The Environmental Response of Marine Bacteria to Waste Disposal Activities at Deep Water Dump Site 106.

Final Report

by

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Summary

Both shipboard and laboratory studies have been conducted to assess the impact of Dupont and American Cyanimid wastes on the native marine bacterial flora. The principle response parameter scrutenized has concerned variations in organic carbon assimilation in the presence of known seawater dilutions of the primary wastes. Readout is provided by the amounts of ^{14}C assimilated from a predetermined concentration of labelled glucose. When used as a bioassay procedure, such observations offer a highly sensitive means of assessing changes in heterotrophic activity under controlled conditions. The response patterns obtained are believed to be indicative of variations in the ability of marine bacteria to convert organic residues into mineralized chemical components essential for maintaining primary production by phytoplankton.

The above bioassay procedure or its appropriate modification has led to the conclusions enumerated below.

1. The principle toxic ingredients in both wastes resemble organic rather than metallic inhibitors.

2. Regarding natural populations of mixed bacterial species, the Dupont waste is typically about eight times more inhibitory than the American Cyanimid waste.

3. During R/V *KNORR*, Cruise 58, Leg II there were no discernable differences between the inhibitory effects on entire population taken from within and outside the designated dump site area.

4. Exposure of mixed populations to enrichment concentrations of Cyanimid

waste resulted in the isolation of pure cultures showing an increase in waste tolerance.

5. Periodically trace amounts of either waste are capable of initiating ^{14}C glucose rates of uptake which significantly exceed those of a control population observed in the absence of any added waste.

Introduction

In 1977 the amounts and variations of wastes products released at Deep Water Dump Site 106 increased significantly. This trend can be expected to continue given continued expansion of the nation's chemical technology. Annually, thousands of new chemical combinations are being produced, many of which are reaching the environment with unknown consequences. Until society learns to cope with all of its wastes there will be a continuing need for remote dump sites of "last resort".

Prior to 1977 two industrial corporations, Dupont and American Cyanimid, accounted for about 85 percent (0.40×10^6 metric tons) of the total waste discharge at Dump Site 106. The inclusion of Dupont acid iron waste and sewage sludge from Camden, New Jersey during the past year has enhanced the possibility of adverse consequences and emphasizes the need for continued monitoring of this impacted area.

A reordering of representative biological species in a manner which maintains community function and continuity is one of nature's characteristic responses to environmental perturbations. Often, however, nature's solutions are not compatable with man's proprietary interests as for example, when species of economic value disappear. Ideally both nature's and man's interests are deserving of protection with regard to the activities at Dump Site 106.

Given a stable environment, bacterial populations in the sea typically exhibit a high degree of diversity, but limited dominance with regard to genetic types. Species whose life processes are most in harmony with a steady state environment tend to attain dominance. However, significant environmental changes tend to modify specie distribution by encouraging the more adaptable species. The result is often an overall increase in community tolerance. We have attempted to characterize changes in tolerance on the part of some representative bacterial species from Dump Site 106 by comparing rates of heterotrophic activity in seawater samples collected from inside and outside of the disposal area. This approach recognizes the fundamental role of bacterial populations in natural waters, i.e. the biodegradation of complex organic substances and the release of the essential nutrients required by the photosynthetic autotrophs.

Primary production by photosynthetic autotrophs is the major source of chemical energy which supports marine food webs. Usually the energy provided by primary production supports all other diverse and more complicated marine populations. However, there is also an alternative pathway, less thoroughly understood, which involves the release of soluble organic matter from the major food chain which is converted into bacterial cells and then cycled through the microzooplankton. Under ordinary circumstances the scope of this secondary pathway amounts to but 10 percent (Parsons and Seki, 1970) to about 50 percent (Andrews and Williams, 1971) of the total particulate carbon production. However, under chemical stress a significantly larger fraction of the phytoplankton organic carbon is lost through leakage and heterotrophic bacterial activity can be markedly stimulated (Vaccaro *et al.*, 1977). Thus it is important that we acquire an improved understanding of this alternative pathway and its reaction to extraneous sources of toxic waste materials.

