Environmental Chemistry

EFFECT OF SEDIMENT TOXICITY ON ANAEROBIC MICROBIAL METABOLISM

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(Received 6 June 2000; Accepted 20 March 2001)

Abstract—Mineralization of a readily biodegradable aromatic compound (benzoate) by intrinsic microorganisms in the anoxic sediment was used to quantify the inhibitory effect of heavily contaminated sediment from the Arthur Kill estuary (NY/NJ Harbor system, USA) on the anaerobic metabolism by naturally present bacterial populations. In anoxic microcosms, the effect of varying ratios of contaminated sediment:site water and contaminated sediment:noncontaminated sediment (Flax Pond, Stony Brook, NY, USA) were investigated. In all cases, increasing the ratio of Arthur Kill sediment in the microcosms showed an inhibitory effect on the rate of 14C-benzoate mineralization as measured by the evolution of 14CO2. This inhibitory effect could be alleviated through dilution of the sediment with noncontaminated sediment, resulting in some cases in mineralization rates that were greater by an order of magnitude. The toxicity of the sediment was confirmed by whole-sediment Microtox® bioassay. Analysis of the sediment revealed high (>200 mg/kg) levels of Pb, Cu, Zn, and Cr, suggesting that heavy metals may contribute to overall sediment toxicity.

Keywords—Toxicity Heavy metals Anaerobic Bacteria Sediment

INTRODUCTION

Since the early 1900s, the transportation and accidental release of petroleum hydrocarbons and other hazardous chemicals have resulted in high levels of contaminants in estuaries with commercial activities. These pollutants often accumulate in the sediments, forming an anoxic zone of persistent xenobiotic compounds. This accumulation and persistence has created significant environmental problems, threats to human health, and complicated disposal issues. One particularly polluted area is the Arthur Kill estuary section of the NY/NJ Harbor system, having received, for example, 18 x 106 gallons of petroleum and hazardous chemical spills over one five-year period [1]. Water quality parameters, as well as metal and organic chemical concentrations in the sediments, have been reviewed and summarized, providing an extensive database of parameters that contribute to the toxicity of this estuary [1–7]. Contaminants in Arthur Kill sediments include high levels of various chlorinated pesticides, dioxin, polychlorinated biphenyls, diesel fuel components including polyaromatic hydrocarbons (PAHs), and heavy metals.

The toxicity of the sediment present in the Arthur Kill has been measured in terms of ecological surveys (e.g., benthic fauna, fish, periphyton, birds, and mammals) as well as toxicity measurements based on standard laboratory tests (e.g., Microtox®, Microbics, Carlsbad, CA, USA; amphipod and bivalve larval viability) [8–12]. These reports indicate severe adverse effects, resulting in a highly stressed aquatic system. Metals and petroleum hydrocarbons (with polyaromatic hydrocarbons among the most toxic components) are repeatedly cited as the two major concerns in terms of toxicity factors.

In eutrophic coastal areas, oxygen is rapidly depleted, and sediments are often anoxic below a few millimeters of the sediment–water interface [13]. It is estimated that more than 90% of the total organic matter buried in ocean sediments lie under anoxic conditions [14]. In these oxygen-depleted areas, anaerobic microbial metabolism naturally occurs under a variety of reducing conditions. Introduction of various contaminants to these systems can have a profound impact on the metabolism of the anaerobic microbial populations, which in turn can affect higher organisms via the flow of energy through the entire ecosystem. In this way, the health of the microbial sediment communities can directly impact and reflect the overall health of a waterway.

This study examined the sediment toxicity in the test site (Arthur Kill) and its impact on naturally occurring anaerobic bacterial populations in contrast to standard tests with laboratory-introduced species, as cited previously. Because of their long-term preexposure to the sediment, intrinsic microbes may react very differently to the imposed sediment toxicity. In this study, toxicity tests using naturally present microbial populations were performed alongside standard tests (Microtox) in order to evaluate the sediment toxicity of the test site.

