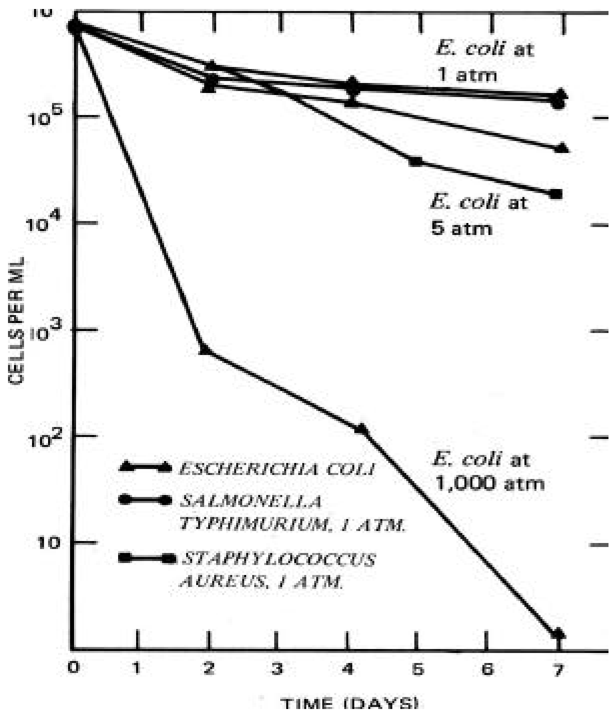
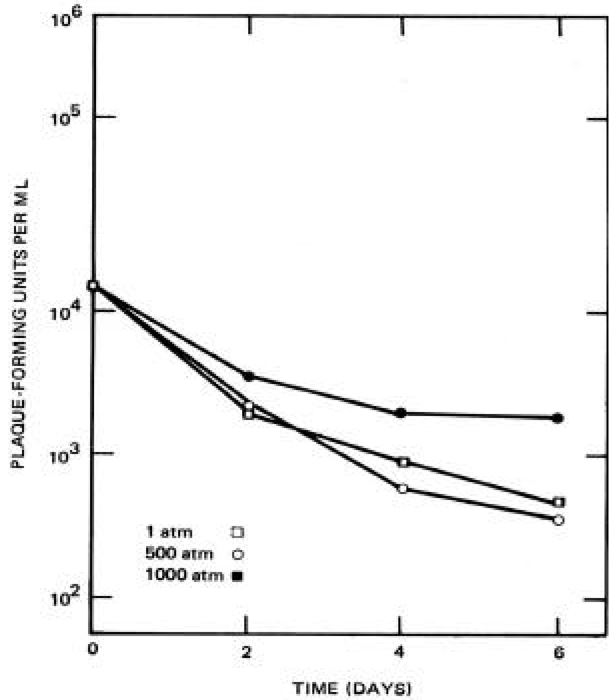


Figure 3.
Inactivation rate of a virus, Bacteriophage ~X 174 in filtered seawater at 25°Cand pressures of 1 to 1, 000 atmospheres.



TABLES

Table 1.
Microbiological criteria and species examined in samples of waters, floatables, and sediments from Santa Monica Bay, 1973-74.

General Criteria	*Staphylococcus aureus*	*Aeromonas hydrophilia*
Total coliform (MPN)	*Salmonella species*	*Aeromonas liquifaciens*
Fecal coliform (MPN)	*Shigella species*	*Aeromonas salmonicida*
Fecal streptococcus (MPN)	*Mycobacterium tuberculois*	*Erysipelothrix insidiosa*
Aerobic marine bacteria (No./sample)	*Esherchia coli*	*Mycobacterium marium*
Anaerobic marine bacteria (No./sample)		*Chondrococcus columnaris*
Aerobic freshwater bacteria (No./sample)	**Specific Marine Bacteria**	*Clostridium botulinum* Type E
Anaerobic freshwater bacteria (No./sample	*Vibrio alginolyticus*	
Specific Human and Patho-genic Bacteria	*Vibrio anguillarum*	**Fungi, Molds, and Yeasts**
Streptococcus pyogenis	*Vibrio piscium*	Total No. fungi/sample
Streptococcus fecalis	*Vibrio ichthyodermis*	Total No. molds/sample
	Vibrio parahaemolyticus	Total No. yeasts/sample
	Vibrio cholerae	No. *Candida* species
	Pseudomonas punctata (Aeromonas)	*Candida albicans*
	Pseudomonas fluorescens	*Cryptococcus neoformans*

Table 2.
Number (MPN) of marine bacteria in the water column and sediments from stations in Santa Monica Bay. Numbers are average values from two cruises (September and November 1973).

		No. of Bacteria/ml	
	Sludge Outfall, 78 m	**Effluent Outfall, 62 m**	**Nearshore Station, 38 m**
Aerobic Bacteria			
Surface waters	58	620	655
Middepth waters	67	2,905	420
Bottom waters	180	285	580
Sediments	37,200	30,200	53,250
Anaerobic Bacteria			
Surface waters	12	89	10
Middepth waters	55	2,007	610
Bottom waters	106	235	41
Sediments	25,750	45,600	33,000

Table 3.
Microorganisms in surface tow-net samples taken during and after discharge of chlorinated secondary effluent in Santa Monica Bay, May 1974.

	During Discharge		7 Days After Discharge
	No. of Organisms/sq m*	% of Total	No. of Organisms/sq m*
Marine aerobic bacteria	1,000-544,000 (28,000)	74	43,000-464,000 (228,000)
Freshwater aerobic bacteria	1,700-56,600 (6,380	17	No Data
Freshwater anaerobic bacteria	425-3,800 (3,200)	8.5	No Data
Marine anaerobic bacteria	425 (425)		425-3,400 (425)
Fecal streptococcus	10-390 (30)		2-5 (2)
Fecal coliform	1-10 (1)		1-10 (1)
Freshwater molds	0.42-2.3 (1.0)		0.86-2.1 (1.4)
Marine molds	0.19-1.8 (0.2)		0.10-2.1 (0.4)
Freshwater yeasts	0.08-1.1 (0.2)		0.1-0.76 (0.4)
Marine yeasts	0.04-0.3 (0.2)		0.1-0.3 (0.24)

*Numbers are range and, in parentheses, median value.