

## Screening Tests for Assessing the Bioreclamation of Dredged Material

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**PURPOSE:** The primary goal of this technical note is to provide guidance on how to determine the suitability of a contaminated dredged material for bioreclamation. Rapid screening protocols are demonstrated with two different sediments, and references are provided to the technical literature. This guidance is designed to serve as the second phase in a two-phased biotreatability management decision guide. It is assumed that the physical and chemical analyses of the dredged material (Winfield and Lee 1999; U.S. Army Corps of Engineers and U.S. Environmental Protection Agency 1998) have been conducted and that a determination has been made that the material is not suitable for open-water disposal. Selection of the appropriate bioreclamation technology and evaluation of its efficacy should be performed in the third phase.

**BACKGROUND:** In the course of completing its mission on maintaining and improving navigation in waters of the United States, the U.S. Army Corps of Engineers must annually handle about 300 million cubic meters of dredged material. Five to ten percent of this material is not suitable for unrestricted open-water disposal. One option for disposal of material that is not suitable for open-water disposal is placement in confined placement facilities (CPFs). However, most CPFs are at or approaching their design capacities, and new sites for CPFs are very difficult to find. As a result, other means of handling contaminated dredged material are needed. Bioreclamation offers a potentially effective and affordable means of decontamination. However, at present it is difficult to determine with certainty if bioreclamation is a viable alternative for treatment of contaminated dredged materials.

**INTRODUCTION:** Of the remediation technologies currently available, bioreclamation may be uniquely suited to decontamination of dredged material (National Research Council (NRC) 1997). From a remediation perspective, dredged material is often characterized by very large volumes of low to moderately contaminated material. Costs of handling large amounts of wet material severely limit economically viable treatment alternatives. Many biotreatment alternatives are relatively inexpensive and require minimal sample handling. However, successful application of a bioreclamation technology requires knowledge of the physicochemical and biological factors that limit contaminant removal, factors that often vary from site to site.

This technical note focuses exclusively on organic contaminants. Although metals are often a problem with dredged materials and microbial biotreatment systems have been shown to effectively remove metals from waste and groundwaters (Marinucci and Bartha 1979; McHale and McHale 1994), practical biotreatment systems for metal-contaminated soils and sediments are still in the development stage (Barkay, Liebert, and Gillman 1989; Harding 1997; Loveley 1993; Summers 1992).

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## SCREENING TESTS USEFUL IN DETERMINING BIOTREATABILITY

For bioreclamation to be successful, the dredged material must support microbial communities and their metabolism (Alexander 1981; Fiorenza, Duston, and Ward 1991; Focht 1994; Madsen 1998; Madsen 1997; Shannon and Unterman 1993; Tiedje 1993). The number of microorganisms present and their potential metabolic activity (Madsen 1996; Ryan, Loehr, and Rucker 1991) can be estimated by assaying samples taken of the material (Norris et al. 1994). Herein, these assays are demonstrated on a highly organic, contaminated sediment from New York Harbor and relatively clean marine sediment from Bartram Island (Jacksonville, FL).

**Cell Enumeration and Biomass Estimates:** There are two basic approaches to quantifying microbial cell densities in environmental samples. The traditional approach requires that the indigenous populations be cultured in an artificial medium. Inferences made on the densities of microorganisms in the original sample are drawn from estimates of microorganisms able to grow on the artificial medium (i.e., plate counts and most probable number estimates). The other approach is based on direct analyses of microbial cells (i.e., epifluorescent microscopic counts) or cell components (i.e., polar membrane lipid fatty acid analyses).

- **Epifluorescent Microscopic Cell Counts** - Sediment samples can be stained with fluorescent dyes, and fluorescing cells can be counted with the aid of a fluorescent microscope (Figure 1). A number of staining protocols are available. The stain illustrated (Figure 1,