Evidence of sediment toxicity was measured in terms of the effect on the rate of 14C-benzoate mineralization by anaerobic bacteria present in the sediment. Benzoate was chosen as a test compound because it is easily degraded in the absence of oxygen and is a key metabolite in the anaerobic degradation of larger aromatic contaminants, such as pesticides and BTX compounds. [15–19]. An extensive review by Harwood and Gibson [15] states that the benzoate degradation pathway has been shown to be the principal route of anaerobic aromatic ring cleavage that leads to the complete oxidation of many diverse aromatic substrates. In our study, measurement of differences in 14C-benzoate mineralization rates under various treatments was used to demonstrate the effect of sediment...
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Environ. Toxicol. Chem. 20, 2001 2407

Toxicity on indigenous anaerobic bacterial populations. Components that are likely to be the most significant toxic factors, as well as possible measures to alleviate the overall toxic effect in order to enhance the anaerobic activity of naturally occurring microbial communities, are proposed.

MATERIALS AND METHODS

Sources of sediment

The test sediment used in this study was collected from the Arthur Kill estuary (AK), a section of the NY/NJ Harbor system. The sampling site was located near the Fresh Kills landfill and was adjacent to a number of petroleum loading docks. The sediment was collected using a gravity core sampler (Benthos, North Falmouth, MD, USA) fitted with plastic liners. Samples were collected from the upper 0 to 60 cm of sediment located beneath approximately 30 m of water. The sediment cores were sealed with rubber caps and kept on ice while being transported to the lab. All sediment samples were refrigerated at 4°C in the dark until use.

Uncontaminated sediment, used as a Microtox test reference and for clean sediment dilutions, was collected from Flax Pond in Stony Brook (NY, USA). Samples were collected using a 2-gallon plastic container with a snap closure plastic cap. This sediment is similar to that of the Arthur Kill in salinity and sand/clay composition (based on unpublished data from ion chromatography analysis and visual observations) and is sulfidogenic but is unimpacted by chronic oil input or landfill leachate. The sediment used in these experiments were all from the same collection event.

Preparation of anoxic microcosms

Sediment slurries (either 50:50 or 10:90 vol%:vol% sediment and site water) were prepared by degassing Arthur Kill site water under N₂:CO₂ (70:30 vol%:vol%) for 1 h, adding the appropriate amount of sediment, and degassing for another 30 min. Prior to dispensing, the pH of the medium was measured to be 7, where it remained for the duration of the experiment. Serum bottles (160 ml) were degassed for 10 min before 80 ml of the sediment slurry were anoxically pipetted into each bottle. The bottles were then capped with blue butyl rubber stoppers and crimped with aluminum seals. Strict anaerobic conditions were maintained and monitored throughout by constant headspace gassing with N₂:CO₂, degassing of any added material, the use of resazurin as a redox indicator when appropriate, and the presence of active sulfidogenesis (indicating a redox potential [Eₚ] of −200mV or lower).

For sediment dilution experiments, sediment:site water slurries (50:50 vol%:vol%) were prepared as described previously. The sediment portion of each was comprised of either 100% Arthur Kill sediment or a mixture of Arthur Kill sediment plus sterile Flax Pond sediment (20:80 vol%:vol%). The Flax Pond sediment was autoclaved three times on consecutive days prior to use. Ion chromatography (as previously described by Kazumi et al. [20]) prior to addition of the radiolabel was used to determine the initial sulfate concentration. Sulfate was added to the Arthur Kill microcosms from an anoxic stock of Na₂SO₄ to standardize sulfate concentrations in all bottles at 25 mM.

Uniformly labeled ¹⁴C-benzoate (1.5 μCi; Sigma, St. Louis, MO, USA) was added to each bottle from a 100-μCi stock solution in toluene. The microcosms were incubated in the dark at room temperature. All experiments were performed in triplicate plus sterile controls for each condition. Sterile controls were autoclaved three times, with 24 h between each autoclaving. All bottles were preincubated for 24 h before substrate addition to ensure anaerobiosis.

Analysis of ¹⁴C-benzoate mineralization

Mineralization of uniformly labeled ¹⁴C-benzoate was monitored by the detection and quantification of ¹⁴CO₂ and ¹⁴CH₄ evolved into the headspace of each bottle. At each time point, gas headspace analysis was performed by withdrawing a 1-ml headspace sample via a Teflon®-sealed, gastight syringe equipped with a sample lock mechanism. The samples were injected into a Varian 3300 gas chromatograph equipped with a 100/120 Porapac Q packed column (Supelco, Bellafonte, PA, USA) and with both a thermal conductivity detector and a radioactive gas analyzer (Raytest, New Castle, DE, USA). Radioactive compounds detected by the radioactive gas analyzer were identified based on comparison to retention times of nonlabeled standards as detected by the thermal conductivity detector. The radiolabeled compounds were quantified at each time point by comparison to standard curves. These curves were derived from identically prepared microcosm standards (without added benzoate), which had varying concentrations of ¹⁴C-bicarbonate (Sigma) added from a 1,000-μCi/ml stock solution.

