

Distribution of Sewage Indicated by *Clostridium perfringens* at a Deep-Water Disposal Site after Cessation of Sewage Disposal†

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Received 29 August 1995/Accepted 1 March 1996

Clostridium perfringens, a marker of domestic sewage contamination, was enumerated in sediment samples obtained from the vicinity of the 106-Mile Site 1 month and 1 year after cessation of sewage disposal at this site. *C. perfringens* counts in sediments collected at the disposal site and from stations 26 nautical miles (ca. 48 km) and 50 nautical miles (ca. 92 km) to the southwest of the site were, in general, more than 10-fold higher than counts from an uncontaminated reference site. *C. perfringens* counts at the disposal site were not significantly different between 1992 and 1993, suggesting that sewage sludge had remained in the benthic environment at this site. At stations where *C. perfringens* counts were elevated (i.e., stations other than the reference station), counts were generally higher in the top 1 cm and decreased down to 5 cm. In some cases, *C. perfringens* counts in the bottom 4 or 5 cm showed a trend of higher counts in 1993 than in 1992, suggesting bioturbation. We conclude that widespread sludge contamination of the benthic environment has persisted for at least 1 year after cessation of ocean sewage disposal at the 106-Mile Site.

Between 1986 and 1992, large amounts of sewage sludge from New York and New Jersey were transported by barge to a deep-water sewage disposal site, located approximately 106 nautical miles (ca. 196 km) southwest of New York Harbor. This site is designated the Deep Water Municipal Sewage Sludge Disposal Site (also called the 106-Mile Site), and water depths at the site are between 2,340 and 2,740 m (5). Approximately 8 million tons (wet) of sewage sludge was discharged in surface waters at the 106-Mile Site between 1988 and July 1992 (17), making up about 50% of the total sewage sludge dumped at sea worldwide during that period (18). Since 1989, a multi-disciplinary research team has been studying the fate of this sludge and its impact on the benthic environment.

The Ocean Dumping Ban Act passed by the U.S. Congress in 1988 required the dumping of sewage sludge from barges to end by 31 December 1991, although there was dumping at the 106-Mile Site until July 1992, with payment of penalties. Cessation of sludge dumping at this site provided an excellent opportunity to study the long-term ecological effects on, and subsequent recovery of, this site. As part of this study, we monitored concentrations of *Clostridium perfringens* at the 106-Mile Site. *C. perfringens* forms highly resistant endospores that can be expected to survive for long periods under the extreme conditions of low temperature and high pressure typically found in the marine benthic environment. We have previously reported *C. perfringens* to be a good indicator of the patterns of sewage contamination at the 106-Mile Site (5, 14).

It was initially expected that sludge material discharged at the surface would be undetectable in the underlying sediments, because of the low settling velocities of sewage particles, the great water depth, and the dilution and degradation that would occur as particles pass through the water column (19). It has been conclusively shown, however, that sewage sludge reaches

the ocean floor in relatively high concentrations directly below the 106-Mile Site and at a considerable distance (ca. 92 km) to the southwest of the site. Contamination of the benthic environment has been confirmed by the presence of increased concentrations of *C. perfringens*, an indicator of sewage contamination (5, 14, 15). *C. perfringens* spores are ubiquitous in sewage sludge at concentrations several orders of magnitude higher than in soil or sediments (11). Silver, linear alkylbenzene, and coprostanol concentrations were also elevated, and all indicators confirmed the distribution of sewage sludge in a pattern beneath and to the southwest of the disposal site (5). This pattern conformed to predictions made with a model of recent sludge settling velocity data and currents measured at this site (10).

Sludge reaching the benthic environment has been shown to have a profound ecological impact. Near-bottom pelagic bacteria in the vicinity of the disposal site differed significantly in their ability to grow under deep-sea pressure and temperature conditions from bacteria in an unimpacted site (15, 20). Megafauna such as holothurians and starfish were an order of magnitude more abundant at the disposal site than at nearby control areas (13). Two species of polychaete worm abundant at the disposal site have not been found previously in the continental slope and were absent from unimpacted continental rise sediments near the disposal site (12).