Methods

Relative rates of bacterial heterotrophic activity have been estimated from a modification of the heterotrophic potential technique described by Wright and Hobbie (1965) and Vaccaro and Jannasch (1966). The organic substrate of choice was ^{14}C labelled glucose at effective concentrations of about 0.20 $\mu g\ C\ell^{-1} \equiv 0.06\ \mu Ci\ell^{-1}$. Incubation periods were varied, but were generally two hours or less at room temperature. Measurements of heterotrophic activity have been made both on mixed and single species bacterial populations obtained from locations both inside and outside (controls) the dumpsite area. Representative single species were obtained from enrichment cultures prepared with increasing concentrations of representative industrial wastes.

Experimental marine bacterial test cultures used in our bioassay studies were grown at room temperature for 24 hours on a broth medium containing 0.6 g tryptone, 0.5 g glucose, 0.1 g yeast extract, 0.5 g NH_4NO_3 and 1000 ml 0.45 μ filtered seawater. Cells were harvested by centrifugation, washed 2 x and resuspended at appropriate concentrations in filtered seawater.

Results

Seawater mixing characteristics, Grasselli and Cyanimid wastes.

Before determining the impact of Dump Site wastes on test microbial populations, an attempt was made to characterize pH and solubility relations for various waste:seawater mixtures. Results with Grasselli waste, shown in Table 1, assign an alkaline pH of 10.8 and a noticeable turbidity at waste concentrations as low as 0.50 percent in seawater. Grasselli waste concentration in excess of 2 percent caused heavy precipitation, hence it must be concluded that a significant fraction of Grasselli waste undergoes precipitation and sedimentation shortly after entry into the ocean. The chemical nature of this precipitate and its ability to coprecipitate and adsorb other waste constituents would appear to be worthy of further examination. Table 2 shows that unlike Grasselli waste Cyanimid waste was acid (pH = 4.42) and remained soluble in seawater at concentrations as high as 10 percent which reduced the pH to 5.30.

Optimal pH levels are an important consideration when biological effects are used to assess potential toxicity. In nature, marine microbial populations are rarely exposed to a pH which exceeds the range 7.75 - 8.30. However, for our bioassay observations we expanded this range to 9.10 - 6.22 in order to accomodate experimentally useful Grasselli and Cyanimid waste concentrations. This provided upper limits of concentration which corresponded to 1.0 percent for Grasselli and 3.0 percent for

Table 1

Dupont Waste

Seawater Mixture Characteristics*

Waste %	Seawater %	pH	Turbidity
0.0	100.0	8.01	none
0.2	99.8	8.30	none
0.5	99.5	8.80	slight
1.0	99.0	9.12	slight
2.0	98.0	9.36	high
10.0	90.0	9.61	very high
100.0	0.0	10.80	none

*Seawater and waste filtered through 0.3 μ membrane filters before mixing.

Table 2

American Cyanimid Waste

Seawater Mixture Characteristics

Waste %	Seawater %	pH	Turbidity
0.0	100.0	8.01	none
0.2	99.8	7.62	"
0.5	99.5	7.30	"
1.0	99.0	6.94	"
2.0	98.0	6.49	"
3.0	97.0	6.22	"
4.0	96.0	6.02	"
6.0	94.0	5.78	"
8.0	92.0	5.50	"
10.0	90.0	5.30	"
100.0	0.0	4.42	"

Cyanimid wastes.

Relative bacterial inhibition of Grasselli and Cyanimid wastes vs that of copper.

