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by

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The Effect of the Chesapeake Corporation on the Water Quality of the Upper York River with Respect to Total and Fecal Coliforms and Fecal Streptococci

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ABSTRACT

Levels of total and fecal coliforms and fecal streptococci in the Mattaponi, Pamunkey and York Rivers were monitored for one year in the vicinity of the town of West Point, Virginia. These parameters were also determined for selected process waste waters and effluents from the Chesapeake Corporation, West Point, Virginia.

Point sources of fecal coliforms and fecal streptococci to the Pamunkey River were detected at the UNOX effluent and in the vicinity of Dozier's Closure. Sources of fecal coliforms in the UNOX effluent appeared related to levels in the paper mill sump discharge since the UNOX system neither generated nor reduced populations of these bacteria.

While certain known sources of fecal contamination were located during the course of this study within the Chesapeake Corporation, removal of the offending discharges did not affect the levels of indicator bacteria in either Dozier's Closure or the UNOX effluent. In the absence of proven sources of fecal matter, we suggest that the observed elevated levels of indicator bacteria were not due to contamination by domestic sewage, but due to saprophytic growth on wood-derived soluble organic compounds and the associated BOD. If this hypothesis is valid, the source of elevated levels of indicator bacteria in certain river samples must be considered with respect to implementation of the fecal coliform standard for shellfish harvesting. However, conditions conducive to the growth/survival of non-pathogenic indicator bacteria may also favor growth of true pathogens. Recommendations to test for the possibility of saprophytic growth are made.

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Introduction

Preliminary bacteriological studies of the upper York River in the vicinity of the town of West Point and the Chesapeake Corporation, a pulp and paper manufacturer, indicated elevated levels of total and fecal coliforms with respect to levels currently acceptable for shellfish harvesting. The results of additional preliminary surveys, which included samples from both river and Chesapeake Corporation plant sites and effluents suggested that some areas within the plant may have been sources of indicator bacteria. Similarly, river water adjacent to a bulk-headed containment area known as Dozier's Closure, exhibited rather high levels of fecal coliforms and fecal streptococci.

Two areas of the plant were of particular interest bacteriologically; the river-fed cooling system and a unique biologically oxygen activated sludge system (UNOX) utilizing inorganic nutrient enrichment for process waste water treatment. Closer examination of these systems was suggested to measure their contribution of indicator bacteria, if any, and their potential for producing conditions favorable for saprophytic growth of these bacteria. Generation of indicator bacteria or conditions favorable to their growth independently of the introduction of fecal material must be assessed for the intelligent use of bacteriological water quality standards.

Therefore, a study was initiated in April 1976 with funds provided by Chesapeake Corporation of Virginia to determine the abundance, distribution and seasonal variation of indicator bacteria with respect to

industrial, municipal, and private-residential sources of bacterial pollution of the upper York River - West Point area and to assess the saprophytic growth potential of both the UNOX waste water treatment and river water cooling systems.

Materials and Methods

In-Plant and River Surveys

Sampling sites were selected in the Mattaponi, Pamunkey, and upper York Rivers to include reference points free of direct effluent from the Chesapeake Corporation in addition to sites within the effluent discharge area (Fig. 1). Site #1, in the Pamunkey River, and site #11, in the Mattaponi River, were located near low density private residential areas. Site #2 was the discharge area for the effluent from the paper mill's active sludge process waste water treatment plant, the UNOX system. Site #4 was located where river water utilized in the plant's major cooling systems re-entered the Pamunkey River. Site #6 was slightly downstream from the plant's intake of water for the cooling system. Sites #3, 5, and 7 were adjacent to the west bank of the Pamunkey where marsh predominated. Site #10 was located inside the mouth of West Point Creek, which received effluent from the West Point sewage treatment plant and direct overflow from several of the sewage plant's pumping stations. In addition, there were private establishments along the creek, at least one of which had been cited in past surveys for contributing kitchen waste to the creek. Sites #8 and 9 were proximate to private residential areas of high density. Immediately upstream from #9 were several homes and marinas also cited in past sanitary surveys. Sites #12 and 13 were in the York River downstream from West Point. Salinity ranged from 2 ~ 5 o/oo at sites 12 and 13, while at sites #1 and 11, the salinity was 0 - 2 o/oo. The entire area is currently "restricted" for the harvesting of shellfish.

Figure 1. Location of sampling stations

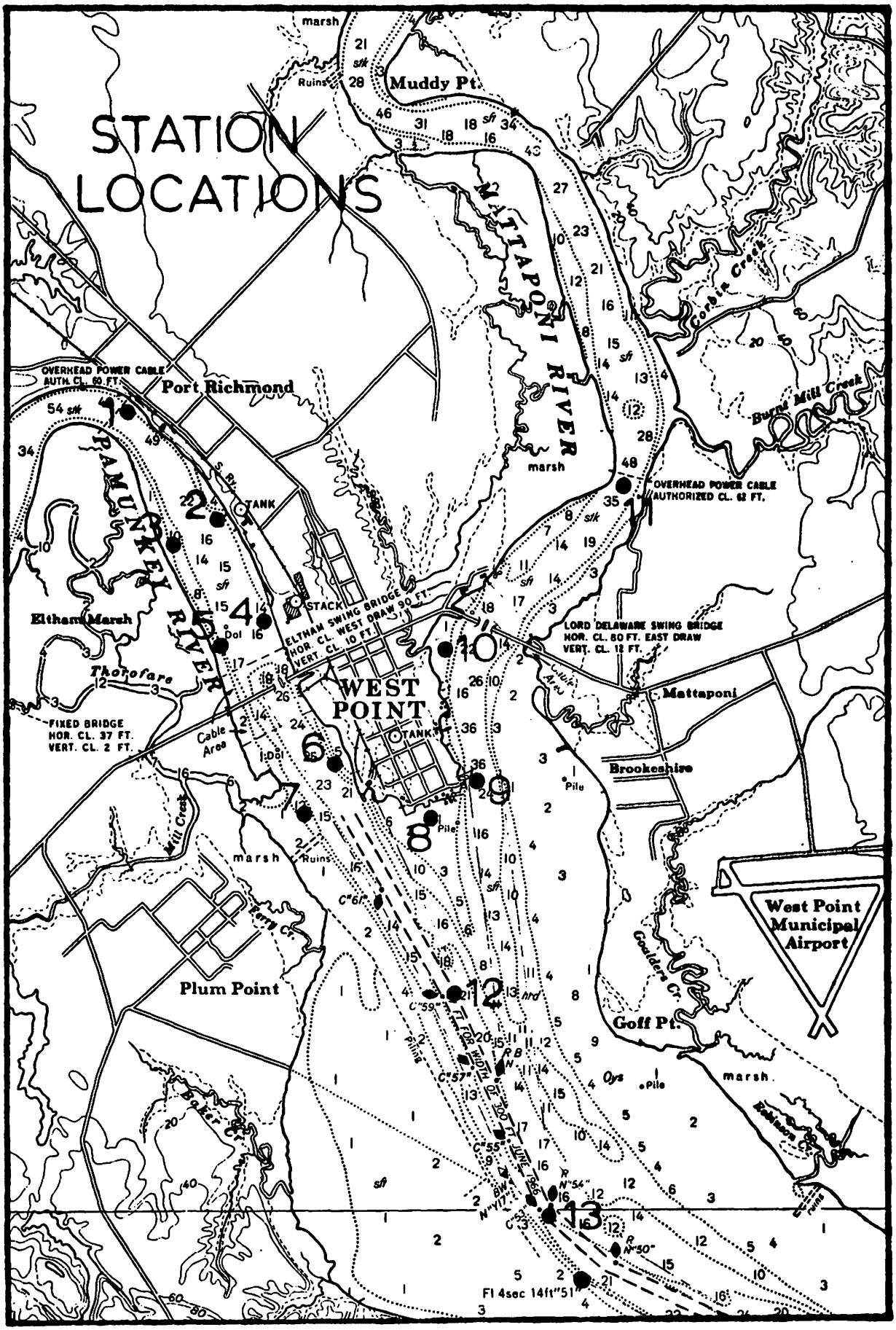

Figure 2 is a diagram of the paper mill's waste water treatment system. All water in the system originated from artesian wells, Samples were regularly taken from effluents of the paper mill, pulp mill, bleach plant and caustic sumps. The combined waste water resulting from these various paper-manufacturing processes was sampled immediately before entry into a primary clarifier, where settling of a portion of suspended particulates occurred. Waste water was next sampled after its passage through a cooling tower and then sampled from the UNOX system. Finally, treated waste water from the UNOX was sampled after emergence from secondary clarifiers prior to discharge into the Pamunkey River at site #2.

A schematic of the salt water cooling system is illustrated in Figure 3. Salt water entered at the salt water intake, slightly upstream from River site #6 and then passed through several condenser units. Samples were obtained at points immediately after passage through blow heat condensers (part of B Set discharge). The heated water next passed through open canals to enter Dozier's Closure, a settling pond originally designed to prevent plant debris, such as wood chips, from entering the river. Samples were regularly taken at depths of 1 m at both the influent and discharge ends of the closure.