By continuing to monitor *C. perfringens* as an indicator of sewage sludge contamination at the 106-Mile Site after cessation of dumping, we were able to obtain valuable information on the fate of sludge upon burial and on resuspension and redistribution of sludge previously accumulated at this site and also to track the movement of sludge to lower levels within the sediment by resuspension and bioturbation events. In addition, data reported here are useful for indicating sites that remain heavily impacted and those at which the concentrations of sewage sludge may have decreased.

MATERIALS AND METHODS

Sample collection. For locations of sample sites, see Tables 1 and 2. RV *Atlantis II* occupied sampling stations in August 1992 and July 1993, 1 month and

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† Contribution 257 from the Center of Marine Biotechnology.

TABLE 1. *C. perfringens* spore counts in tube core samples

Yr collected	Station	Dive no.	Latitude (north)	Longitude (west)	Sample	No. of subsamples	Mean counts (CFU/cm ²) ± SE
1992	Ref	2557	39°20.07'	70°40.19'	TC1	3	186 ± 50
		2558	39°20.02'	70°39.18'	TC1	2	106 ± 27
	Max	2551	38°49.08'	72°8.06'	TC1	3	1,501 ± 257
			TC2	3	1,578 ± 592		
		2552	38°49.23'	72°8.12'	TC1	1	661
			TC2	1	3,982		
			TC10	3	529 ± 327		
	26-Mile	2554	38°34.34'	72°29.47'	TC1	3	2,046 ± 391
					TC2	2	1,317 ± 349
			TC9	4	3,744 ± 1,399		
				TC10	2	2,009 ± 964	
	50-Mile	2555	38°15.05'	72°52.75'	TC1	3	1,315 ± 579
					TC4	2	2,330 ± 76
					TC9	4	1,265 ± 624
					TC10	3	642 ± 400
1993	Ref	2626	39°20.16'	70°39.91'	TC2	3	145 ± 56
					TC10	3	154 ± 46
		2629	39°20.97'	70°40.41'	TC3	3	129 ± 27
					TC4	2	151 ± 25
	Max	2630	38°48.85'	72°9.37'	TC2	3	5,028 ± 611
					TC5	2	2,372 ± 349
					TC1	1	4,547
					TC5	2	1,370 ± 364
		2633	38°48.96'	72°9.30'	TC8	3	6,078 ± 1,652
					TC3	1	3,127
					TC7	1	2,994
					TC8	1	1,788 ± 371
	26-Mile	2635	38°30.00'	72°25.00'	TC1	3	2,695 ± 850
					TC5	3	3,113 ± 686
		2636	38°34.34'	72°29.47'	TC1	1	848
					TC2	3	1,137 ± 299
	50-Mile	2637	38°15.02'	72°52.85'	TC1	4	3,865 ± 853
					TC6	2	630 ± 419

1 year, respectively, after cessation of dumping at the 106-Mile Site. Sediment samples were collected by the DSRV *Alvin*, using an acrylic corer 50 cm long and with an internal diameter of 15 cm. These samples were designated tube cores. Additional sediment samples were collected with surface-deployed box corers (0.5 by 0.5 m in cross section) containing 25 subcores (each 0.1 by 0.1 m in cross section). These samples were designated box cores. Both corers were designed to retrieve sediment to a depth of ca. 0.5 m.

Immediately upon recovery of each sampling apparatus, the water overlying each core was carefully removed. The two types of cores were sampled in an identical manner, with 10-ml syringes modified by removal of end flanges to form open cylinders. The modified syringes were inserted into the sediment sample to a depth of ca. 6 cm, to obtain undisturbed depth profiles, and were sealed, maintained at 4°C, and transported to a land-based laboratory for analysis.