Our prior interest in the effects of heavy metals on bacterial populations led to some early studies on the comparative effects of Grasselli and Cyanimid wastes with that of copper. The test bacterial population common to these studies was a pure culture of a short, gram negative, euryhaline, motile rod. This organism is highly sensitive to copper, to the extent that the addition of as little as 15 parts per billion (ppb) in seawater causes a 50 percent inhibited response (IR-50) in terms of heterotrophic activity. To obtain an effect comparable to that of copper it was necessary to dilute Grasselli and Cyanimid waste with seawater at the 0.33 and >2.80 percent levels respectively. On the other hand the IR-50 for a 50:50 mixture of the above two wastes gave an intermediate value of 2.00 percent. These data indicate that, in this instance, the inhibitory effect from Grasselli waste exceeded that of Cyanimid waste by a factor of 8 or more, that is:

0.33% Grasselli : >2.80% Cyanimid : 15 ppb Cu.

To what extent these results characterize the long term or average properties of these wastes, remains uncertain since we have little or no information on their day-to-day variation.

Dump Site studies on R/V *KNORR*, Cruise 58, Leg II. A mixed population study.

An opportunity to compare ocean characteristics inside and outside the dump site area was provided by R/V *KNORR*, Cruise 58. Here, priority was given to a comparison of the biological effects of Grasselli and Cyanimid wastes on the resident mixed bacterial flora. Other objectives, only partly realized, included an attempt to differentiate between the role of metallic as opposed to organic components in these wastes and the preparation of bacterial enrichment cultures aimed at the isolation of bacterial species of high tolerance.

To differentiate between metallic and organic influences, parallel observations on the inhibition of bacterial activity were made in the presence and absence of 0.80 mM ethylenediaminetetracetic acid (EDTA). The enrichment cultures were prepared by inoculating a complete liquid media containing varying amounts of waste to eleminate all but tolerant species. Final analysis of these isolates was performed following our return to Woods Hole.

The resulting information on mixed populations as summarized in Table 3 has been interpreted as follows:

(1) The resident bacterial flora from inside and outside the dump site area showed a similar response to Grasselli waste.

(2) The fact tha EDTA did not lessen the impact of Grasselli waste suggests a non-metallic impact for this waste.

(3) Again, the Grasselli waste was measurably more inhibitory than the Cyanimid waste.

Comparable information available for pure culture isolates is summarized in Table 4. Once again the increased toxicity of the Grasselli as compared

Table 3

Effect of Grasselli and American Cyanimid Wastes on mixed microbial populations sampled outside and inside Dump Site area

Type Waste	EDTA Added	Microbial Source	IR-50 percent*
Grasselli	no	Outside Dump Site	0.50
"	no	" " "	0.54
"	no	Inside Dump Site	0.52
"	no	" " "	0.54
"	yes	" " "	0.43
"	no	" " "	0.65
Cyanimid	no	" " "	1.70

*Percent waste in seawater which reduces heterotrophic activity to 50 percent of waste-free control.

Table 4

Some effects of Grasselli and Cyanimid wastes on bacterial isolates from the Dump Site area

Type Enrichment	EDTA Added	Waste Assayed	IR-50* Percent
none	no	Cyanimid	1.3
Cyanimid	no	Cyanimid	3.2
none	no	Cyanimid	1.6
none	no	Cyanimid	1.9
none	no	Grasselli	0.44
none	no	Cyanimid	1.80

*Percent waste in seawater which reduces heterotrophic activity to 50 percent of a waste-free control.

with Cyanimid waste is apparent. Also, there is a suggestion that Cyanimid resistant bacterial strains may, indeed, occur within the dump site area.

Chemical characteristics of acid-iron waste from Edgemoor Plant of Dupont Corporation.

General acid-iron waste characteristics:

A representative sample of the acid-iron waste barged on June 27, 1977 was obtained from Mr. James Dixon, Environmental Coordinator at the Edgemoor Plant of the Dupont Corporation. This sample provided preliminary information on heavy metal composition and on distilled and seawater mixing characteristics pertinent to the experimental design of bacterial bioassay studies.