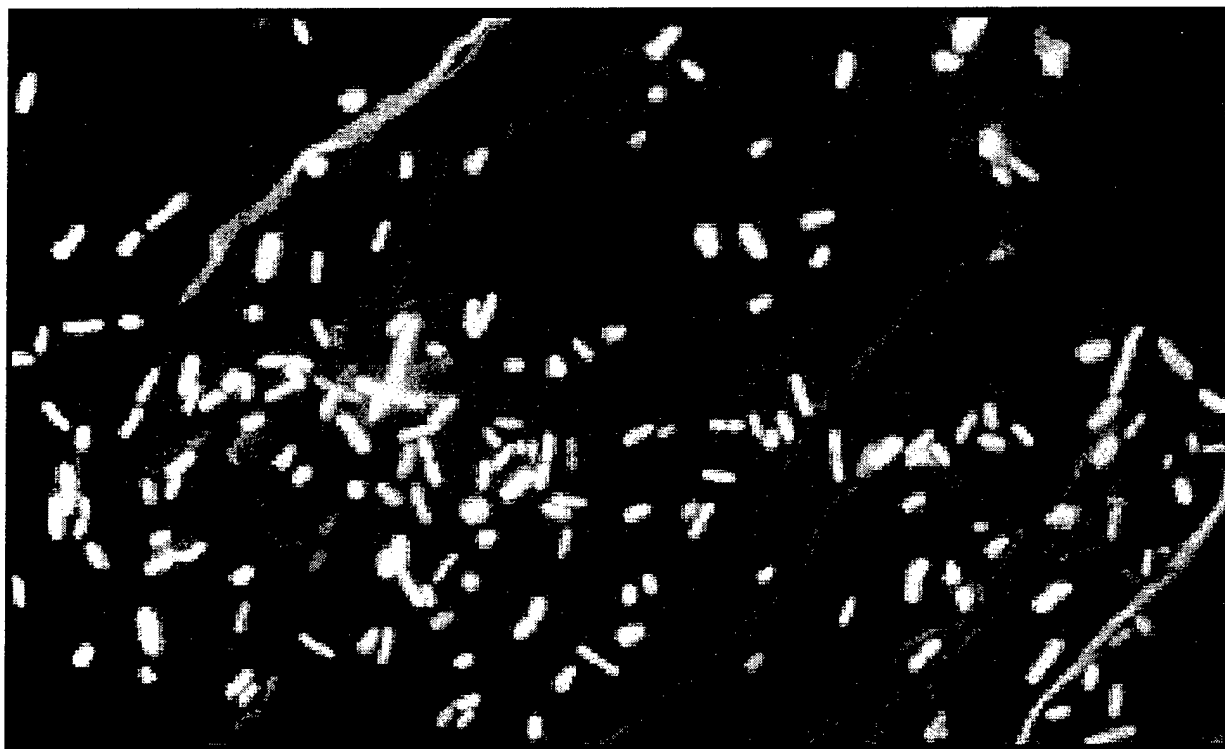


Figure 1. Live/dead (Molecular Probe, Inc.) fluorescent staining of soil bacteria observed with a scanning laser confocal microscope (Samples were observed with a 100x objective and excited with an argon laser (line at 488 nm). Green emissions were recorded at 515 nm and red at 635 nm)

Molecular Probe's live/dead stain) results in living cells fluorescing green and dead cells fluorescing yellow. This staining procedure can be completed in less than 1 hr. The cells in 10 randomly selected microscopic fields of known volume are counted and cell counts averaged. Specificity for targeted taxonomic functional groups can be obtained by using fluorescent targets linked to nucleic acid hybridization probes and antibodies. Although such microscopic observations can quickly yield information on the morphological types and quantities present in a sample, the information is not quantitative. Many microorganisms tend to live in pore spaces and are not readily detached from particles.

- **Colony-Forming Units on Agar Plates** - Another means of ascertaining microbial cell densities in a dredged material is cultivation of the indigenous microbes on nutrient media. Perhaps the most familiar cultivation method is to serially dilute a known amount of dredged material in sterile water and spread the water from the dilutions on nutrient media solidified with agar in Petri dishes (Clesceri, Greenberg, and Rhodes 1989). Counting the colonies that develop on the Petri dishes gives an estimate (i.e., colony-forming units) of the number and types of bacteria present in the original sample (Figure 2). Specific information can be generated by using mineral salts media with only a single source of carbon (Kiyohara, Nagao, and Yana 1982) and energy (i.e., phenanthrene) to support microbial growth. This type of media can be used to estimate the potential of the native microbial community to degrade a specific contaminant. While the data generated with cultivation methods can produce useful estimates, these estimates are not quantitative. Only 0.1 to 1 percent of the cells present in an environmental sample can actually be cultivated on nutrient media. Fastidious microorganisms do not form colonies. On the other hand, germination of resting stages (i.e., spores) and fragmentation of fungal hyphae with each fragment producing a separate colony can lead to overestimations of the numbers of some microbial groups.