Enumeration of *C. perfringens*. *C. perfringens* counts were obtained as described previously (14). Briefly, sediment was extruded from sampling syringes in 1-cm divisions, to a total depth of 5 cm, and diluted in 1% (wt/vol) sterile saline. Dilutions were plated on modified mCP medium (2), a more economical version of mCP medium (2, 4), designed for isolation of *C. perfringens*. The media were incubated for 24 h in an anaerobic atmosphere at 45°C, and *C. perfringens* colonies were subsequently enumerated. The dry weight of sediment in each 1-cm division was determined, and *C. perfringens* counts were expressed in CFU per gram of dry sediment. For between-station comparisons of *C. perfringens* counts in entire 5-cm cores, counts obtained from each 1-cm division were added. This total was divided by the cross-sectional area of the core to give a count per unit area (in CFU per square centimeter).

Sampling strategy. Sample stations were grouped into categories and designated as follows: Ref (reference station more than 20 km to the northeast of the 106-Mile Site and northeast of the Hudson Canyon), Max (sites of expected

maximum impact, within and no more than 10 km to the southwest of the 106-Mile Site), 26-Mile Station (ca. 48 km [26 nautical miles] to the southwest of the 106-Mile Site), and 50-Mile station (ca. 92 km [50 nautical miles] to the southwest of the 106-Mile Site). Sample stations were selected to the southwest of the disposal site because this is the predicted direction of sewage sludge movement from the site (10) and because previous studies had indicated sludge settling to the southwest of the 106-Mile Site (5, 14).

In general, three syringe subcores were obtained from each tube and box core. Counts obtained from each depth of these syringe subcores were averaged to obtain *C. perfringens* values for each tube and box core. Replicate cores were obtained from each station, and *C. perfringens* counts from these replicate cores were averaged to obtain a *C. perfringens* count and standard error for that station.

Statistical analyses. To compare the means and standard error of total *C. perfringens* counts by site, by year, and by sample method, counts were integrated over the 5-cm depth sampled and expressed as CFU per square centimeter. *C. perfringens* counts for all sites within each of the four categories designated above, for research cruises in 1992 and 1993, were statistically analyzed by a three-factor analysis of variance with the SuperANOVA program (Abacus Concepts) and were considered to be statistically different at $P < 0.05$.

RESULTS

Distribution among sites of *C. perfringens* in surface sediments. Means and standard errors of *C. perfringens* counts in the top 5 cm of individual core samples are shown in Tables 1 (tube cores) and 2 (box cores). *C. perfringens* counts in the top

TABLE 2. *C. perfringens* spore counts in box core samples

Yr	Station	Latitude (north)	Longitude (west)	Sample	No. of subsamples	Mean counts (CFU/cm ²) ± SE	
1992	Ref	39°20.03'	70°40.04'	BC1	4	30 ± 9	
		39°15.08'	71°10.89'	BC2	7	25 ± 14	
		39°20.16'	70°40.04'	BC13	6	66 ± 22	
		39°19.77'	70°39.75'	BC14	2	12 ± 6	
		39°20.04'	70°40.02'	BC15	5	52 ± 25	
	Max	38°43.13'	72°7.25'	BC6	5	1,454 ± 729	
		38°43.47'	72°6.95'	BC7	3	1,428 ± 868	
		38°43.39'	72°7.33'	BC8	6	1,056 ± 652	
	26-Mile	38°34.28'	72°29.54'	BC11	6	1,444 ± 262	
		38°25.01'	72°25.22'	BC12	7	930 ± 155	
	50-Mile			ND ^a		ND	
	1993	Ref	39°19.76'	70°39.28'	BC1	3	77 ± 12
			39°20.04'	70°40.03'	BC2	3	315 ± 240
			39°20.12'	70°39.62'	BC3	3	305 ± 72
		Max	38°42.85'	72°7.03'	BC5	3	3,327 ± 350
38°42.86'			72°7.06'	BC6	3	1,519 ± 731	
38°42.98'			72°7.04'	BC7	2	6,173 ± 2,386	
26-Mile		38°46.42'	72°33.55'	BC9	3	577 ± 290	
		38°25.03'	72°25.54'	BC10	3	761 ± 241	
50-Mile		38°11.10'	72°44.71'	BC13	2	166 ± 52	
		38°15.08'	72°52.72'	BC15	3	1,303 ± 654	
		38°16.19'	72°52.44'	BC16	3	240 ± 87	
		38°15.04'	72°52.58'	BC17	3	1,083 ± 315	

^a ND, not done.