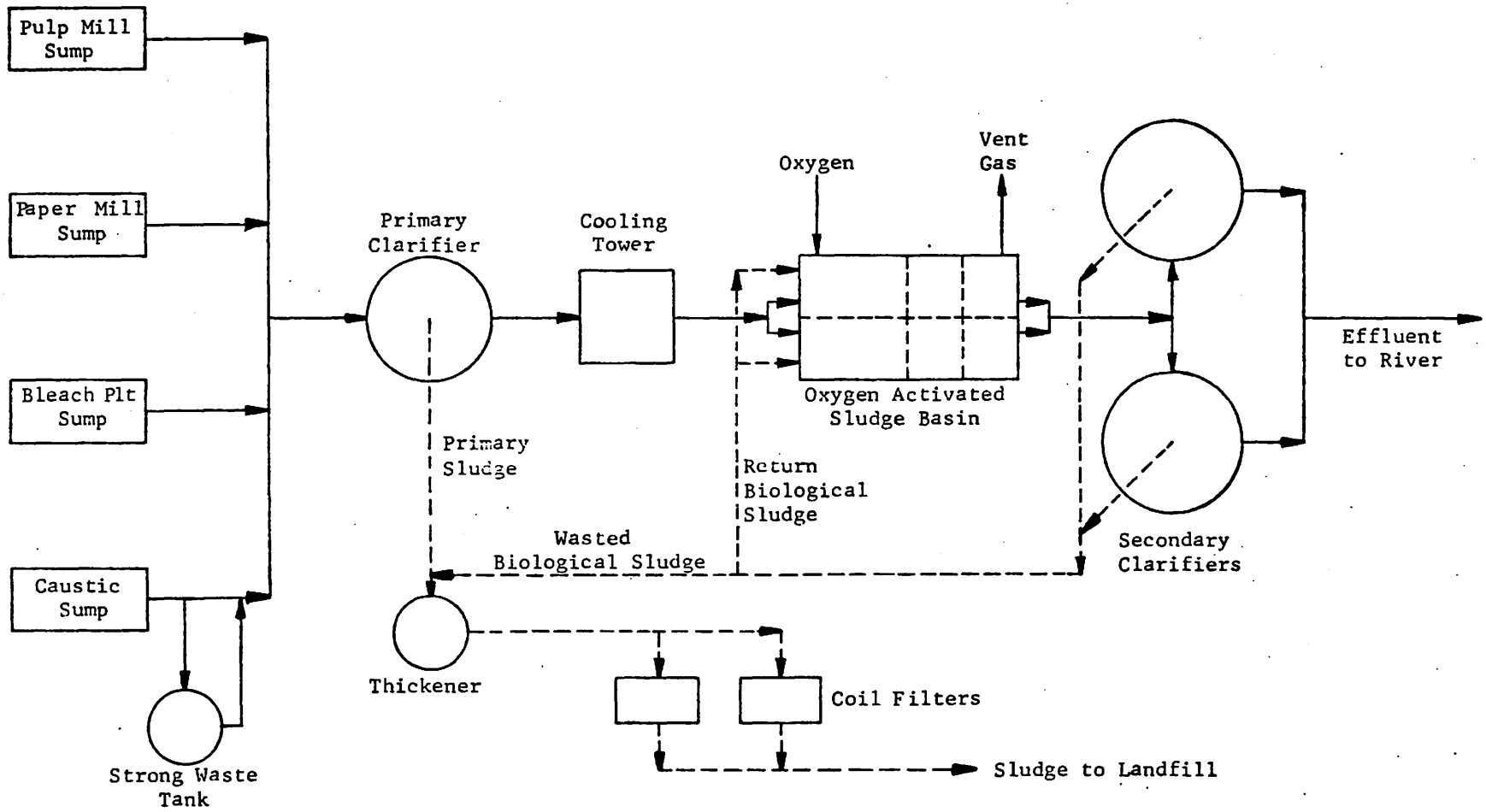

Figure 2. Flow diagram of waste water treatment plant.

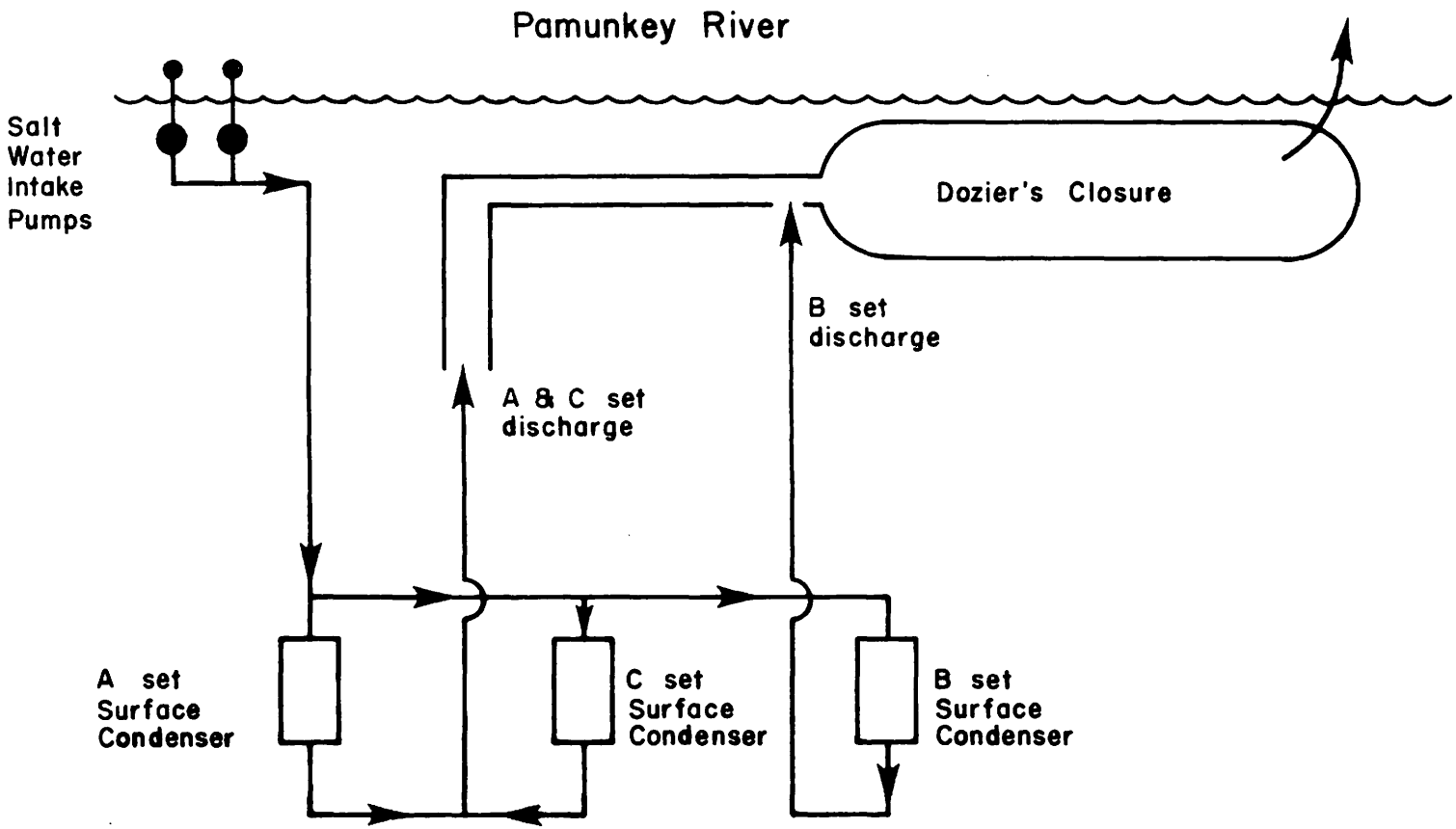

Figure 3. Flow diagram of salt water cooling system.

Plant and river surveys were performed at approximately bi-monthly intervals over a period of one year in order to assess the effects of season on bacterial populations. Surveys were never performed earlier than 3 days following rainfall. River samples were collected in sterile milk dilution bottles during low slackwater at a depth of 1 m. All water samples were transported on ice to the laboratory and processed within 2 hrs of collection.

UNOX Surveys The UNOX influent, two compartments within the UNOX basin, UNOX effluent, and secondary clarifier effluent were sampled on 3 occasions during the year. On May 3, 1976, stations were sampled 3 times (morning, noon, afternoon) each according to a schedule allowing for the retention time of a parcel of water in each phase of the treatment system.All stations were sampled twice on June 23, 1976, first as described above following flow through the system, and second, taking all samples simultaneously. Simultaneous sampling, using 3 replicate samples per station, took place on January 20, 1977.

Bacteriological Methods

The most probable number technique (five tube) was employed to enumerate populations of total coliforms, fecal coliforms, fecal streptococci, and total heterotrophic bacteria (APHA, 1975). Total coliform levels were determined using lactose and brilliant green bile broth as the presumptive and confirmatory media respectively. Inoculated media was incubated at $35 \pm 0.5^{o}C$ and examined for gas production after 48 ± 3 hrs. Fecal coliforms were enumerated by inoculating EC medium

from positive lactose tubes and incubating at 44.5 ± 0.2°C for 24 ± 2 hrs. Azide dextrose broth and ethyl violet broth were used as presumptive and confirmatory media for the enumeration of fecal streptococci and incubated at 35 ± 0.5°C for 48 ± 3 hrs. Isolates from selected surveys of the UNOX system were subjected to the following tests to verify that they belonged to the fecal streptococci group: gram stain, catalase production, growth in 0.1% methylene blue milk and 6.5% NaCl, growth at 45°C and 10°C, and starch hydrolysis.

Total heterotrophic populations were enumerated by inoculation of tryptone glucose yeast broth and examined for growth after 2 weeks incubation at ambient room temperature.

Analysis of coliform species composition was initiated by streaking EC positive tubes on eosin methylene blue agar. All representative colony types were isolated and reinoculated into EC broth. Isolates established as EC + were identified using the API-20 Enterobacteriaceae system (AnalyTab Products, Inc.).

Salmonella detection

A combination of membrane filtration and most probable number techniques were used to detect and enumerate Salmonella sp. One hundred ml volumes of water were filtered through sterile membrane filters (0.45 microns). Replicate filters were transferred to 50 ml volumes of selenite cysteine and m-tetrathionate enrichment broths and incubated at 41.5 ± 0.5°C for 48 hrs. Replicate 10 ml, 1 ml, and 0.1 ml quantities were also inoculated into the above media. All enrichment cultures were streaked on brilliant green sulfa agar and suspected Salmonella

colonies identified using triple sugar iron agar and API 20 E biochemical strips.

In Situ Growth Studies

Although it was possible that undetected sanitary discharges were still being introduced into Dozier's Closure at the termination of this research, it was decided to test the hypothesis that Dozier's Closure provided conditions conducive to the saprophytic growth of indicator bacteria. Therefore, the following experiment was designed. Water was collected from the discharge end of Dozier's Closure and sterilized by either autoclaving or membrance filtration (0.45 micron). Both types of sterilized water samples were then inoculated with indicator bacteria obtained from recent in-plant surveys. Escherichia coli, Klebsiella pneumoniae and Streptococcus sp. cultures were grown for 24 hrs in trypticase soy broth at 35°C, harvested by centrifugation, and washed three times in PBS (phosphate buffered saline). Washed suspensions were incubated at 35°C for 4 hrs in PBS to reduce endogenous metabolism and then stored for 18 hrs at 4°C before the experiment. These cultures were then combined and suspended in closure water sterilized by either autoclaving or filtration, ambient closure water, or sterile PBS yielding final concentrations of approximately 5.0×10^1/ml E. coli, 1.0×10^1/ml K. pneumoniae, and 5.0×10^1/ml Streptococcus sp. Volumes of inoculated water from each treatment were transferred to replicate dialysis bags, sealed tightly and suspended in Dozier's Closure.

Individual bags representing each treatment were removed from the closure at 0, 12, 24, 36, 48 and 96 hour intervals following initial

submersion. Bags were swabed, opened aseptically with an alcohol-sterilized razor blade and the contents transferred aseptically to sterile tubes. Samples were iced and returned immediately to the laboratory for processing. Bacterial densities were determined by the spread plate technique using trypticase soy agar with 0.3% yeast extract, Pfizer enterococcus agar, and eosin methylene blue agar (enumerated after 24 - 48 hrs at 35 $\pm$ 0.5^{o}C).