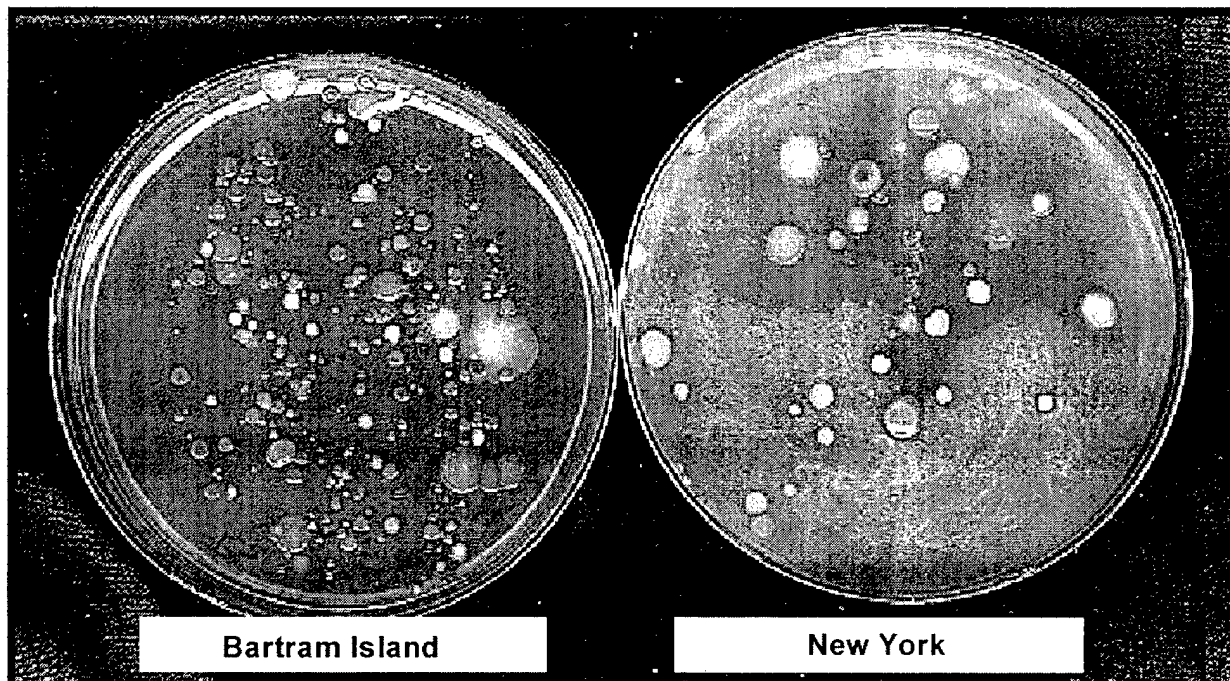


Figure 2. Dilution and plate count estimates of the microorganisms culturable on nutrient agar

For example, the total number of colony-forming units (CFU) produced from Bartram Island was slightly less than from New York Harbor, but Bartram Island sediment showed a greater diversity of colony types. Petri dishes containing material from New York were cloaked with many colonies that spread across the plate, making counting difficult.

- **Most Probable Numbers** - Microorganisms from dredged material can also be cultivated in liquid media and estimated using the most probable number (MPN) technique (Clesceri, Greenberg, and Rhodes 1989). A known amount of dredged material is serially diluted directly in test tubes containing the liquid cultivation medium. After incubation, the tubes in the dilution series that become turbid as a result of microbial growth are recorded (Figure 3). The number of microorganisms present in the original sample are estimated from MPN tables.