5 cm of sediment were generally more than 10-fold higher in the vicinity of the disposal site (at the station designated Max) than at the reference site (Ref), for samples collected during 1992 and 1993. Counts in tube core samples collected at Ref ranged between 106 and 186 CFU/cm², compared with a range of 661 to 6,078 CFU/cm² in tube core samples collected at Max. Counts in box core samples collected at Ref ranged between 12 and 315 CFU/cm² compared with a range of 1,056 to 6,173 CFU/cm² in box core samples collected at Max. Counts at the 26-Mile Station and 50-Mile Station were intermediate between the high counts at Max and the low counts at Ref. The range of counts in tube core samples from the 26-Mile Station was between 848 and 3,744 CFU/cm², and the range of counts in box core samples was between 577 and 1,444 CFU/cm². Counts at the 50-Mile Station ranged between 630 and 3,865 CFU/cm² for tube cores and 166 and 1,303 CFU/cm² for box cores.

Means of counts at each station are given in Table 3. Mean counts at Max were more than 20-fold higher than at Ref, and counts at the 26-Mile and 50-Mile Stations were more than 10-fold higher than at Ref. A three-factor analysis of variance indicated that the variation in *C. perfringens* counts between sites was significant ($P < 0.001$), whereas no significant variation in counts was found between samples collected in surface-deployed box corers and samples collected in tube corers by DSRV *Alvin* ($P > 0.05$), or between samples collected in 1992 and 1993 ($P > 0.05$).

Temporal changes in the distribution of *C. perfringens*. *C. perfringens* counts were not significantly different between samples collected in 1992 and those collected in 1993 ($P > 0.05$).

C. perfringens counts in sediments from the 106-Mile Site have been reported previously for the top 1 cm of sediment, expressed in terms of *C. perfringens* CFU per gram (dry weight) of sediment. *C. perfringens* counts obtained in this study for the top 1 cm of sediment were compared with previously reported results in Table 4. Samples from reference stations gave low (less than 10² CFU/g [dry weight]) *C. perfringens* counts in all studies, and there was no clear trend of changes in *C. perfringens* counts between 1989 and 1993 at Max and the 26- and 50-Mile Stations. In all cases, *C. perfringens* counts at Max and at the 26- and 50-Mile Stations were at least five times higher than those at Ref.

Distribution of *C. perfringens* with depth in the top 5 cm of sediment. *C. perfringens* counts in subsamples taken at 1-cm divisions in the top 5 cm of sediment were determined for both tube core and box core samples. Data for tube core samples

TABLE 3. Mean *C. perfringens* counts obtained in the vicinity of the 106-Mile Site in 1992 and 1993

Station	No. of samples	<i>C. perfringens</i> counts ^a		
		Mean	SD	SE
Ref	14	125	95	25
Max	19	2,554	1,755	414
26-Mile	12	1,718	1,016	293
50-Mile	10	1,283	1,104	349

^a *C. perfringens* counts are expressed in CFU per square centimeter and are integrated over the top 5 cm of sediment.

TABLE 4. *C. perfringens* counts obtained in the vicinity of the 106-Mile Site between August 1989 and July 1993

Station	<i>C. perfringens</i> counts (CFU/g) ^a obtained on:					
	Aug. 1989 ^b	Aug. 1990 ^c	Nov. 1990 ^c	Oct. 1991 ^d	Aug. 1992 ^e	July 1993 ^e
Ref	9.5×10^1 (3)	5.2×10^1 (2)	8.9×10^1 (1)	$(1.0-5.0) \times 10^2$	4.0×10^1 (5)	1.6×10^2 (3)
Max	1.2×10^3 (10)	4.1×10^3 (11)	3.3×10^3 (1)	$(3.1-10) \times 10^3$	1.0×10^3 (3)	2.2×10^3 (3)
26-Mile	ND ^f	ND	3.5×10^3 (1)	$(1.6-3.1) \times 10^3$	2.1×10^3 (2)	7.1×10^2 (2)
50-Mile	ND	ND	6.3×10^2 (3)	$(1.0-1.6) \times 10^3$	ND	7.8×10^2 (4)

^a Mean or range of *C. perfringens* counts are given in CFU per gram (dry weight) of sediment in samples obtained from the top 1 cm of sediment, unless otherwise stated. The number of samples analyzed is given in parentheses.