Results

Bacteriological quality of selected river and in-plant stations with respect to heterotrophic and indicator bacteria.

Data collected during six surveys of river stations are presented in Tables 1-5. In general, the Pamunkey River site #4, proximate to Dozier's Closure, was characterized by elevated levels of indicator organisms. During the study, a major break in a high pressure pipeline was discovered leaking sanitary sewage into Dozier's Closure. Correction of this leak did not, however, result in an overall decrease of bacterial indicators at site #4 as assessed by the last three surveys (with the exception of fecal coliform levels during the extremely cold winter). Levels of total and fecal coliforms were occassionally elevated at sites #7 and #8. Previous hydrographic studies of the Pamunkey River indicated that plant effluents tend to "hug" the east bank where these stations were located. Populations of fecal coliforms and streptococci were generally higher at station #1 than downstream at station #2 near the discharge pipe from the secondary clarifier.

Levels of indicator bacteria in the area of station #10 (located in the creek which received effluent from the West Point sewage treatment plant) suggested the presence of sporadic fecal pollution. However, several businesses located on this creek have been cited in past sanitary surveys and, therefore, the West Point STP cannot be unequivocally considered as the source. Additional sources of pollution were probably located upstream from station #11 as evidenced by coliform and streptococci levels generally as high or higher than those observed at site #10.

Table 1. Total heterotrophic bacterial densities per 100 ml at selected river sampling sites.

Location	Sampling Date					
	4-6-76	6-7-76	8-2-76	11-15-76	2-10-77	4-11-77
River #1	2.8×10^5	4.9×10^6	2.3×10^6	9.2×10^6	7.0×10^6	3.3×10^6
River #2	1.6×10^7	$\geq 2.4 \times 10^8$	4.5×10^6	1.7×10^7	7.9×10^6	7.9×10^6
River #3	7.0×10^5	1.3×10^7	7.9×10^6	3.3×10^6	7.0×10^6	7.0×10^6
River #4	3.5×10^6	1.8×10^6	3.3×10^6	2.3×10^6	2.2×10^7	3.3×10^6
River #5	3.3×10^5	7.0×10^6	4.9×10^6	4.6×10^6	1.3×10^7	3.3×10^6
River #6	2.4×10^6	2.4×10^7	3.3×10^6	1.1×10^7	3.5×10^7	7.0×10^6
River #7	1.7×10^6	1.7×10^7	9.3×10^5	4.9×10^6	2.8×10^6	4.9×10^6
River #8	7.0×10^5	7.9×10^6	4.6×10^6	3.1×10^6	3.3×10^6	4.6×10^6
River #9	1.3×10^6	1.7×10^6	1.1×10^6	2.8×10^6	2.3×10^6	1.3×10^6
River #10	1.6×10^7	4.3×10^6	4.5×10^5	3.5×10^6	3.3×10^6	7.9×10^6
River #11	1.1×10^6	1.7×10^6	1.4×10^6	1.7×10^6	2.2×10^5	2.4×10^6
River #12	3.5×10^6	4.6×10^6	1.7×10^6	3.5×10^6	7.9×10^6	2.2×10^6
River #13	1.7×10^6	4.9×10^6	1.7×10^6	3.5×10^6	3.3×10^6	3.3×10^6

Table 2. Total coliform densities per 100 ml at selected river sampling sites.

Location	Sampling Date					
	4-6-76	6-7-76	8-2-76	11-15-76	2-10-77	4-11-77
River #1	1.6×10^3	4.9×10^3	9.5×10^2	1.3×10^2	2.8×10^2	2.2×10^2
River #2	7.9×10^1	4.6×10^3	1.3×10^3	1.3×10^2	2.2×10^2	4.9×10^2
River #3	7.9×10^1	6.4×10^3	1.7×10^3	3.3×10^2	3.1×10^2	1.7×10^2
River #4	3.5×10^4	7.9×10^3	5.4×10^4	4.9×10^2	2.3×10^3	3.3×10^3
River #5	7.0×10^1	4.9×10^3	7.9×10^2	1.3×10^2	1.8×10^2	2.3×10^2
River #6	2.4×10^3	1.3×10^4	2.2×10^3	3.1×10^3	7.9×10^1	1.3×10^2
River #7	7.0×10^1	4.9×10^3	4.9×10^2	1.4×10^2	4.7×10^1	4.6×10^2
River #8	4.6×10^3	6.4×10^3	1.3×10^3	4.9×10^2	7.9×10^1	1.7×10^2
River #9	1.8×10^2	1.1×10^3	7.9×10^2	3.3×10^2	1.3×10^2	2.2×10^2
River #10	3.5×10^3	1.1×10^4	7.0×10^2	4.9×10^2	7.9×10^1	7.9×10^1
River #11	2.1×10^2	1.7×10^4	2.3×10^3	3.5×10^2	7.9×10^1	1.3×10^2
River #12	1.8×10^2	2.4×10^3	4.9×10^2	1.1×10^2	4.6×10^1	2.3×10^2
River #13	1.7×10^2	4.6×10^3	3.3×10^2	4.9×10^2	3.3×10^1	2.3×10^2

Table 3. Fecal coliform densities per 100 ml at selected river sampling sites.

Location	Sampling Date					
	4-6-76	6-7-76	8-2-76	11-15-76	2-10-77	4-11-77
River #1	1.7×10^1	3.3×10^1	7.9×10^1	1.7×10^1	7.8×10^0	2.2×10^2
River #2	7.8×10^0	2.3×10^1	7.9×10^1	1.7×10^1	2.3×10^1	3.3×10^2
River #3	1.3×10^1	3.3×10^1	3.3×10^1	1.4×10^1	4.5×10^0	1.3×10^2
River #4	3.1×10^2	3.3×10^2	1.3×10^4	3.3×10^1	6.4×10^1	7.9×10^2
River #5	1.4×10^1	4.9×10^1	1.1×10^2	1.3×10^1	6.8×10^0	7.9×10^1
River #6	7.9×10^1	7.9×10^1	4.9×10^2	1.1×10^2	n.d.[a]	2.3×10^1
River #7	6.8×10^0	3.3×10^1	1.3×10^2	1.7×10^1	4.5×10^0	1.1×10^2
River #8	1.7×10^1	7.9×10^1	4.9×10^2	1.3×10^1	2.0×10^0	3.3×10^1
River #9	3.3×10^1	4.5×10^0	3.3×10^1	4.5×10^0	2.0×10^0	7.9×10^1
River #10	3.1×10^2	4.9×10^1	4.6×10^1	1.3×10^1	n.d.[a]	3.3×10^1
River #11	2.3×10^1	3.3×10^1	4.6×10^1	1.3×10^1	2.0×10^0	3.3×10^1
River #12	7.8×10^0	3.3×10^1	1.7×10^2	1.3×10^1	2.0×10^0	1.3×10^2
River #13	1.3×10^1	7.9×10^1	7.0×10^1	7.8×10^0	n.d.[a]	7.9×10^1

[a] n.d. = not detected

Table 4. Fecal streptococcus densities per 100 ml at selected river sampling sites.

Location	Sampling Date					
	4-6-76	6-7-76	8-2-76	11-15-76	2-10-77	4-11-77
River #1	6.1×10^0	1.7×10^3	4.9×10^2	7.0×10^1	2.1×10^1	2.3×10^1
River #2	1.3×10^1	3.5×10^3	7.9×10^1	3.3×10^1	1.1×10^2	1.3×10^2
River #3	4.5×10^0	2.8×10^2	3.3×10^2	6.8×10^0	4.6×10^1	1.1×10^2
River #4	1.1×10^3	1.7×10^3	1.3×10^4	1.8×10^2	7.9×10^2	2.8×10^2
River #5	n.d.[a]	7.9×10^2	7.9×10^2	6.8×10^0	4.5×10^0	8.4×10^1
River #6	4.5×10^0	3.5×10^2	3.3×10^2	7.9×10^2	1.1×10^1	4.3×10^1
River #7	4.5×10^0	2.1×10^2	1.3×10^3	1.1×10^2	7.8×10^0	3.3×10^1
River #8	2.3×10^1	4.9×10^2	3.3×10^2	3.3×10^1	4.0×10^0	1.7×10^1
River #9	7.8×10^0	7.9×10^1	4.9×10^1	1.1×10^1	6.8×10^0	3.3×10^1
River #10	7.9×10^1	4.9×10^2	3.3×10^1	2.0×10^0	n.d.[a]	7.8×10^0
River #11	6.8×10^0	7.9×10^2	3.3×10^1	1.4×10^1	n.d.[a]	1.7×10^1
River #12	7.8×10^0	4.9×10^2	1.7×10^2	5.6×10^0	1.3×10^1	1.7×10^1
River #13	1.4×10^1	7.9×10^2	9.5×10^1	2.6×10^1	7.8×10^0	2.1×10^1

[a] n.d. = not detected

Table 5. Temperature and salinity measurements at selected river sampling sites.

Location	Sampling Date											
	4-6-76		6-7-76		8-2-76		11-15-76		2-10-77		4-11-77	
	T °C	Salinity ‰	T °C	Salinity ‰	T °C	Salinity ‰	T °C	Salinity ‰	T °C	Salinity ‰	T °C	Salinity ‰
River #1	15.0	0.0	23.5	2.0	28.0	—[a]	7.5	2.0	1.5	2.5	19.0	0.6
River #2	15.0	1.0	23.5	2.0	28.0	—	7.5	4.0	2.0	4.0	19.0	1.0
River #3	16.0	0.0	28.0	2.0	28.5	—	7.5	4.0	3.0	3.0	19.0	1.0
River #4	21.5	0.0	24.0	2.5	39.0	—	7.5	4.0	24.5	4.0	31.0	1.8
River #5	17.0	0.0	28.0	2.2	28.0	—	7.5	4.5	3.0	3.5	19.0	1.0
River #6	15.5	0.0	23.5	3.8	28.5	—	7.5	6.0	3.0	5.5	19.0	2.0
River #7	16.0	1.0	23.5	4.0	28.0	—	7.5	6.0	3.0	6.0	19.0	2.4
River #8	16.0	1.8	23.5	3.8	28.0	—	7.5	6.5	2.5	7.5	19.0	2.2
River #9	17.0	0.0	24.0	3.8	28.5	—	7.5	3.0	2.5	5.0	18.0	2.0
River #10	17.0	0.2	24.0	2.0	29.0	—	7.5	2.0	3.0	4.5	19.0	1.0
River #11	15.5	0.0	23.0	2.0	28.5	—	7.5	2.0	2.5	4.0	19.0	0.6
River #12	15.0	1.8	23.5	4.0	28.0	—	7.5	6.0	2.0	6.2	19.0	3.0
River #13	16.0	2.0	23.5	5.0	28.0	—	7.0	5.5	3.0	7.5	19.0	4.0

[a] refractometer not available

Stations located farthest downstream, #12 and #13, were characterized by levels of indicator bacteria similar in magnitude to those observed at the residential areas located near the convergence of the Pamunkey and Mattaponi River, sites #8 and #9. Except for levels observed during the colder months, fecal coliform densities generally exceeded the state shellfish harvesting standard of $1.4 \times 10^1/100$ ml.