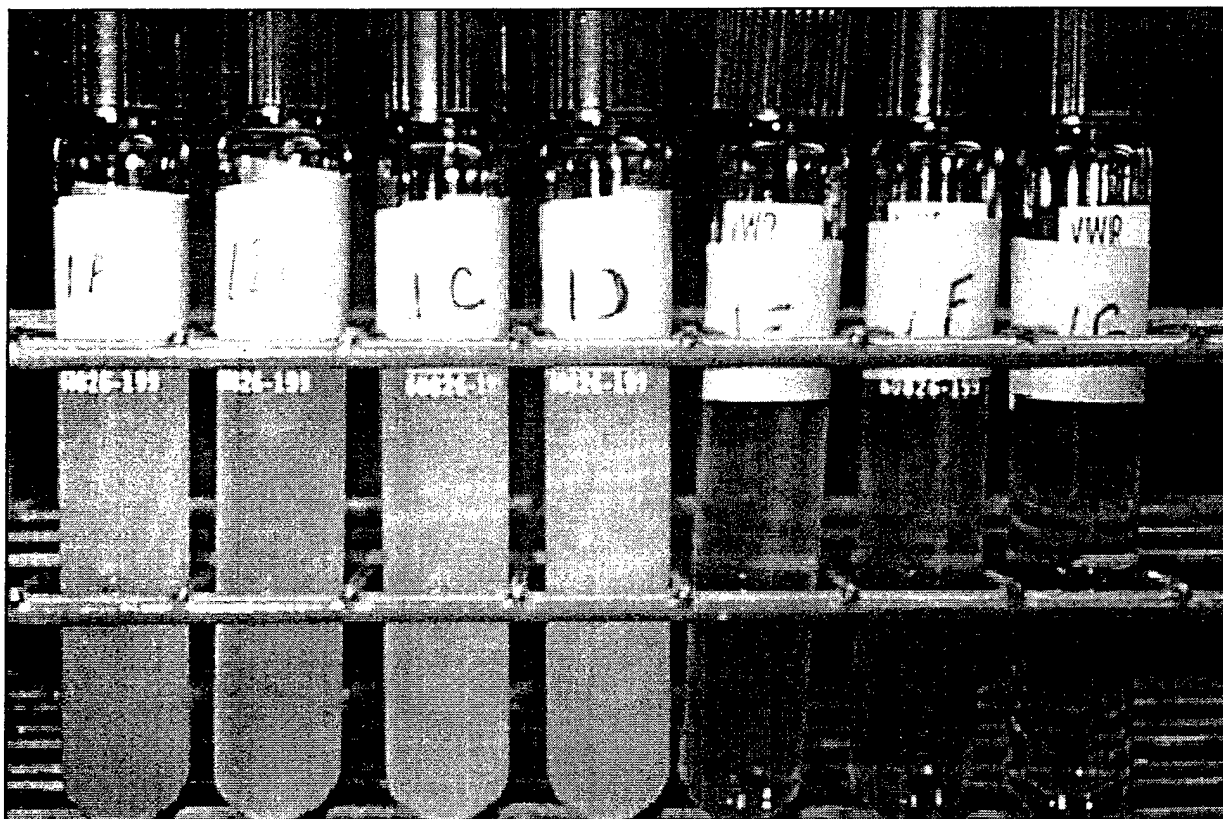


Figure 3. A seven tube most probable number dilution series showing turbidity and gas production as a result of microbial growth

The MPN method is less laborious than the plate count method and is more amenable to automation. Specific information on the potential of the native microbial community to use a contaminant as a sole source of carbon and energy can also be obtained. This method is also not quantitative. Although liquid media is often more effective in cultivating fastidious microorganisms, it suffers from many of the biases stated in the previous section.

- **Polar Lipid Fatty Acid Analyses** - Biological membranes that surround cells are comprised of a lipid bi-layer, which in turn is comprised of complex polar lipids. The fatty acids are components of these polar lipids. Gas chromatographic analysis of the polar lipid fatty acids (PLFA) yields a direct quantitative measure of the biomass present in dredged