^b Data from reference 5. Tube core samples were collected by the DSRV *Alvin*. Ref samples were obtained in September 1989 by using surface-deployed box corers.

^c Data from reference 14.

^d Data from reference 21. The top 0.5 cm of sediment was sampled.

^e This study.

^f ND, not determined.

are shown in Fig. 1; the same general trends were found for box core samples (results not shown). Generally, at stations where *C. perfringens* counts were elevated, i.e., stations other than Ref, counts were higher in the top 1 cm and decreased at depths down to 5 cm. In some cases, *C. perfringens* counts in the bottom 4 or 5 cm showed a trend of higher counts in 1993 than in 1992.

DISCUSSION

Sewage sludge, whose presence is indicated by *C. perfringens* spores, persists in the benthic environment at the 106-Mile Site, and redistribution and bioturbation processes did not cause marked change in large-scale distribution patterns during the period of approximately 1 year following cessation of dumping. Sewage sludge dumping at the 106-Mile Site was stopped in July 1992, 1 month before the 1992 sampling cruise and 1 year before the 1993 sampling cruise, for which data are reported here. It is striking that there was no significant change in *C. perfringens* counts during this period. In addition, *C. perfringens* counts obtained in the vicinity of the disposal site and at stations 26 and 50 nautical miles to the southwest have been remarkably similar in all studies reported since 1989. These data clearly indicate widespread contamination of the benthic environment in the vicinity of the 106-Mile Site as a result of sewage dumping in surface waters at this site. The small numbers of *C. perfringens* spores found at Ref may be present as a result of sediment transport from coastal waters (3), deposition of fecal material by marine birds (22), or atmospheric transport of soil particles (6).

It was shown previously that sewage disposal at this site had an effect on the bacterial community present at the site, and it was hypothesized that the rate at which sewage sludge was degraded in the epibenthic environment may be decreased by changes in the bacterial community (20). It is striking that *C. perfringens* counts obtained between 1989 and 1993 by different investigators are remarkably consistent and show a clear pattern of more than 10-fold greater *C. perfringens* counts at Max than at Ref. The persistence of large numbers of *C. perfringens* spores for the period between 1989 and 1993 and for over 1 year after cessation of dumping supports the hypothesis that degradation rates of sludge in this environment are very low.

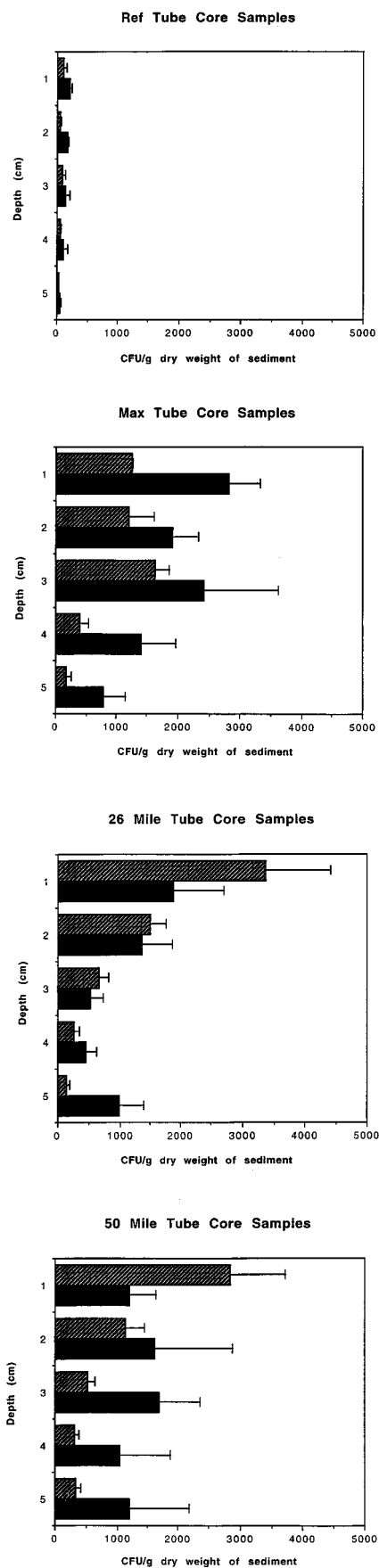
One possible explanation for the persistence of high *C. perfringens* counts in surficial sediments at the Max station after cessation of dumping is that sludge continued to settle through the water column for an extended period after cessation of dumping in July 1992. However, this can be discounted. Fry and Butman (10) predicted, from computer modeling, that only sludge particles with a settling velocity of greater than 0.45