To assess the seasonal stability of bacterial populations, a non-parametric statistical test was used to determine the uniformity of counts as a function of sampling date. Test results indicated that the counts were not statistically uniform and the null hypothesis,i.e., bacterial levels at any given station were the same for all surveys, was rejected for heterotrophic as well as indicator bacteria (Table 6). In the case of total heterotrophs, variations were small, although significant at alpha = 0.01, with levels generally ranging between $10^6 - 10^7/100$ ml. Relatively elevated heterotroph levels were observed on two occasions at river station #2 which was in the discharge area of the plant's UNOX facility. Higher densities were anticipated since large populations of heterotrophic bacteria are generated in the UNOX system, all of which do not settle out in the secondary clarifier (Table 7). Greater variations in populations of indicator bacteria appeared to be related to seasonal effects. The highest levels of total coliforms, fecal coliforms and fecal streptococci were generally observed during the warmer months of June and August when water temperatures were ca. 24-28° C (Table 5). In contrast, the lowest levels of indicator organisms were observed during the February survey when the water temperature was ca. 2-3°C.

Table 6. Values of the Friedman Test Statistic calculated for total heterotrophs, total coliforms, fecal coliforms, and fecal streptococci at river sampling sites for all surveys.

Bacterial Parameter	Summation of ranks with respect to survey date						Calculated T Value[a]	Critical T Value[b] (alpha = 0.01) for Rejection of H_o	H_o: Results from all surveys are identical
	4-6-76	6-7-76	8-2-76	11-15-76	2-10-77	4-11-77			
Total Heterotrophs	31.5	62.5	30.5	50.5	52.5	45.5	17.25	15.09	Rejected
Total Coliforms	39	76	60	39	22.5	36.5	40.35	15.09	Rejected
Fecal Coliforms	38.5	54	69	32	16.5	63	44.07	15.09	Rejected
Fecal Streptococci	26	73.5	64.5	39	26	44	42.90	15.09	Rejected

a Friedman nonparametric test statistic. The Friedman test statistic compares the sums of the ranks of mutually independent variables (e.g. total coliform counts at a given station sampled 6 times per year)and yields a statistical measure of the variation in these values over various seasons assuming non-normality.(Practical Nonparametric Statistics, W. J. Conover, John Wiley and Sons, Inc., 1971).

b Critical T Value must be exceeded by calculated T to reject null hypothesis.

Table 7. Total heterotrophic bacterial densities per 100 ml at selected in-plant locations.

Location	Sampling Date					
	4-6-76	6-7-76	8-2-76	11-15-76	2-10-77	4-11-77
Paper Mill Sump	4.9×10^7	5.4×10^8	6.4×10^7	9.2×10^8	1.3×10^8	7.0×10^8
Pulp Mill Sump	3.3×10^5	2.4×10^7	1.8×10^5	4.9×10^6	2.8×10^7	$<1.8 \times 10^5$
Bleach Plant Sump	n.d.[a]	7.9×10^2	3.3×10^3	1.7×10^2	___[b]	7.9×10^3
Caustic Sump	n.d.	$\geq 2.4 \times 10^5$	$\geq 2.4 \times 10^6$	7.0×10^6	1.3×10^7	3.3×10^5
Primary Clarifier	9.2×10^6	3.3×10^8	$\leq 1.8 \times 10^5$	4.9×10^7	7.9×10^7	1.7×10^8
UNOX Effluent	$\geq 2.4 \times 10^7$	5.4×10^{11}	4.9×10^{10}	3.3×10^{10}	4.6×10^{10}	2.2×10^{10}
Secondary Clarifier	$\geq 2.4 \times 10^7$	4.9×10^9	7.9×10^9	1.7×10^9	2.3×10^9	3.3×10^9
Salt Water Intake	3.5×10^6	1.3×10^7	7.0×10^6	1.3×10^7	1.3×10^7	3.3×10^6
Blow Heat Condensers	6.4×10^6	1.3×10^7	7.9×10^5	5.4×10^6	___[b]	7.9×10^6
Inlet Dozier's Closure	4.9×10^6	1.3×10^7	1.4×10^6	1.6×10^7	2.4×10^7	1.3×10^7
Discharge Dozier's Closure	7.9×10^6	3.5×10^7	4.6×10^6	9.2×10^6	1.1×10^7	7.9×10^6

[a] n.d. = not detected

[b] not in operation

Data obtained from in-plant sampling sites are presented in Tables 7 - 11. Of the various plant process waste waters, the paper mill sump contributed most significantly to total and fecal coliform densities in the primary clarifier. Fecal streptococci were derived in large number not only from the paper mill sump but also from the pulp mill and to a lesser extent from the caustic sump. On the basis of completed biochemical tests, fecal streptococcus densities from these samples were not due to false positives. Samples of source artesian water used in various plant process waters were negative for the presence of indicator organisms. UNOX effluent and secondary clarifier samples were characterized by relatively high total coliform and fecal streptococcus densities, i.e. 10^2 - 10^3/100 ml as opposed to fecal coliform densities generally less than 10^1/100 ml.

Also included in Tables 7 - 10 are levels of heterotrophic and indicator bacteria prior to and after passage through the plant's river-fed salt water cooling system. Although the system had the potential for accelerated saprophytic growth due to the elevated temperature, populations of bacterial indicators remained relatively constant across the system.

Enumeration results from the various plant process wastes prior to and after treatment in the UNOX system were also analyzed using the Friedman test for uniformity (Table 12). Results from the bleach plant were excluded since coliforms were detected on only one occasion and at very low levels, i.e. 2 x 10^0/100 ml. Levels of total heterotrophs obtained during the first survey were also not included in computations

Table 8. Total coliform densities per 100 ml at selected in-plant locations.

Location	Sampling Date					
	4-6-76	6-7-76	8-2-76	11-15-76	2-10-77	4-11-77
Paper Mill Sump	1.4×10^3	1.6×10^5	9.5×10^3	2.4×10^4	1.7×10^4	1.6×10^5
Pulp Mill Sump	n.d.[a]	7.8×10^0	2.0×10^0	7.9×10^1	2.8×10^2	n.d.
Bleach Plant Sump	n.d.	n.d.	n.d.	n.d.	___ [b]	2.0×10^0
Caustic Sump	n.d.	1.1×10^1	$\geq 2.4 \times 10^1$	4.9×10^1	1.8×10^0	1.3×10^1
Primary Clarifier	4.9×10^1	7.9×10^1	n.d.	3.3×10^1	1.3×10^1	$\geq 2.4 \times 10^4$
UNOX Effluent	4.6×10^3	4.9×10^3	1.3×10^3	7.0×10^3	4.9×10^2	1.6×10^5
Secondary Clarifier	9.2×10^2	4.6×10^2	2.3×10^2	1.3×10^3	2.3×10^2	1.6×10^5
Salt Water Intake	2.4×10^4	4.9×10^3	$\geq 2.4 \times 10^1$	4.9×10^3	7.8×10^1	7.9×10^2
Blow Heat Condensers	5.4×10^3	7.9×10^3	$\geq 2.4 \times 10^1$	5.8×10^3	___ [b]	4.9×10^2
Inlet Dozier's Closure	1.3×10^5	1.3×10^4	1.6×10^5	1.6×10^5	2.2×10^3	3.3×10^2
Discharge Dozier's Closure	5.4×10^5	2.3×10^4	5.4×10^4	2.3×10^4	3.1×10^3	3.3×10^3

[a] n.d. = not detected

[b] not in operation

Table 9. Fecal coliform densities per 100 ml at selected in-plant locations.

Location	Sampling Date					
	4-6-76	6-7-76	8-2-76	11-15-76	2-10-77	4-11-77
Paper Mill Sump	3.5×10^2	7.0×10^3	4.6×10^2	4.9×10^3	4.9×10^2	2.2×10^4
Pulp Mill Sump	n.d.[a]	2.0×10^0	n.d.	2.3×10^1	2.2×10^1	n.d.
Bleach Plant Sump	n.d.	n.d.	n.d.	n.d.	___ [b]	2.0×10^0
Caustic Sump	n.d.	2.0×10^0	$\geq 2.4 \times 10^1$	1.7×10^1	n.d.	2.0×10^0
Primary Clarifier	n.d.	1.7×10^1	n.d.	1.1×10^1	n.d.	1.7×10^2
UNOX Effluent	2.0×10^0	7.8×10^0	6.1×10^0	1.1×10^1	7.8×10^0	1.6×10^5
Secondary Clarifier	4.0×10^0	4.5×10^0	1.8×10^0	4.0×10^0	2.0×10^0	1.6×10^5
Salt Water Intake	1.6×10^3	4.9×10^1	$\geq 2.4 \times 10^1$	1.7×10^2	$< 1.8 \times 10^1$	7.9×10^1
Blow Heat Condensers	7.9×10^1	1.7×10^2	$\geq 2.4 \times 10^1$	2.2×10^2	___ [b]	7.9×10^1
Inlet Dozier's Closure	4.9×10^3	1.8×10^3	7.9×10^3	3.3×10^3	3.3×10^1	4.9×10^2
Discharge Dozier's Closure	4.9×10^3	4.9×10^3	1.1×10^4	3.3×10^3	4.6×10^1	1.3×10^3

[a] n.d. = not detected

[b] not in operation

Table 10. Fecal streptococcus densities per 100 ml at selected in-plant locations.