material, as well as a profile of the microbial community structure (Phelps et al. 1988; Ringelberg et al. 1989; Smith et al. 1986; Vestal and White 1989; White et al. 1997a,b). Biomass estimates based on microbial lipids (Figure 4) showed the New York Harbor materials to contain  $5.8 \times 10^8$  cells/g and the Bartram Island materials to contain  $4.8 \times 10^8$  cells/g. Microbial community structure was different in the two dredged materials. Evidence for the presence of obligate anaerobes was detected in both materials, but greater percentages were evident in the Bartram Island material (in particular for the *Desulfobacter* sp.). Both materials showed a diversity of membrane lipids indicating the presence of a complex microbial community. Evidence of toxic exposure to hazardous substances, *trans* fatty acids (16:1w7t and 18:1w7t), was also observed in both sediments, but at a greater magnitude in the New York Harbor materials. In this way, the absence or presence of specific microbial groups can be determined prior to the initiation of a biotreatment regime. Bioreclamation processes that would require complete change in the resident microbial community would be expensive to implement and likely to fail. They can also be used to monitor the status of a microbial community through at treatment process.

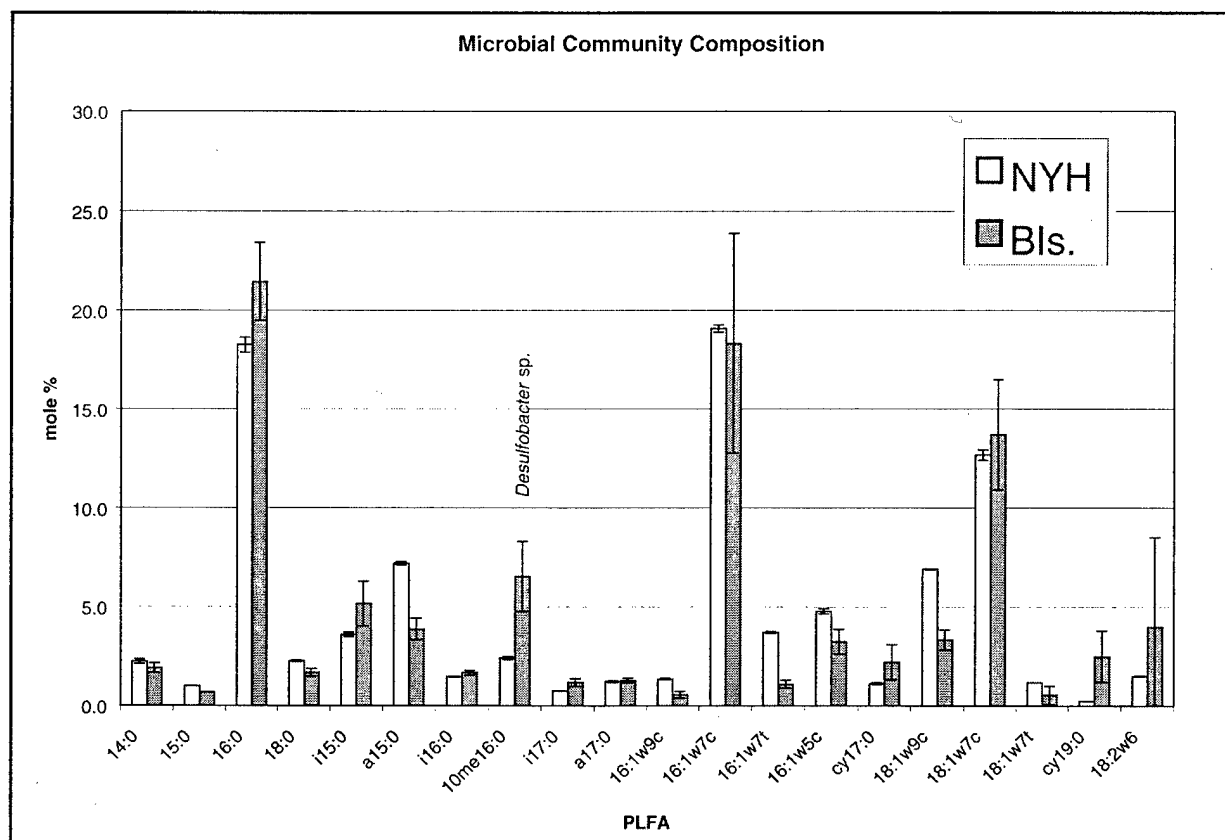


Figure 4. Polar lipid fatty acid profiles of dredged material from New York Harbor and Bartram Island