Titration of the acid-iron waste with seawater showed that a 1:10,000 dilution of the acid iron waste lowered seawater pH from 8.0 to 7.5 and resulted in the formation of a brown flocular precipitation of ferric hydroxide. However, repetition of the above with distilled water showed no precipitation at a 1:100 dilution (pH: 2.2) of the waste. These observations provided some useful guidelines regarding the chemical attenuation of acid-iron waste in the sea and have also been used to define an upper limit for the range of experimental concentrations employed for our studies of marine bacterial sensitivity. It is generally accepted that heavy metal precipitation and sinking plays an important role in minimizing biological toxicity within the water column.

A third characteristic of Dupont acid-iron waste and one that we are further exploring, concerns the unexpected chemical changes which occur with time in a distilled water dilution series. These are manifest by progressive changes in spectral characteristics which lead to a "yellowing" three hours after solution preparation. For reasons unknown, "yellowing" is more pronounced in a 1:1,000 dilution (pH: 2.8) than in a more concentrated 1:100 dilution. Preliminary indications are that "yellowing" may be accompanied by a decrease in toxicity toward our bacterial test populations.

Our laboratory sample of acid-iron waste has been analyzed for heavy metal content and the results compared with similar measurements which appear in the Dupont Corporation's Discharge Permit Application. The results, Table 5, compare reasonably well and indicate that the waste sample being used as a source of our laboratory studies is not atypical. Specifically, our sample is considerably richer in vanadium while the Application sample contains somewhat greater amounts of chromium and copper.

We have endeavored to apply the chemical oxygen demand (COD) analysis described in Standard Methods, Water and Wastewater (1971) for assessing variations in organic carbon contents of seawater. Unfortunately, we have been unable to overcome the difficulty introduced by salt interference which seriously reduces the precision of the method. Consequently we are currently using persulfate oxidation followed by infrared analyses of the liberated carbon dioxide to detect variations in total dissolved organic carbon.

Current laboratory studies:

Seawater samples collected during Cruise No. 77-06 of *ALBATROSS IV* from a variety of locations in and out of the Dump Site provide a source of bacterial isolates for conducting acid-iron waste tolerance studies. Common to

Table 5

Comparison of selected heavy metals in Dupont

acid-waste barged to Site 106 on June 27, 1977

vs analyses given in Discharge Permit Application.

	Discharge Permit mg/liter	Barge Sample mg/liter
Cadmium	--	0.5
Chromium	208	110
Copper	--	3.6
Iron	50,400	45,000
Lead	41	20
Nickel	--	8
Vanadium	200	450

these observations is the use of trace amounts of radioactive ^{14}C organic substrate to measure rates of heterotrophic activity by these organisms under various experimental conditions. The objectives of these studies as outlined below.

1. To describe differences in the heterotrophic response (rate of ^{14}C uptake) by Dump Site as opposed to control marine bacterial populations.

2. To demonstrate the possibility of tolerance differences toward acid-iron waste according to population source.

3. To compare the time-related differences in the heterotrophic response associated with known dilutions of acid-iron waste in distilled water and in seawater.

4. To determine how the presence of added metal binding capacity provided by synthetic chelators such as ethylenediaminetetracetic acid (EDTA) alter the biological affects of acid-iron waste.

Recent observations in a similar vein, suggest that time-related changes in waste toxicity may occur which are independent of the total amount of waste present in a diluted sample. As described earlier, we have noted a pronounced tendency toward "yellowing" with time in non-precipitating dilutions of acid iron waste. Early indications are that the occurrence of "yellowing" corresponds to a reduction in the toxic effect attributable to acid-iron waste. In this regard, the possibility of oxidation shifts, due to an increased dissolved oxygen supply or pH change, which favor the appearance of less toxic heavy metal species may be a controlling factor.