Location	Sampling Date					
	4-6-76	6-7-76	8-2-76	11-15-76	2-10-77	4-11-77
Paper Mill Sump	3.3×10^2	1.3×10^4	3.3×10^2	3.3×10^3	2.4×10^4	4.3×10^4
Pulp Mill Sump	1.7×10^2	1.7×10^3	7.0×10^1	1.6×10^4	3.5×10^4	2.7×10^1
Bleach Plant Sump	n.d.[a]	n.d.	n.d.	n.d.	___[b]	n.d.
Caustic Sump	n.d.	4.5×10^0	2.2×10^2	2.4×10^3	1.1×10^1	2.2×10^2
Primary Clarifier	5.4×10^2	1.7×10^3	1.3×10^1	7.9×10^2	2.8×10^3	4.3×10^3
UNOX Effluent	1.5×10^3	1.1×10^2	1.7×10^2	1.4×10^2	4.6×10^2	$\geq 2.4 \times 10^5$
Secondary Clarifier	1.7×10^3	2.3×10^2	1.4×10^2	2.2×10^2	4.9×10^2	1.6×10^5
Salt Water Intake	5.4×10^2	4.9×10^2	1.4×10^3	4.6×10^2	2.2×10^1	9.5×10^2
Blow Heat Condensers	1.4×10^1	7.9×10^2	4.6×10^2	7.9×10^2	___[b]	1.4×10^2
Inlet Dozier's Closure	7.0×10^3	1.3×10^3	1.3×10^4	7.9×10^3	1.3×10^3	1.3×10^3
Discharge Dozier's Closure	1.6×10^4	4.6×10^3	7.9×10^3	3.3×10^3	2.2×10^4	2.3×10^3

[a] n.d. = not detected

[b] not in operation

Table 11. Temperature measurements at selected in-plant locations. Values in °C.

Location	Sampling Date					
	4-6-76	6-7-76	8-2-76	11-15-76	2-10-76	4-15-77
Paper Mill Sump	44.0	45.0	46.0	49.0	43.0	46.0
Pulp Mill Sump	51.0	53.0	51.0	49.0	43.0	53.0
Bleach Plant Sump	53.0	58.0	58.0	54.0	— [a]	52.0
Caustic Sump	48.0	50.0	51.0	47.0	35.0	47.0
Primary Clarifier	49.0	50.0	50.0	48.0	42.0	44.0
UNOX Effluent	36.0	36.0	36.5	39.0	36.0	36.0
Secondary Clarifier	36.0	34.0	34.0	36.0	34.0	36.0
Salt Water Intake	16.0	24.0	23.0	9.0	2.0	16.5
Blow Heat Condensers	34.0	22.2	25.0	27.0	— [a]	45.0
Inlet Dozier's Closure	38.0	42.0	39.0	30.0	27.0	30.0
Discharge Dozier's Closure	39.0	36.0	39.0	29.0	28.0	30.0

[a] not in operation

Table 12. Values of the Friedman Test Statistic calculated for total heterotrophs, total coliforms, fecal coliforms and fecal streptococci in process waste waters for all surveys.

Bacterial Parameter	Summation of ranks with respect to survey date						Calculated T Value[b]	Critical value of T for rejection of null hypothesis H_o		H_o: Results from all surveys are identical	
	4-6-76	6-7-76	8-2-76	11-15-76	2-10-77	4-11-77		alpha=0.01	alpha=0.05	alpha=0.01	alpha=0.05
Total Heterotrophs	-	22	16	17	20	15	2.28	13.28	9.49	Accepted	Accepted
Total Coliforms	14.5	24.5	14.5	28	15.5	29	11.52	15.09	11.07	Accepted	Rejected
Fecal Coliforms	11	26	15	27.5	17	29.5	14.02	15.09	11.07	Accepted	Rejected
Fecal Streptococci	17.5	18	13	21	27	29.5	9.31	15.09	11.07	Accepted	Accepted

[a] Calculations based on data obtained from samples representing the paper mill sump, pulp mill sump, caustic sump, primary clarifier, UNOX effluent and secondary clarifier.

[b] Friedman nonparametric test statistic (Practical Nonparametric Statistics, W.J. Conover, John Wiley and Sons, Inc., 1971).

because results of treated wastes were indeterminately high. Statistical analysis revealed that heterotrophic and indicator bacterial populations were relatively stable over the various sampling periods and the null hypothesis was accepted at a significance level of alpha = 0.01. When alpha = 0.05, the null hypothesis was rejected for both total and fecal coliforms. The data indicate rejection of the null hypothesis was primarily due to elevated total coliform and fecal coliform levels from UNOX effluent and secondary clarifier samples collected during the last survey (April 11, 1977). At this time total coliform populations were ca. 2 orders of magnitude higher than previously observed and fecal coliforms were ca. 4-5 orders of magnitude larger. Higher coliform levels were also accompanied by fecal streptococcus populations elevated over previous surveys 2 - 3 orders of magnitude. Corresponding temperature at the time of sampling (Table 11) indicated that the temperature ranges were fairly uniform over all sampling periods. It is possible that the changes in the indicator populations during the last survey were related to the elevated BOD level. Increased bacterial densities were accompanied by a BOD value of 54 mg/1 (Table 13) in the secondary clarifier as compared to a normal average value of 30 mg/1. However, BOD levels were elevated on two other occasions (Table 13) and bacterial densities were not characterized by elevated fecal coliform and fecal streptococcus populations.

Table 13. BOD levels of the secondary clarifier[a].

Date of Bacterial Sampling	BOD(mg/1)		
	Day of Sampling	Day prior to Sampling	Day after Sampling
4-6-76	33	48	29
6-7-76	99	71	64
8-2-76	28	16	21
11-15-76	72	69	36
2-10-77	32	35	28
4-11-77	54	59	43

[a] Average BOD = 30 mg/1 (Chesapeake Corporation data).

Bacteriological analysis of the UNOX treatment system with respect to heterotrophic and indicator bacteria.

Influent to the UNOX waste water system was characterized by relatively high levels of total coliforms and fecal streptococci with median values of 4.6×10^3/100 ml and 3.5×10^2/100 ml respectively whereas fecal coliform populations were substantially lower with a median value of 2.2×10^1/100 ml (Tables 14-16). There was no evidence that multiplication of indicator bacteria occurred across the system. Total coliform populations decreased approximately 2 orders of magnitude while fecal coliform and streptococcus densities remained relatively unchanged. In contrast, heterotrophic bacterial populations increased approximately 2 orders of magnitude within the UNOX system and remained elevated after passage through the secondary clarifier. Measurements taken on one occasion and routine observations during sampling showed that pH and temperature were relatively stable within the UNOX system (Table 17). Comparison of data shown in Tables 14 - 16 indicated little difference in bacterial counts using an interval sampling regime designed to compensate for the residence time of a given parcel of water through the UNOX system as opposed to sampling all UNOX components simultaneously.

Although saprophytic growth of indicator bacteria did not occur in the UNOX system, significant residual levels of these bacteria were ultimately discharged into the Pamunkey River. It is difficult to isolate the impact of the UNOX effluent on the river quality due to the presence of fecal coliforms at Station #1 as well as in the discharge from Dozier's Closure (Tables 3 & 9). Calculations using an average of 12.5 MGD as the UNOX discharge volume and median levels of indicator bacteria

Table 14. Total heterotrophic and indicator bacterial densities in waste waters prior to and after treatment in the UNOX system on May 3, 1976.[a]

Location	Total Heterotrophs /100ml.	Total Coliforms /100ml.	Fecal Coliforms /100ml.	Confirmed Streptococci /100ml.	Completed Streptococci /100ml.
UNOX influent					
W025	7.9×10^{7}	1.1×10^{4}	2.3×10^{1}	9.5×10^{1}	9.5×10^{1}
W030	9.5×10^{7}	4.6×10^{3}	0.0	3.5×10^{2}	3.5×10^{2}
W035	1.3×10^{8}	2.3×10^{3}	2.0×10^{0}	2.3×10^{3}	2.3×10^{3}
1st Chamber					
W026	$\geq 2.4 \times 10^{10}$	1.1×10^{4}	4.5×10^{0}	7.9×10^{1}	7.9×10^{1}
W031	$\geq 2.4 \times 10^{10}$	1.3×10^{4}	6.8×10^{0}	3.5×10^{3}	3.5×10^{3}
W036	$\geq 2.4 \times 10^{10}$	1.3×10^{4}	2.0×10^{0}	2.3×10^{3}	2.3×10^{3}
2nd Chamber					
W027	$\geq 2.4 \times 10^{10}$	3.3×10^{4}	4.0×10^{0}	1.1×10^{2}	1.1×10^{2}
W032	$\geq 2.4 \times 10^{10}$	1.7×10^{5}	1.7×10^{1}	1.1×10^{3}	1.1×10^{3}
W037	$\geq 2.4 \times 10^{10}$	9.2×10^{5}	1.3×10^{1}	1.6×10^{3}	1.6×10^{3}
UNOX effluent					
W028	1.6×10^{10}	3.3×10^{3}	1.1×10^{1}	2.2×10^{2}	1.4×10^{2}
W033	$\geq 2.4 \times 10^{10}$	3.3×10^{3}	0.0	9.2×10^{2}	9.2×10^{2}
W038	1.6×10^{10}	2.3×10^{3}	1.8×10^{0}	2.8×10^{3}	2.8×10^{3}
2nd Clarifier effluent					
W029	3.5×10^{9}	2.3×10^{3}	7.8×10^{0}	4.9×10^{2}	4.9×10^{2}
W034	7.9×10^{8}	3.1×10^{2}	1.7×10^{1}	4.9×10^{3}	4.9×10^{2}
W039	2.8×10^{9}	7.9×10^{2}	1.7×10^{1}	1.4×10^{3}	1.4×10^{3}

[a] Samples were collected at ca. 1/2 - 1 hr intervals to compensate for the calculated residence time of a parcel of water passing through the UNOX system.

Table 15. Total heterotrophic and indicator bacterial densities in waste waters prior to and after treatment in the UNOX system on July 23, 1976.[a]

Location	Total Heterotrophs /100ml.	Total Coliforms /100ml.	Fecal Coliforms /100ml.	Confirmed Streptococci /100ml.
UNOX Influent				
W067	3.5×10^{8}	1.3×10^{2}	1.3×10^{1}	7.0×10^{1}
W072	3.5×10^{8}	2.3×10^{2}	2.2×10^{1}	3.3×10^{1}
1st Chamber				
W068	3.3×10^{10}	1.3×10^{2}	4.5×10^{0}	2.3×10^{2}
W073	7.9×10^{10}	3.3×10^{2}	1.3×10^{1}	1.3×10^{2}
2nd Chamber				
W069	4.9×10^{10}	2.2×10^{3}	1.4×10^{1}	1.7×10^{2}
W074	4.6×10^{10}	7.9×10^{2}	1.7×10^{1}	3.3×10^{2}
UNOX Effluent				
W070	4.9×10^{10}	1.3×10^{2}	4.5×10^{0}	7.9×10^{1}
W075	7.9×10^{10}	3.1×10^{1}	4.5×10^{0}	1.1×10^{2}
2nd Clarifier Effluent				
W071	1.7×10^{9}	1.3×10^{1}	0.0	2.7×10^{1}
W076	7.9×10^{9}	3.3×10^{1}	0.0	4.9×10^{1}

[a] The first set of samples (W067- W076)was collected at ca. 1/2 - 1 hr intervals to compensate for the calculated residence time of a parcel of water passing through the UNOX system. The second set of samples (W072 - W076) were collected simultaneously).