**Table 1**

**Most Probable Number (MPN), Heterotrophic Plate Count, and Polar Lipid Fatty Acid Estimations of Microbial Cell Numbers in New York Harbor and Bartram Island Dredged Materials**

	Heterotrophic Plate Count CFU/g	Most Probable Number MPN/ml	Polar Lipid Fatty Acids Cells/g
New York Harbor	$9.28 \times 10^5$ $1.18 \times 10^5$	$>1.6 \times 10^8$	$5.77 \times 10^8$
Bartram Island	$9.24 \times 10^4$ $5.25 \times 10^4$	$>1.6 \times 10^8$	$4.85 \times 10^8$

Estimates of microbial biomass in an environmental sample vary because of the bias imposed by the method chosen (Table 1). Cultivation-based methods generally tend to underestimate total biomass and overestimate those microorganisms that rapidly grow on the artificial media. In general, estimates of microbial cell densities under  $10^4$  cells per gram dry weight of sediment indicate the sediment does not support microbial communities very well.

**Measuring Microbially Mediated Activities:** From a functional point of view, it is probably less important to have information on the biomass and community composition of dredged material and more important to have information on the potential biochemical capability of a system to degrade contaminants. The screening tests or assays outlined below are designed to estimate the biochemical potential of the microbial communities in dredged material to degrade contaminants.

- **Abatement of Contaminants** - Perhaps the most intuitively direct means of determining the potential of dredged material to biologically degrade a contaminant is to measure the levels of the contaminant before and after treatment in a microcosm (Bartha and Pramer 1996) designed to simulate pertinent aspects of a bioreclamation system (Figure 5). The Bligh and Dyer (Bligh and Dyer 1959) extraction method can be used for analysis of PLFA and most organic contaminants.

The dichloromethane extraction procedure used for polar lipid fatty acid (above) will concomitantly yield hydrophobic contaminants. The dredged material from New York Harbor contained polynuclear aromatic hydrocarbon (PAH) and polychlorinated biphenyl (PCB) contamination (Figure 6), while contamination in the Bartram sediment was below the detection limits. After 9 days of continually mixing the sediment slurries, the analyzable levels of PAH often increase, particularly in those microcosms amended with mM amounts of nitrogen in the form of  $\text{NH}_4\text{NO}_3$  and phosphate in the form of  $\text{K}_2\text{HPO}_4$ . Similar trends were seen for PCBs (data not shown). Physical erosion of particulate aggregates affects contaminant extraction efficiencies. Stimulation of microbial growth and/or activity often results in the production of organic acids and/or biosurfactants resulting in a lowering of the pH and an increase in the bioavailability of the contamination. When incubation times are short (i.e., 9 days), net loss of extractable hydrophobic contaminants are often not realized and sometimes show net increases in contaminant levels. Some indications of degradation of some compounds can be seen. For example, the abundance of phenanthrene relative to