cm/s would settle at Max. Particles with these settling velocities would reach the bottom in 7 days or less at the water depths of ca. 2,600 m present at the disposal site. Persistent high concentrations of *C. perfringens* in the top 1 cm of sediment at Max are therefore not due to continued settling of sludge particles through the water column.

In general, *C. perfringens* counts were highest in the top 1 cm of sediment. In previous studies, the highest counts of *C. perfringens* were also found in the top 1 cm of sediment (5, 14) and a similar distribution of other sewage indicators, i.e., linear alkylbenzenes, coprostanol, and silver, was found (i.e., concentrations of these indicators were generally highest in the top 1 cm of core samples) (5, 19). However, there appeared to be a trend of movement of *C. perfringens* spores to greater depths in the sediment column between 1992 and 1993, possibly as a result of movement of *C. perfringens* spores to greater depths by bioturbation. Bioturbation has been observed at sites about 100 km from the 106-Mile Site, at similar water depths (1). Bioturbation may be enhanced in the vicinity of the disposal site. Two species of polychaete worms, not previously found in nearby sediments, were abundant at the disposal site (12), and foragers such as ophurids, starfish, and holothurians were an order of magnitude more abundant at the disposal site than at control sites (13).

The general trend of reduction in *C. perfringens* counts in the top 1 cm of sediment observed in samples from the 26- and 50-Mile Stations between 1992 and 1993 may also be caused by deposition of a surface layer of uncontaminated sediment during this period. This uncontaminated sediment could result from flux of material through the water column or from resuspension and subsequent transport of bottom sediments. Hourly averaged currents measured in the vicinity of the 106-Mile Site were occasionally strong enough to resuspend bottom sediments (5). Sediment trap measurements indicated that the flux of sediment was greater near the bottom (6 m above bottom) than at 107 m above bottom, suggesting that resuspension of bottom sediment was occurring (5). These oceanographic data are consistent with the observed reduction of *C. perfringens* counts in the top 1 cm having resulted from fresh deposition of bottom sediment. This may be a factor in the movement of *C. perfringens* spores to greater depths, particularly at the 26- and 50-Mile Stations. It seems unlikely that sedimentation is a major factor, however, especially at Max, since *C. perfringens* counts in the top 1 cm remained approximately the same between 1992 and 1993 and sedimentation of uncontaminated sediment would be expected to decrease these concentrations.

Three years after cessation of dumping at the shallow-water (ca. 30-m-deep) New York Bight disposal site located 12 nau-



tical miles (ca. 22 km) offshore, *C. perfringens* counts were generally elevated in subsurface samples (1 to 10 cm) but were markedly reduced in surficial samples (0 to 1 cm) (16). It will be interesting to see whether a similar pattern is observed at the 106-Mile Site 3 years after cessation of dumping. It is concluded from this study that bioturbation and settling rates are insufficient to have a major effect on *C. perfringens* depth profiles in a 1-year period, although small changes are apparent.