Table 16. Total heterotrophic and indicator bacterial densities in waste waters prior to and after treatment in the UNOX system on January 20, 1977.[a]

Location	Total Heterotrophs /100 ml	Total Coliforms /100 ml	Fecal Coliforms /100 ml	Confirmed Streptococci /100 ml
UNOX Influent				
W128	3.5×10^{9}	1.1×10^{4}	2.2×10^{2}	1.3×10^{3}
W129	2.8×10^{9}	4.6×10^{3}	2.3×10^{2}	1.3×10^{3}
W130	2.8×10^{9}	1.1×10^{4}	7.9×10^{1}	2.4×10^{3}
1st Chamber				
W131	7.0×10^{9}	3.3×10^{3}	2.2×10^{2}	3.3×10^{3}
W132	3.3×10^{9}	1.3×10^{4}	2.3×10^{2}	2.4×10^{3}
W133	1.7×10^{10}	7.9×10^{3}	1.5×10^{2}	4.9×10^{2}
2nd Chamber				
W134	1.3×10^{10}	3.3×10^{3}	1.1×10^{2}	1.8×10^{3}
W135	4.9×10^{9}	7.9×10^{3}	1.7×10^{2}	2.4×10^{3}
W136	4.9×10^{9}	2.4×10^{4}	2.2×10^{2}	4.9×10^{2}
UNOX Effluent				
W137	1.7×10^{10}	2.3×10^{3}	1.4×10^{2}	1.3×10^{3}
W138	7.9×10^{9}	2.3×10^{3}	3.3×10^{2}	1.3×10^{3}
W139	4.9×10^{10}	3.3×10^{3}	7.9×10^{1}	1.3×10^{3}
2nd Clarifier Effluent				
W140	1.7×10^{9}	2.2×10^{3}	7.9×10^{1}	1.3×10^{3}
W141	2.3×10^{9}	1.7×10^{3}	1.7×10^{2}	3.1×10^{3}
W142				

[a] All samples were collected simultaneously.

Table 17. Mean pH and temperature of UNOX system on May 3, 1977.

Location	Mean pH
UNOX Influent	9.13
1st Chamber	6.81
2nd Chamber	6.75
UNOX Effluent	6.61
2nd Clarifier Effluent	6.72

The mean temperature throughout the system was 36.6°C.

from secondary clarifier effluents (Tables 8-10, excluding survey 4-11-77) suggested daily bacterial loading of the river was ca. 218 billion total coliforms, 1.9 billion fecal coliforms and 109 billion fecal streptococci.

Species composition of fecal coliform samples from representative river, in-plant and UNOX system samples.

Positive fecal coliform tubes were analyzed for species present which would give a positive elevated temperature reaction in pure culture. Bacterial genera isolated from samples representing river stations, Dozier's Closure, the plant cooling system, mill sumps, and the primary clarifier are listed in Table 18.

Of the species identified, E. coli and K. pneumoniae predominated. The ratio of E. coli to K. pneumoniae appeared to be positively correlated with temperature (Table 19). A Kendall non-parametric correlation coefficient (Conover, 1971) calculated for this ratio with temperature at river stations yielded a significant positive correlation coefficient (alpha = 0.01) of $\tau = .46$. Despite the fact that these numbers were derived from MPN values, the data suggested that E. coli levels increased with temperature while the population of K. pneumoniae giving positive elevated temperature tests remained fairly constant. When greater numbers of both organisms were observed in the relatively constant warm water in Dozier's Closure, populations of E. coli always exceeded those of K. pneumoniae.

Analysis of samples from the UNOX waste water system (Table 20) revealed similar bacterial types with E. coli and K. pneumoniae being the species most frequently isolated. However, as the absolute

Table 18. Species compositional analysis of positive fecal coliform samples representing river and in-plant sites.

Location	Date Sampled	Species Present	Organisms/100ml	Station Temp. °C
River #1	11-15-76	E. coli K. pneumoniae	4.5×10^0 1.1×10^1	7.5
River #2	8-2-76	E. coli K. pneumoniae	7.9×10^1 4.5×10^0	28.0
River #3	11-15-76	E. coli K. pneumoniae	1.1×10^1 1.8×10^0	7.5
River #4	6-7-76	E. coli K. pneumoniae	3.3×10^2 8.3×10^0	24.0
	11-15-76	E. agglomerans E. coli K. pneumoniae	2.0×10^0 1.3×10^1 1.1×10^1	7.5
River #5	2-10-77	E. coli	6.8×10^0	3.0
River #6	6-7-76	E. cloacae E. coli K. pneumoniae	2.0×10^0 7.9×10^1 2.0×10^0	23.5
	11-15-76	E. coli K. pneumoniae	4.9×10^1 1.2×10^1	7.5
River #7	2-10-77	E. coli	4.5×10^0	3.0
River #8	2-10-77	E. coli	2.0×10^0	2.5
River #9	2-10-77	E. coli	2.0×10^0	2.5
River #10	8-2-76	E. coli K. pneumoniae	4.6×10^1 4.5×10^0	29.0

Table 18. cont'd.

Location	Date Sampled	Species Present	Organisms/100ml	Station Temp. °C
River #11	11-15-76	*E. coli*	1.3×10^1	7.5
River #12	2-10-77	*E. coli*	2.0×10^0	2.0
River #13	4-11-77	*E. coli* *K. pneumoniae*	7.9×10^1 2.0×10^0	19.0
Paper Mill Sump	8-2-76	*E. cloacae* *E. coli* *K. pneumoniae*	3.7×10^0 2.1×10^1 2.1×10^1	46.0
Pulp Mill Sump	2-10-77	*E. coli* *K. pneumoniae*	2.0×10^0 2.2×10^1	43.0
Bleach Plant Sump	No EC+ organisms isolated			
Caustic Sump	6-7-76	*E. coli*	2.0×10^0	50.0
Primary Clarifier	4-11-77	*E. coli* *K. pneumoniae*	2.0×10^0 3.4×10^1	
Salt Water Intake	6-7-76	*E. coli* *K. pneumoniae*	4.9×10^1 2.0×10^0	24.0
Blow Heat Condensers	6-7-76	*E. coli* *K. pneumoniae*	4.6×10^1 8.3×10^0	22.2

Table 18. cont'd.

Location	Date Sampled	Species Present	Organisms/100ml	Station Temp. °C
Inlet-Dozier's Closure	6-7-76	E. coli	1.4×10^3	42.0
		K. pneumoniae	8.2×10^0	
	8-2-76	E. cloacae	1.8×10^0	39.0
		E. coli	9.2×10^3	
		K. pneumoniae	2.6×10^2	
Discharge-Dozier's Closure	6-7-76	E. coli	4.3×10^2	36.0
		K. pneumoniae	1.2×10^1	
	8-2-76	E. coli	1.1×10^3	39.0
		K. pneumoniae	2.1×10^2	
	11-15-76	C. freundii	4.0×10^0	29.0
		E. agglomerans	8.1×10^0	
		E. coli	2.8×10^2	
		K. pneumoniae	5.2×10^1	
Discharge-Dozier's Closure (Bottom)	6-7-76	C. freundii	2.0×10^0	36.0
		E. cloacae	1.8×10^0	
		E. coli	2.2×10^3	
		K. pneumoniae	9.2×10^0	
River #4a[a]	6-7-76	E. cloacae	4.0×10^0	36.0
		E. coli	4.8×10^2	
		K. pneumoniae	1.7×10^1	

[a] River station #4a - replicate sample taken in the vicinity of discharge from Doziers Closure.

Table 19. Ratio of Escherichia coli to Klebsiella pneumoniae with respect to temperature at river and in-plant sampling sites

Location	Date Sampled	Temp. °C	E. coli/K. pneumoniae
River #1	11-15-76	7.5	0.41
River #2	8-2-76	28.0	17.56
River #3	11-15-76	7.5	6.11
River #4	6-7-76	24.0	39.76
	11-15-76	7.5	1.18
River #5	2-10-77	3.0	6.8/0
River #6	6-7-76	23.5	39.50
	11-15-76	7.5	4.08
River #7	2-10-77	3.0	4.5/0
River #8	2-10-77	2.5	2.0/0
River #9	2-10-77	2.5	2.0/0
River #10	8-2-76	29.0	10.22
River #11	11-15-76	7.5	13.0/0
River #12	2-10-76	2.0	2.0/0
River #13	4-11-77	19.0	39.5
Paper Mill Sump	8-2-76	46.0	1.00
Pulp Mill Sump	2-10-77	43.0	0.09
Caustic Sump	6-7-76	50.0	2.0/0
Primary Clarifier	4-11-77	40.0	0.06
Salt Water Intake	6-7-76	24.0	24.5
Blow Heat Condensers	6-7-76	22.2	5.54
Inlet-Dozier's Clo.	6-7-76	42.0	170.73
	8-2-76	39.0	35.38
Discharge-Dozier's Clo.	6-7-76	36.0	35.38
	8-2-76	39.0	5.24
	11-15-76	29.0	5.38
River 4a	6-7-76	36.0	28.24

Table 20. Species compositional analysis of positive fecal coliform samples representing the UNOX waste water system.

Location	Date Sampled	Species Present	Organisms /100 ml.	E. coli/ K. pneumoniae
UNOX Influent	5-3-76(morning)	E. cloacae	2.0×10^0	6.50
		E. coli	1.3×10^1	
		K. pneumoniae	2.0×10^0	
	5-3-76 (noon)	none detected		-
	5-3-76 (late afternoon)	E. coli	2.0×10^0	2.0/0
	7-23-76	K. pneumoniae	1.3×10^1	0/13.0
	7-23-76	E. coli	1.4×10^1	1.79
		K. pneumoniae	7.8×10^0	
	1-20-77	C. freundii	4.5×10^0	1.69
		E. coli	2.2×10^1	
		K. pneumoniae	1.3×10^1	
	1-20-77	C. freundii	2.0×10^0	1.47
		E. aerogenes	1.8×10^0	
		E. coli	2.2×10^1	
		K. pneumoniae	1.5×10^1	
	1-20-77	C. freundii	4.0×10^0	1.81
		E. coli	4.9×10^1	
		K. pneumoniae	2.7×10^1	
UNOX 1st Chamber	5-3-76(morning)	E. coli	4.5×10^0	2.25
		K. pneumoniae	2.0×10^0	
	5-3-76 (noon)	E. coli	4.0×10^0	4.0/0
		K. pneumoniae	2.0×10^0	
	5-3-76 (late afternoon)	E. coli	2.0×10^0	2.0/0
	7-23-76	E. coli	2.0×10^0	1.00
		K. pneumoniae	2.0×10^0	
	7-23-76	E. coli	7.8×10^0	1.73
		K. pneumoniae	4.5×10^0	
	1-20-77	C. freundii	4.0×10^0	0.53
		E. coli	2.1×10^1	
		K. pneumoniae	4.0×10^1	

Table 20. cont'd.