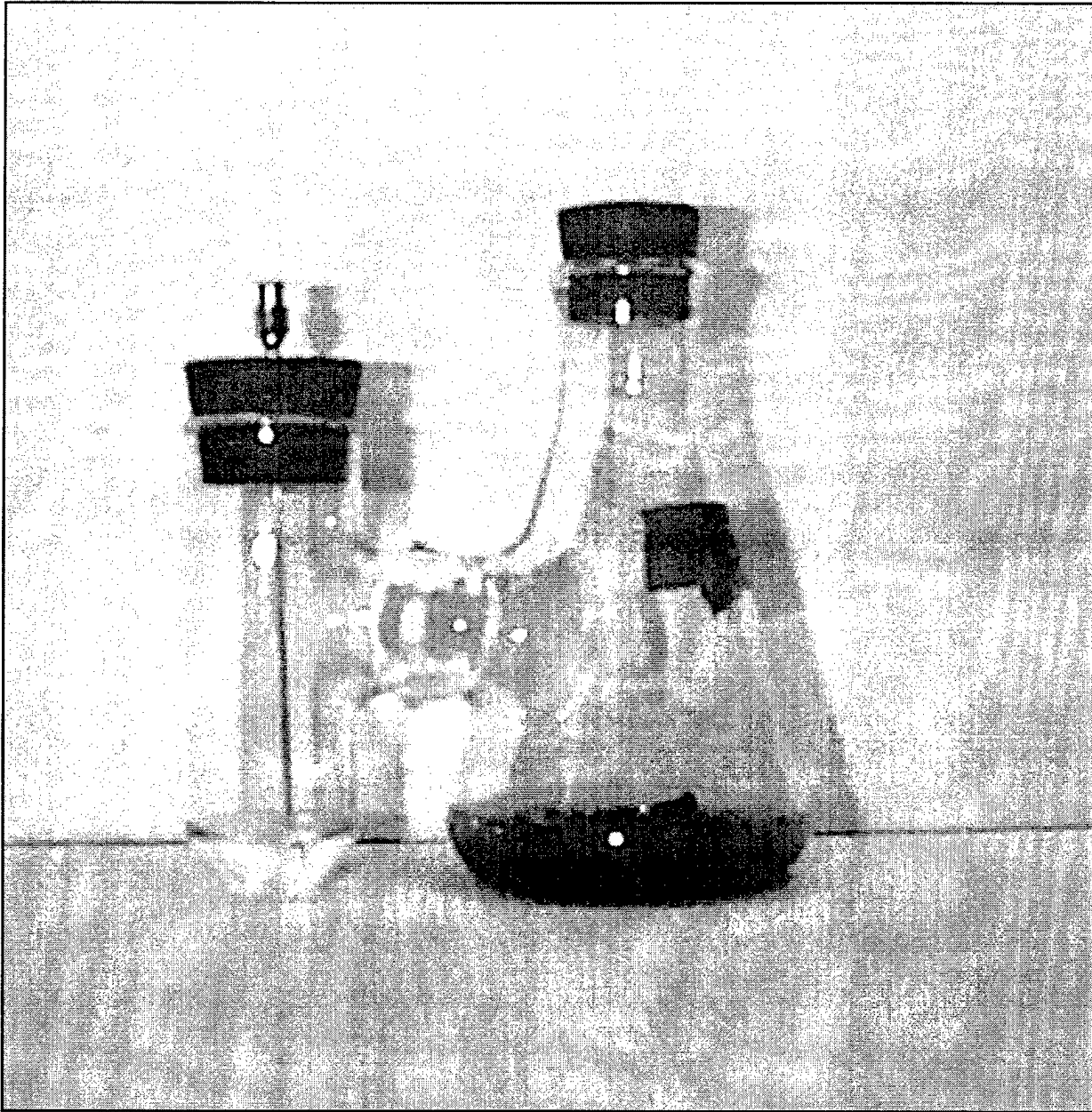


Figure 5. Biotreatability micocosm consisting of a 250-ml biometer flask containing 50 ml of a sediment slurry (30 percent wet/vol) (The flask is placed on an orbital shaker to continually mix and suspend solids. The same vessel can be used for radiorespirometry studies. In these studies, approximately 800,000 cpm of the radio-labeled contaminant is mixed into the sediment slurry, and 2 ml of 1 M KOH is placed in the side arm well to trap  $^{14}\text{CO}_2$ )

total extractable PAH showed a decrease as a result of treatment (Figure 6B and 6C). Phenanthrene is also the most easily biodegradable contaminant listed. When compared with the other higher molecular weight PAHs, a consistent and specific loss of phenanthrene, as a result of nutrient addition, became apparent. Similar results were obtained on the levels of PCBs (data not shown). In summary, the most intuitively direct approach does not always produce the clearest information.