Microtox bioassays

Whole-sediment solid-phase Microtox bioassays were used to assess acute toxicity of the sediment. This was determined by measuring light emission produced by the test organism Vibrio fischeri when exposed to dilutions of the sediment. Reduced light emission indicated greater toxicity of the sediment. The tests were performed according to the procedure described by Microbics, with a Microtox Model 2055 instrument. Toxicity of sediments were reported as EC₅₀ values (an estimate of the concentration, in mg/L, which reduced the light output from the test organism by 50%) as calculated by instrument software (Microbics Basic Test software). Uncontaminated site water from Flax Pond was used as the diluent for all sediment dilutions. Background levels of light emissions were determined using Flax Pond site water with no added sediment. Validity of the experimental procedure was verified through the use of a ZnSO₄ method standard following the procedure outlined in the Microbics bioassay test manual.

Metals analysis

Energy dispersive x-ray fluorescence spectrometry was used to determine the bulk metals concentrations in sediment samples from Arthur Kill and Flax Pond sites. From each location, a sediment sample, which had been dried previously, was ground with a mortar and pestle in preparation for analysis. Each of the ground samples was divided into three replicate subsamples (triplicates) and analyzed. Triplicate analyses were performed in order to evaluate the sample homogenization process. The samples were analyzed using a QuanX energy dispersive x-ray fluorescence spectrometer (TN Spectrace, Sunnyvale, CA, USA). The QuanX was calibrated using semi-standardless fundamental parameters calibration. Five standard reference materials (National Institute of Standards and Technology 2704, 2709, 2710, and 2711 and CRC PACS-1) were used for standardization. Instrument software computed contaminant concentrations using the matrix-correcting funa-
Fig. 1. $^{14}$C-benzoate mineralization in anaerobic microcosms established with either 10 or 50% sediment slurries with site water. Data normalized for grams dry weight of sediment. Both of the autoclaved controls (10 and 50%) and both of the no-substrate-added controls (10 and 50%) showed no activity over the 25-d period.

Table 1. Initial mineralization rates of $^{14}$C-benzoate in microcosms with different Arthur Kill sediment:site water ratios. Rates were calculated for the time period of days 2 to 9.

<table>
<thead>
<tr>
<th>Sediment</th>
<th>Sediment: site water (vol%:vol%)</th>
<th>Initial rate$^a$ ($\mu$Ci $^{14}$C$O_2$/gdw)/d$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthur Kill</td>
<td>50:50</td>
<td>7</td>
</tr>
<tr>
<td>Arthur Kill</td>
<td>10:90</td>
<td>29</td>
</tr>
<tr>
<td>Controls: autoclaved</td>
<td>50:50</td>
<td>0</td>
</tr>
<tr>
<td>Controls: no substrate added</td>
<td>50:50</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
+-------------------+----------------------------------+---------------------------+---

$^a$Initial rates were calculated as

$$\text{Rate} = \frac{^{14}\text{CO}_2 \text{ produced/dry weight of sediment}}{\text{time}}$$

$^b$gdw = gram dry weight.

Measurement of sediment toxicity

Whole-sediment solid-phase Microtox bioassays assessed the relative toxicity of the Arthur Kill and Flax Pond sediments and the experimental sediment dilution. The EC50 value was at least five times lower in Arthur Kill than in Flax Pond sediment samples, confirming the high toxicity of the contaminated Arthur Kill sediment (Table 3). Furthermore, by decreasing the ratio of Arthur Kill:Flax Pond sediments, a concomitant increase in EC50 values and, hence, a decrease in toxicity was observed.