So far we have been unable to obtain unambiguous results from the use of metal chelators in acid-iron bioassay experiments, but there is reason to believe that the experimental conditions may not have been optimal. Our expectations are that an addition of EDTA should reduce the toxicity of the heavy metals present in Dupont Edgemoor Waste providing non-metal toxins are not present.

Shipboard studies, Cruise No. 77-06, *ALBATROSS IV*.

Acid-iron waste:

The above cruise provided an opportunity to scrutinize two distinctly different waste disposal operations. One concerned the release of primary sewage sludge originating from Camden, N.J. while the other involved acid-iron waste from Dupont's Edgemoor Plant. Besides the observations and sampling within the Dump Site, a control site was also sampled to obtain comparable observations remote from the disposal area.

At the control location ($39^{o}15.2'N$, $66^{o}54.8'W$), 5 liter volumes of seawater were collected from above and below the thermocline. For concentration purposes, the bacterial contents of these samples were removed onto 0.2 μ membrane filters and resuspended into 50 ml of filtered seawater. A portion of this cell concentrate was used to prepare enrichment cultures wherein the mixed populations were exposed to previously prepared dilutions of acid-iron waste in seawater. After one week, cells which remained viable were streaked and grown on solid seawater nutrient agar for the isolation and recovery of individual species intended for future laboratory studies.

The remainder of the cell suspension was used on shipboard to measure potential rates of heterotrophic activity based on the uptake of ^{14}C glucose over known periods of time. Equal numbers of bacteria were added to replicate subsamples of seawater and a 24 hour incubation period provided before the cells were removed on membrane filters and prepared for ^{14}C scintillation counting. Results from this study, which appear in Table 6, show no significant differences in the ability of either of these bacterial populations to utilize glucose. Apparently at the time of sampling the attenuation and dilution of the acid-iron waste had progressed sufficiently so that any prevailing change was indistinguishable from that of the waste-free control population.

Sludge waste:

During the release of Camden primary sewage sludge surface samples of seawater were collected from the waste plume and analyzed for total and fecal coliform bacteria. The presence of these organisms provides an index of the extent of fecal pollution of the sludge receiving waters. Fecal coliform analyses are believed to provide a more definitive test for recent pollution than the total coliform test and are therefore now preferred over total coliform measurements by many laboratories. Fecal coliforms (*Escherichia coli, E. cole,* var. II, *E. intermediens*) grow mainly in the intestines of warm-blooded animals including man. On the other hand, members of the total coliform group can come from a variety of non-fecal sources, including soil and soil run-off.

In this instance surface samples were taken with a plastic bucket lowered from the side of the ship during the first hour of the sludge dump. Subsurface samples were recovered from the 8 liter Nisken bottles associated with the salinity-temperature-depth (STD) apparatus which selected strategic depths for sampling. Subsurface samples were taken up to 21 hours after the dump.

Positive results were limited to the first hour of surface sampling from within the plume area. Regarding total coliforms, 75 percent of the samples collected proved positive and gave a most probable number range of 1-240 total cells per 100 ml. Measurements on these same samples for fecal coliforms were positive at the 25 percent level and provided a range of 1-120 cells per 100 ml.

No positive results from either test were obtained from any of the subsurface samples. Possibly these results might have differed given the opportunity for continuous sampling over the entire plume. However, the necessary gear was not available at this time and we had to rely on a stationary ship to acquire water samples from beneath the surface.

There are strong indications that the bacterial population associated with sewage sludge is rapidly dispersed by the turbulence and sinking associated with sludge release. Most of the bacterial load appears to remain associated with solid material which rapidly descends to the deeper portions of the water column where a positive sampling becomes highly dubious.

Table 6

Percent uptake of ^{14}C glucose in seawater by bacterial populations of different origin.

Source	Uptake, % glucose supplied
Control location	
Above thermocline	4.4
Below thermocline	5.0
Dump Site 106	
Surface, before dump	5.3
Surface, after dump, in plume	6.6

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