C. perfringens counts varied significantly between replicate core samples taken at individual stations, indicated by the wide range of mean *C. perfringens* spore counts from individual sites. Counts from replicate samples taken within a single core also varied significantly, as shown by the high standard errors in many of the counts. Replicate plating of single samples gave much smaller variation, generally <10% (results not shown). These data suggest that the large variation found in *C. perfringens* counts is not a result of experimental variation but, rather, of great spatial heterogeneity of the counts. This is consistent with coarser sludge particles being the predominant input of sludge, since individual larger particles will contain many *C. perfringens* spores and may result in heterogeneous distribution of spores in the benthic environment, on a scale of centimeters. Smaller particles, which would tend to deposit uniformly, will not reach the bottom in the vicinity of the 106-Mile Site but will be widely dispersed by currents. Fry and Butman (10) predicted from computer modeling that only the coarser fraction (particles with settling velocities of greater than 0.04 cm s⁻¹), about 23% of the sludge discharged, would settle on the sea floor within 350 km of the site. Variation in *C. perfringens* counts on a small scale also suggests redistribution, caused by resuspension or bioturbation, after sludge particles settle on the bottom and may be affected by local topological features such as small mounds or burrows.

C. perfringens has proven to be a reliable indicator of sewage contamination in the marine environment at the 106-Mile Site (5, 14, 21) and the New York Bight disposal site (7, 16). Although other clostridia have been isolated from oceanic sediments (9), it was previously shown that putative *C. perfringens* colonies isolated from sediments at the 106-Mile Site by using mCP medium were confirmed as *C. perfringens* in over 95% of isolates tested, on the basis of 21 biochemical characteristics such as the ability to utilize particular sugars and other substrates (14).

Two important questions remain unanswered about *C. perfringens* as a marker of sewage contamination in the benthic environment. First, what is the long-term survival of *C. perfringens* under the extreme conditions of the marine benthic environment? *C. perfringens* spores were shown to persist at unchanged concentrations in marine sediments from water depths of approximately 80 m for about 2 months (8). A study in progress has shown no decrease in *C. perfringens* counts in marine sediments stored for over 3 years at 4°C and 250 atm (ca. 25.3 MPa), the pressure at a water depth of 2,500 m (the depth at the 106-Mile Site) (14a). It appears, therefore, that *C. perfringens* spores retain viability for long periods under conditions found in the benthic environment.

The second question is, how well does *C. perfringens* track the persistence of sewage sludge in the marine benthic envi-

FIG. 1. Depth profiles of *C. perfringens* counts in tube core samples from stations in the vicinity of the 106-Mile Site. *C. perfringens* counts are expressed as mean ± standard error. Counts are indicated by shaded bars for samples obtained in 1992 and solid bars for samples obtained in 1993.

ronment? *C. perfringens* spores will be trapped within sewage particles and therefore should be useful in tracing the movement of these particles. However, it is possible that *C. perfringens* spores persist after sewage is subjected to microbial degradation. Although this possibility cannot be excluded, it appears that autochthonous bacteria at the 106-Mile Site are inhibited by sewage sludge and that allochthonous bacteria introduced with the sludge are inhibited by deep-sea conditions of low temperature and high pressure (20). These factors could result in extremely slow degradation of sludge in the benthic environment. We cannot exclude, however, the possibility that *C. perfringens* spore counts in benthic samples remain elevated even after degradation of sewage sludge has occurred. This is a point meriting further study.

In conclusion, the 106-Mile Site provides an excellent opportunity to monitor long-term effects of sewage disposal in the deep-ocean environment. Results of the study reported here indicate that sewage sludge may persist for very long periods and may gradually be transported to greater depths in the sediment column of the ocean.

ACKNOWLEDGMENTS

We thank Fred Grassle for his leadership in the multidisciplinary study of the DWD106-Mile Site, of which this work forms a part. Rose Petrecca played an indispensable role in sample collection, as did the crews of the RV *Atlantis II* and the DSRV *Alvin*. The contributions of Rebecca Sack and Michael Tangrea in sample analysis are gratefully acknowledged.

This work was supported by National Oceanographic and Atmospheric Administration NURP contract NA16RU0217-01 through the Institute of Marine and Coastal Sciences, Rutgers University.

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