In order to further examine whether toxic components in Arthur Kill sediments were affecting the metabolism of the naturally occurring anaerobic microorganisms, a dilution experiment was performed using Arthur Kill sediment and uncontaminated sediment of similar geochemistry. Figure 2 shows benzoate mineralization in anoxic microcosms with different ratios of contaminated (Arthur Kill) sediment to sterilized, uncontaminated (Flax Pond) sediment. The Flax Pond sediment was autoclaved in order to serve as a supporting and diluting matrix in the sediment dilution experiments and to eliminate any effect of Flax Pond microorganisms on benzoate mineralization by Arthur Kill microbial communities. The amount of benzoate mineralized in microcosms where slurries were established with 100% Arthur Kill sediment was compared to the amount mineralized in microcosms of 20% Arthur Kill sediment and 80% autoclaved, uncontaminated sediment. As was seen in the previous experiment, increasing the ratio of Arthur Kill sediment in the microcosms resulted in an inhibitory effect on the rate of benzoate mineralization. This inhibitory effect, however, was alleviated through dilution of the sediment with uncontaminated sediment, resulting in an order of magnitude rate increase in benzoate mineralization (Table 2).

RESULTS

Mineralization of $^{14}$C-benzoate in sediment microcosms

The mineralization of $^{14}$C-benzoate in anoxic microcosms established with either 10 or 50% sediment slurry with site water is shown in Figure 1. As is evident from the evolution of $^{14}$CO$_2$, benzoate was mineralized in both instances without a significant lag period. Sterile controls and no-substrate-added controls did not have detectable mineralization products in their headspace. No radiolabel was incorporated into methane, which is consistent with the fact that sulfate is the predominant electron sink in marine and estuarine habitats [21]. Initial benzoate mineralization rates were based on data from days 2 to 9 in order to minimize the effect of added benzoate on any shifts in the microbial community and subsequent activity. The initial mineralization rates under the tested conditions were calculated using the following formula: ($^{14}$CO$_2$ produced/dry wt of sediment)/time. These values are reported in Table 1. In microcosms established with the 10% sediment slurry, the initial mineralization rate is four times higher than that with the 50% slurry, despite the five times greater population of microorganisms that would be present in the 50% slurry.

In order to further examine whether toxic components in Arthur Kill sediments were affecting the metabolism of the naturally occurring anaerobic microorganisms, a dilution experiment was performed using Arthur Kill sediment and uncontaminated sediment of similar geochemistry. Figure 2 shows benzoate mineralization in anoxic microcosms with different ratios of contaminated (Arthur Kill) sediment to sterilized, uncontaminated (Flax Pond) sediment. The Flax Pond sediment was autoclaved in order to serve as a supporting and diluting matrix in the sediment dilution experiments and to eliminate any effect of Flax Pond microorganisms on benzoate mineralization by Arthur Kill microbial communities. The amount of benzoate mineralized in microcosms where slurries
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**Table 2. Initial mineralization rates of ^{14}C-benzoate in microcosms with different contaminated/uncontaminated sediment ratios. Rates were calculated for the time period of days 0 to 6**

<table>
<thead>
<tr>
<th>Sediment</th>
<th>Initial rate[^a] (μCi × 10^{-3}/gdw)/time</th>
<th>Sediment ratio (vol%: vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthur Kill only</td>
<td>100</td>
<td>9</td>
</tr>
<tr>
<td>Arthur Kill + autoclaved Flax Pond</td>
<td>20:80</td>
<td>69</td>
</tr>
<tr>
<td>Controls: autoclaved</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Controls: no substrate added</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

[^a]: Initial rates were calculated as

\[
\text{Rate} = \frac{^{14}\text{CO produced/dry weight of live sediment}}{\text{time}}
\]

[^b]: gdw = gram dry weight.

... and certain heavy metals. While the sediments did not contain unusually high PAH concentrations (data not shown), the results from the Arthur Kill exceeded the effects range low ERL concentration for Cr and the effects range medium ERM ranges for Cu, Pb, and Zn.

Evaluation of our method standard (1 M ZnSO\textsubscript{4} solution) yielded an EC\textsubscript{50} value of 4.029 mg/L with a 95% confidence range of 3.035 to 5.347. This is within the acceptable standard EC\textsubscript{50} range of 3 to 10 mg/L.

**DISCUSSION**

Numerous factors contributing to the toxicity of the NY/NJ Harbor system have been extensively surveyed and reviewed. Noted contaminants present in the harbor sediment include chlorinated pesticides, dioxin, polychlorinated biphenyls, diesel fuel components including PAHs, and heavy metals [1–7]. The resulting toxicity of these sediments has been measured with respect to standard laboratory test organisms as well as specific ecological surveys of higher organisms [8–12]. The effects of sediment toxicity on intrinsic bacterial populations and their metabolic activities, however, have not been examined thus far. Microbial metabolism and respiration, both aerobic and anaerobic, have far-reaching effects on many aspects of the estuarine ecosystem, including dissolved oxygen levels, energy flow, elemental transformations, and nutrient cycling (e.g., carbon, nitrogen, sulfur) and various other alterations of site geochemistry. Bacterial diversity, number, and interactions largely influence these parameters and can affect commercial and recreational use of impacted sites as well as biodegradation potential of contaminated areas. Indeed, the overall health of a body of water can be directly proportional to the health of its primary and secondary producers.