Location	Date Sampled	Species Present	Organisms /100 ml.	E. coli/ K. pneumoniae
UNOX 1st Chamber	1-20-77	E. coli	7.9×10^1	2.93
		K. pneumoniae	2.7×10^1	
	1-20-77	C. freundii	1.8×10^0	0.38
		E. coli	4.6×10^1	
		K. pneumoniae	1.4×10^2	
UNOX 2nd Chamber	5-3-76(morning)	E. coli	1.8×10^0	0.45
		K. pneumoniae	4.0×10^0	
	5-3-76 (noon)	C. freundii	1.3×10^0	1.13
		E. coli	4.5×10^0	
		K. pneumoniae	4.0×10^0	
	5-3-76 (late afternoon)	E. coli	1.3×10^1	6.50
		K. pneumoniae	2.0×10^0	
	7-23-76	E. coli	2.0×10^0	0.22
		K. pneumoniae	9.3×10^0	
	7-23-76	E. coli	4.0×10^0	0.51
		K. pneumoniae	7.8×10^0	
	1-20-77	E. coli	1.1×10^1	0.52
		K. pneumoniae	2.1×10^1	
	1-20-77	C. freundii	1.8×10^0	2.75
		E. coli	3.3×10^1	
		K. pneumoniae	1.2×10^1	
	1-20-77	E. coli	3.3×10^1	1.94
		K. pneumoniae	1.7×10^1	
UNOX Effluent	5-3-76 (morning)	C. freundii	2.0×10^0	6.8/0
		E. coli	6.8×10^0	
	5-3-76 (noon)	none detected		-
	5-3-76 (late afternoon)	E. cloacae	1.8×10^0	-
	7-23-76	E. coli	2.0×10^0	0.44
		K. pneumoniae	4.5×10^0	
	7-23-76	K. pneumoniae	2.0×10^0	0/2.0
	1-20-77	E. coli	7.0×10^1	3.33
		K. pneumoniae	2.1×10^1	

Table 20. cont'd

Location	Date Sampled	Species Present	Organisms /100 ml.	*E. coli*/ *K. pneumoniae*
UNOX Effluent	1-20-77	*C. freundii* *E. coli* *K. pneumoniae*	4.5×10^{0} 1.7×10^{1} 7.0×10^{1}	0.24
	1-20-77	*E. agglomerans* *E. coli* *K. pneumoniae*	1.8×10^{0} 2.3×10^{1} 2.7×10^{1}	0.85
2nd Clarifier Effluent	5-3-76 (morning)	*E. coli*	7.8×10^{0}	7.8/0
	5-3-76 (noon)	*C. freundii* *E. coli*	2.0×10^{0} 1.1×10^{1}	11.0/0
	5-3-76 (late afternoon)	*E. cloacae* *E. coli* *K. pneumoniae*	1.8×10^{0} 9.3×10^{0} 2.0×10^{0}	4.65
	7-23-76	none detected		-
	7-23-76	none detected		-
	1-20-77	*C. freundii* *E. coli* *K. pneumoniae*	4.0×10^{0} 2.7×10^{1} 1.1×10^{1}	2.45
	1-20-77	*C. freundii* *E. coli* *K. pneumoniae*	3.6×10^{0} 7.9×10^{1} 6.8×10^{0}	11.62

populations of these two genera were very low and similar in magnitude, it was not possible to draw definitive conclusions with respect to their relative numbers.

Occurrence of pathogenic organisms

Salmonella sp. were not detected in six samples analyzed from the UNOX system, secondary clarifier and paper mill sump (Table 21). However Salmonella enteritidis and Aeromonas shigelloides, both of which can cause gastroenteritis in humans, were isolated from river stations #1, #2 and #4 in the Pamunkey River. At the time of pathogen isolation, fecal coliform levels at the above stations ranged between $1.7 \times 10^1 - 7.9 \times 10^1$/100 ml.

Saprophytic growth studies in Dozier's Closure

Results of in situ growth studies using various indicator organisms are shown in Table 22. Treatment A, representing the control to which no bacteria were added showed that contamination of the dialysis bags from the surrounding water apparently occurred. By twelve hours following submersion, total counts from EMB and TSA plates representing the control approximated those observed in Dozier's Closure water (treatment E) at the onset of the experiment. Due to overcrowding from various morphological types it was not possible to enumerate E. coli densities. Even if countable, it would not have been possible to determine if they represented the original inocula. Likewise the presence of streptococcus colonies and colonies resembling Klebsiella sp. or Enterobacter sp. from uninoculated controls precluded conclusions related to the saprophytic growth potential of these bacterial types. Dialysis bags at the time of sampling

Table 21. Detection of pathogenic bacteria using *Salmonella* enrichment media at selected sampling sites.

Locations Tested	Date	Results
UNOX Influent	7-23-76	No *Salmonella* detected.
UNOX - 1st Chamber	7-23-76	None detected.
UNOX - 2nd Chamber	7-23-76	None detected.
UNOX Effluent	7-23-76 8-2-76	None detected. None detected.
Secondary Clarifier	7-23-76	None detected.
Paper Mill Sump	8-2-76	None detected.
River #1	11-15-76	None detected. (*Aeromonas shigelloides* = 8.3/100 ml)
River #2	8-2-76	*Salmonella enteriditis* isolated. (3.6/100 ml)
River #3	11-15-76	None detected.
River #4	11-15-76	*Salmonella enteriditis* isolated. (*Aeromonas shigelloides* = 2.0 /100 ml)
River #6	11-15-76	None detected.

Table 22. Enumeration of bacterial densities as a function of time submerged in Dozier's Closure

Treatment	Time Sampled	Streptococci/ml	Klebsiella/ml	Total bacteria on EMB/ml	Total bacteria on TSA/ml
A	0 hr.	$<1.0 \times 10^1$	$<1.0 \times 10^1$	1.0×10^1	2.1×10^2
	12 hr.	4.0×10^1	3.6×10^1	$> 3.0 \times 10^4$	$>3.0 \times 10^4$
	24 hr.	6.9×10^1	2.5×10^1	7.3×10^3	6.7×10^5
	36 hr.	7.1×10^2	1.6×10^1	1.3×10^6	8.0×10^6
	48 hr.	$<1.0 \times 10^1$	$<1.0 \times 10^2$	1.6×10^5	1.7×10^6
	4 days	1.0×10^1			1.8×10^5
B	0 hr.	2.4×10^1	5.6×10^1	1.9×10^2	9.6×10^2
	12 hr.	1.0×10^1	6.0×10^2	$>3.0 \times 10^5$	$>3.0 \times 10^5$
	24 hr.	$<1.0 \times 10^1$	3.6×10^1	9.8×10^4	1.8×10^6
	36 hr.	3.9×10^1	1.0×10^2	2.0×10^6	$3/6 \times 10^6$
	48 hr.	$<1.0 \times 10^1$	5.6×10^1	2.3×10^5	1.6×10^6
	4 days	$<1.0 \times 10^1$			7.8×10^4
C	0 hr.	1.0×10^1	3.6×10^1	9.8×10^1	3.4×10^2
	12 hr.	1.4×10^1	5.9×10^1	2.8×10^5	$>3.0 \times 10^5$
	24 hr.	2.2×10^1	1.0×10^1	3.0×10^5	1.3×10^6
	36 hr.	$<1.0 \times 10^1$	$<1.0 \times 10^2$	1.8×10^6	6.7×10^6
	48 hr.	2.0×10^1	1.0×10^1	1.6×10^5	1.6×10^6
	4 days	$<1.0 \times 10^1$			1.7×10^5
D	0 hr.	1.0×10^1	6.2×10^1	3.0×10^2	1.5×10^3
	12 hr.	1.7×10^1	1.8×10^2	$>3.0 \times 10^5$	$>3.0 \times 10^5$
	24 hr.	$<1.0 \times 10^1$	3.4×10^2	1.7×10^5	1.8×10^6
	36 hr.	6.9×10^2	2.3×10^2	2.8×10^6	8.8×10^6
	48 hr.	5.6×10^1	$<1.0 \times 10^0$	9.8×10^4	1.6×10^6
	4 days	1.4×10^1			2.3×10^5
E	0 hr.	8.4×10^1	8.4×10^2	1.8×10^4	6.6×10^4
	12 hr.	2.9×10^2	3.7×10^4	$>3.0 \times 10^5$	$>3.0 \times 10^5$
	24 hr.	5.2×10^1	3.5×10^4	8.6×10^5	2.4×10^6
	36 hr.	3.6×10^2	6.6×10^3	7.9×10^5	2.0×10^6
	48 hr.	1.0×10^1	$<1.0 \times 10^3$	4.9×10^3	2.0×10^4
	4 days	3.5×10^1			2.7×10^5
F	48 hr.	1.0×10^1	$<1.0 \times 10^2$	2.8×10^3	4.2×10^3
	4 days	1.4×10^1			2.6×10^4

Table 22. cont'd (legend)

Treatment A: Autoclaved Dozier's closure water, not inoculated, to act as a sterile control.

Treatment B: Phosphate Buffer Solution (PBS), inoculated with 5.0×10^1/ml Strep, 5.0×10^1/ml E. coli, 1.0×10^1/ml K. pneumoniae.

Treatment C: Filtered (pore size- .45 micron) Dozier's closure water, inoculated with 5.0×10^1/ml Strep, 5.0×10^1/ml E. coli, 1.0×10^1/ml. K. pneumoniae.

Treatment D: Autoclaved Dozier's closure water, inoculated with 5.0×10^1/ml Strep, 5.0×10^1/ml. E. coli, 1.0×10^1/ml K. pneumoniae.

Treatment E: Doziers closure water, not sterile, collected at zero hour of the study, to act as a procedural control.

Treatment F: Grab samples taken at various times during the study, to monitor levels in Dozier's closure.

Plating Media: PSE agar - Pfizer Enterococcus Agar, specific for enumeration of streptococci.

TSA + .3% yeast - Trypticase Soy Agar supplemented with yeast, to monitor growth levels of all organisms.

EMB - Levine Eosin Methylene Blue agar, to monitor levels of E. coli and K. pneumoniae, inhibits growth of non-coliform organisms. This media was not used with the 4 day samples.

were securely closed. It was possible that leakage from the environment may have resulted from cellulytic bacteria acting on the methyl cellulose dialysis bags at the elevated temperature of the closure. Industrial wastes from the paper and pulp manufacturing processes would result in environmental conditions favorable for the development of cellulytic bacterial populations.