This study examined the sediment toxicity of the test site (the Arthur Kill estuary) and its impact on the naturally occurring anaerobic bacterial population. The inhibitory effect of the heavily contaminated sediment was measured in terms of its effect on the rate of anaerobic benzoate mineralization. Benzoate was used as a test substrate because it is an important intermediate in the degradation of more complex aromatic compounds and is easily degraded in the absence of oxygen. In anoxic microcosms, lower benzoate mineralization rates were observed in microcosms with 50% Arthur Kill sediment, despite the five times greater microbial population expected, than in microcosms established with 10% sediment. Furthermore, in all cases, increasing the ratio of Arthur Kill sediment in the microcosms decreased the rate of ^{14}C-benzoate mineralization. These observations suggest that some components in the Arthur Kill sediment have an inhibitory effect on anaerobic metabolism of benzoate specifically and perhaps anaerobic carbon turnover in general. This inhibitory effect was alleviated through dilution of the sediment with uncontaminated sediment, resulting in an order of magnitude rate increase in benzoate mineralization.

The observed inhibition of anaerobic microbial activity in Arthur Kill sediments may be due to any of a number of compounds listed in the literature. Metals and petroleum hydrocarbons (with PAHs comprising the most toxic component), however, are repeatedly cited as the two major contributors of sediment toxicity. The sediment samples used in this study were thus specifically analyzed for both PAHs and certain heavy metals. While the sediments did not contain unusually high PAH concentrations (data not shown), the results from the metals analyses were more notable. The Arthur Kill samples showed very high levels (>200 mg/kg sediment) of Cu, Zn, Pb, and Cr. Indeed, the ERMs were exceeded for Cu, Zn, and Pb, indicating that the measured metal concentrations were high enough to cause probable biological effects and that these metals were at least partly responsible for the observed decrease in benzoate mineralization. Furthermore, in highly reducing environments, such as in Arthur Kill sediments, heavy...
metals tend to mobilize from inorganic matrices and become more bioavailable and perhaps toxic to microorganisms.

The potential for metabolic inhibition caused by heavy metals has been shown in other studies. For example, metals added to salt marsh sediments were shown to decrease the amount of sulfate reduction and methanogenesis by the resident bacterial populations [21]. In another study, 3H-thymidine incorporation, which can be a measure of overall growth of a microbial population, was also inhibited in the presence of heavy metals [22]. Said and Lewis [23] concluded that heavy metals, including Cu, Hg, Zn, Cd, and Cr, were considerably more inhibitory to the biodegradation of organic chemicals than high concentrations of known microbially toxic organics (e.g., phenol, toluene, n-butanol, benzene, acetone, and methanol, in the range of 2–100 mM). In a recent study, Kuo and Genthner [24] observed the effect of heavy metal ions on the biotransformation and biodegradation of 2-chlorophenol and 3-chlorobenzoate by an anaerobic bacterial consortia. They observed three effects: extended acclimation periods, reduced dechlorination and biodegradation rates, and failure to dechlorinate or biodegrade the target compound. These studies support our observation that heavy metals are likely contributory factors in the repression of bacterial metabolism in the Arthur Kill.

The inhibitory effect caused by the Arthur Kill sediment could be alleviated through clean sediment dilution. In the case of the Arthur Kill sediment, this could be a useful finding in the study of biodegradation potential. The idea of clean sediment dilution could be applied to the treatment of contaminated hot spots within the estuary. Identified areas of high contamination potential could be treated with uncontaminated sediments as a means of promoting the natural or intrinsic degradation activity.

Acknowledgement—We thank Beau Ranheim and the crew of the Osprey (NYC-DEP) as well as Craig Phelps for assistance in sediment collection. We also thank Keith Cooper and Dawn Raskin for use of equipment and technical assistance in conducting the Microtox bioassays. This work was partially supported by the Office of Naval Research (Grant N00149311008) and Department of Energy (065711) and U.S. Environmental Protection Agency (R825303-01).

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