Discussion

Elevated fecal coliform levels in the Pamunkey, Mattaponi and upper York rivers have resulted in the closure of shellfish grounds in the area of West Point as well as downstream. With respect to contributions from the Chesapeake Corporation, two sources of fecal coliforms were identified: the UNOX waste water treatment system and Dozier's Closure. In addition, unidentified pollution sources upstream on the Pamunkey and Mattaponi rivers as well as non-point source pollution from the creek receiving effluent from West Point sewage treatment plant contribute to fecal coliform loading in the upper York River.

Approximately 200 billion coliforms per day, of which less than 1% are operationally defined as fecal (according to the elevated temperature test) were calculated entering the river from the UNOX system. Fecal coliform densities on a per 100 ml basis were 2 - 3 orders of magnitude higher in Dozier's Closure compared to effluents from the UNOX system. Assuming maximum displacement of closure water equivalent to the 17MGD entering from the cooling water system (Chesapeake Corporation), calculations based on median levels of indicator bacteria in Dozier's closure discharge area suggest maximum total and fecal coliform inputs of 15,000 and 2,600 billion respectively per day. In perspective, the coliform contribution per capita is estimated on the order of 2 billion per day (Geldreich, et al. 1962).

The major source of fecal coliforms to the UNOX system is process waste from the paper mill sump. Fecal streptococci are derived from both paper and pulp mill sumps. Measuring large populations of indicator bacteria in these waters is not unique. Other investigations have

reported similar findings for pulp and paper wastes (Bordner and Carroll, 1972; Huntley, Jones and Cabelli, 1976; Knittell, 1975; NCASI, 1971; NCASI, 1975). A significant proportion of the elevated coliform population in mill wastes has been attributed by these workers to K. pneumoniae. Knittell (1975) reported that K. pneumoniae comprise up to 80% of the total coliform bacterial population. While the origin of K. pneumoniae in pulp and paper waste water effluents is difficult to determine, this bacterium has been isolated from botanical milieux including vegetables, seeds and trees (Bordner and Carroll, 1972; Brown & Seidler, 1973). Seidler, Morrow and Bagley (1977) noted that Klebsielleae can multiply extensively on water soluble nutrients which leach from wood. Similarly, Menon and Bedford (1973) suggested that fecal streptococci in paper and pulp mill effluents reflect the presence of a particular strain, Streptococcus faecalis var. liquifaciens. The ability of this organism to establish commensal growth with plants and a similar adaptation to insects has been proposed (Geldreich, Kenner and Kabler, 1964; Mundt, Coggin and Johnson, 1962).

Industry and regulatory agencies have debated the significance of high fecal coliform levels in effluents characterized predominantly by K. pneumoniae and not E. coli. (Bordner and Carroll, 1972; NCASI, 1975). In the current study both genera were recovered at similar absolute levels from treated effluents. However, it must be recognized that the reported K. pneumoniae densities represent a proportion of the total K. pneumoniae population since only 16% of environmental strains give a

positive fecal coliform reaction at an elevated temperature (Bagley and Seidler, 1977).

Although E. coli was isolated from various mill sumps entering the UNOX system, its presence does not necessarily reflect domestic sewage. Geldreich, Kenner, and Kabler (1964) have described the isolation of IMVIC type - - + + (E. coli. type I) from vegetation and insects. Therefore, while the source water used for mill processes was coliform free, it is feasible that total and fecal coliforms and fecal streptococci entering the process water via raw materials multiplied. Pulp and paper mill wastes are a nutritionally rich medium for most Enterobacteriaceae including E. coli and Klebsielleae (Bordner and Carroll, 1972; NCASI, 1971).

Numbers of fecal coliform and streptococci from the combined mill wastes entering the UNOX remained relatively constant during passage through the waste treatment system. BOD levels of UNOX secondary effluents averaged 30 mg/l (Chesapeake Corporation). Effluent standards for nutritive wastes have been recommended in the range of 14 - 30 mg/l (Bordner and Carroll, 1972). Although BOD levels significantly exceeded this value on three occasions, saprophytic growth of indicator bacteria was only observed once.

Potential inputs of coliforms and streptococci to Dozier's Closure were the plant's cooling water system, runoff, sanitary leakage and river water entering the system during tidal excursions. Multiplication of heterotrophic bacteria (and to a lesser extent E. coli and fecal streptococci) during passage through river-fed cooling systems has been reported (Verstrate, Voets and Vanstaen, 1975). However, there was no evidence during the present study that such saprophytic growth

occurred. Rainwater runoff could not account for the observed levels since sampling took place following a three day interval during which no precipitation had occurred. (The only exception to the above statement was in the November survey when light rain mixed with sleet was observed during sampling.) Although sanitary leakage was known to be a significant source during the initial surveys, levels of indicator organisms remained elevated more than six months after the leak was discovered and corrected. Thus, it was hypothesized that either an undetected sewage leak existed and/or multiplication of indicator bacteria occurred. The latter possibility seemed plausible since recent dye studies have failed to reveal further sanitary sewage contamination.

As discussed previously, saprophytic multiplication of fecal coliforms is possible. The presence of elevated fecal streptococcus levels in Dozier's Closure is disturbing since multiplication of this group of organisms has not been observed in nutrient rich waters associated with kraft mills (Menon and Bedford, 1973; NCASI, 1971). However, the possibility of a particular streptococcus strain capable of saprophytic growth cannot be eliminated. Unfortunately, in the absence of a valid saprophytic growth experiment, conclusions can not be drawn with respect to the reason(s) for elevated populations of indicator bacteria in Dozier's Closure.

Seasonal variations in total and fecal coliform and fecal streptococcus densities were noted in river sample surveys. Both increased abundances of coliforms in the warmer months as well as the absence of seasonal variations have been reported in the literature (Bardsley,

1934; Carney, Carty and Colwell, 1975; Taylor, 1940; Velz, 1970). Departures from data reported herein were probably due to differences in physico-chemical factors which vary from one environment to the next. In contrast to field studies showing increases in indicator bacteria during the warmer months, are in situ growth studies which reveal that E. coli survival is negatively correlated with increasing water temperature (Faust, Aotaky, and Hargadon, 1975; Vasconcelos and Swartz, 1976). However, the fate of E. coli in the aquatic environment can be the result of a variety of factors (Faust, Aotaky and Hargadon 1975). Sediments from estuarine and marine waters have been shown to enhance the survival of E. coli due to organic matter content (Gerba and MacLeod, 1976). Numerous reports exist as to the growth potential of coliforms including E. coli in fresh water and seawater if organic material is present (Allen, Pasley and Pierce, 1972; Dutka, 1973; Hendricks, 1972; Prescott, Winslow and McCrady, 1946; Slanetz and Bartley, 1962). When nutrient starvation occurs, microbial sensitivity to secondary stress is increased (Klein and Wu, 1974; and Wu and Klein, 1976). We may hypothesize as was suggested by Prescott, Winslow and McCrady (1946) that seasonal variations observed in the present study were related to the availability of nutrients such that temperature favored bacterial multiplication rather than contributed to their dieoff.

Based on a limited number of river samples, species compositional analysis indicated that K. pneumoniae did not significantly contribute to observed fecal coliform populations. With one exception, E. coli was detected more frequently and at higher densities than K. pneumoniae.

The occurrence of elevated fecal streptococcus densities in all river samples during the summer months is more difficult to explain since multiplication of this bacterial group has not been reported to occur in polluted water (Geldreich and Kenner, 1969; Slanetz and Bartley, 1965). According to Geldreich (1970) fecal streptococci persist for extended periods of time, particularly in waters with high electrolyte content. Fecal streptococci were characterized as being composed of a wide spectrum of strains with different survival rates and including biotypes of limited sanitary significance. Specifically, S. faecalis var. liquifaciens was considered ubiquitous in nature. In the present study, fecal streptococcus isolates were not tested biochemically beyond those characteristics necessary for confirmation as fecal streptococci. Thus, the contribution of S. faecalis var. liquifaciens to fecal streptococcus populations from representative river and in-plant samples was unknown.

Isolation of pathogenic organisms from selected river samples when fecal coliform densities were relatively low, i.e.< 100/100 ml. concurs with reports by other workers related to prediction of pathogens at low fecal coliform levels (Claudon, et al, 1971; Dutka, 1973; Slanetz, Bartley and Stanley, 1968). Although Salmonella was isolated from the river near Dozier's Closure it is not possible to state that the sample reflected effluent from the closure. However, in the presence of elevated BOD levels, saprophytic growth of Salmonella may occur (Gallagher and Spino, 1968; Bordner and Carroll, 1972; Hendricks, 1972) and, therefore, the UNOX discharge may be indirectly encouraging pathogen growth or survival in this region of the river.

Conclusion

Results from the first year's data have been extremely valuable in providing a baseline of the bacteriological quality of the upper York, Mattaponi and Pamunkey rivers. Point sources of fecal coliform populations have been detected at the UNOX effluent of the Chesapeake Corporation and Dozier's Closure. The UNOX system, itself however, does not appear to generate fecal coliforms. Sources of these fecal coliforms (and fecal streptococci) in mill waste water influents to the UNOX remain to be elucidated. Most importantly, the presence of fecal coliforms in the UNOX effluent in the absence of known sources of domestic sewage, questions the application of the fecal coliform standard to waters receiving this type of industrial effluent. However, Bordner and Carroll (1972) suggest that although fecal coliforms may be growing saprophytically and not related to domestic sewage, conditions favorable to their growth may also facilitate pathogen multiplication. Non-point sources apparently exist upstream of the plant on both the Pamunkey and Mattaponi rivers. However, the possibility that the plant is providing conditions conducive to the growth/survival of fecal coliforms, fecal streptococci, or Salmonella sp. in the river through discharge of elevated BOD effluents and heated water is still a viable one and should be evaluated. Observations that Dozier's Closure continues to exhibit elevated levels of fecal coliforms in the absence of known sanitary discharges should provide sufficient impetus for continued studies. Finally, based on species composition analysis of river samples, Klebsiella sp. did not substantially contribute to the populations of bacteria passing through the elevated temperature fecal coliform test.

We recommend, therefore, that results from the first year's work be amplified by specific experiments designed to assess the bacterial growth potential of plant effluents containing high BOD levels at elevated temperatures. Furthermore, the dynamic significance of Chesapeake Corporation point source discharges on the overall river quality should be assessed using a computer simulated model of this area. Using such a model (available at VIMS) in conjunction with dieoff/survival curve data, it may be possible to determine the contribution of these effluents to the elevated levels of indicator bacteria in the West Point area.

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