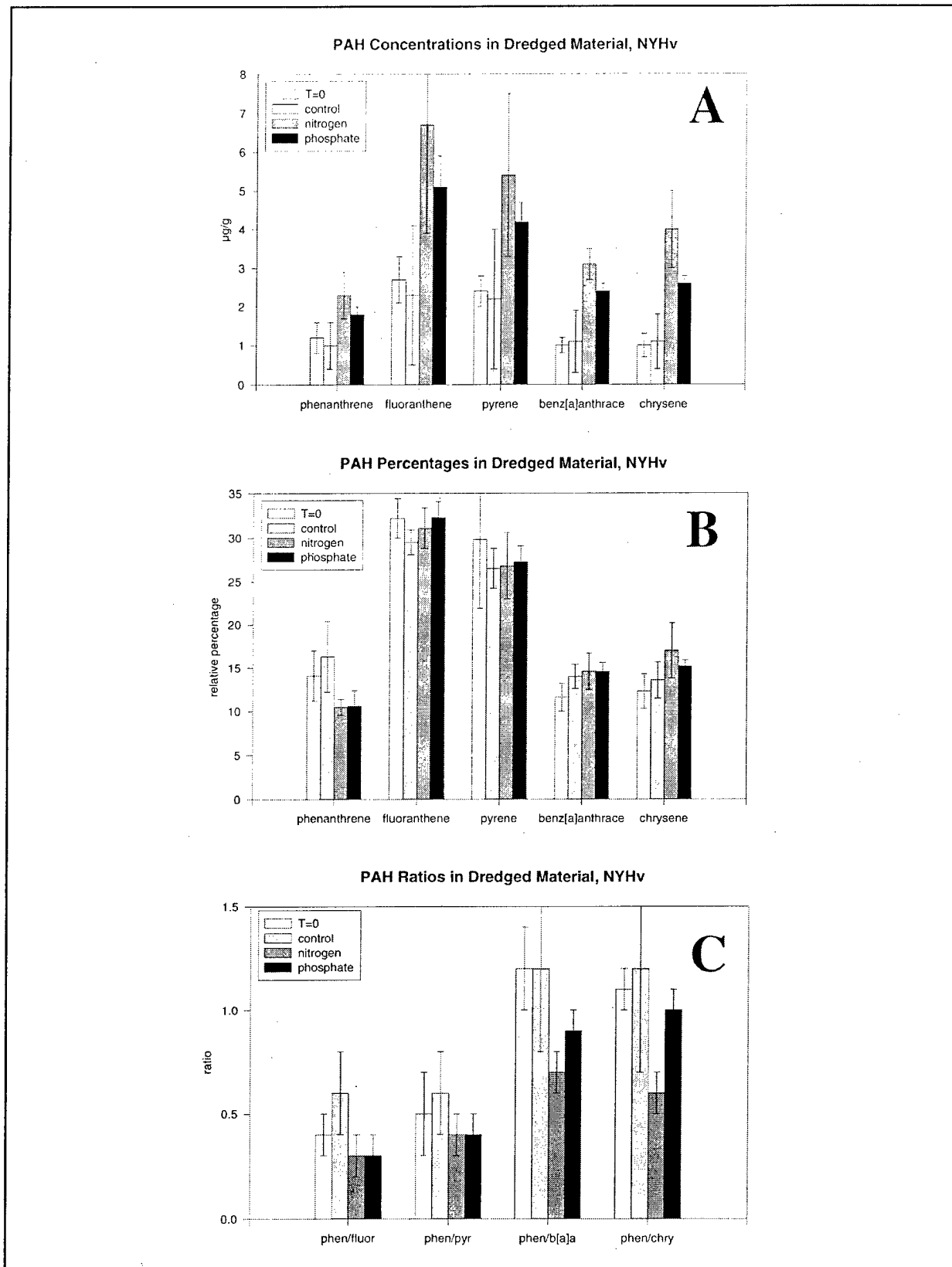


Figure 6. Organic contaminant profiles extracted from dredged material collected from New York Harbor and Bartram Island



- **Oxygen Consumption** - Microbially mediated oxidation of organic contaminants that use molecular oxygen as the terminal electron acceptor provides the most energy to the microbial cell and thus the strongest selection pressure to favor the contaminant-degrading populations (Anderson 1982). Measures of biological oxygen consumption give useful information on the net aerobic metabolism of the sedimentary community.

The original rate of oxygen-dependent respiration in the Bartram sediment was very low, but it was greatly stimulated by the addition of nitrogen and phosphorous (Figure 7). This would indicate fertilization may be required to achieve acceptable kinetics of contaminant degradation. In contrast, the New York Harbor sediment exhibited a rapid rate of oxygen consumption that was not inhibited by formaldehyde. This was due to the high chemical oxygen demand of these highly reduced sediments. Strongly reducing conditions are advantageous for the degradation (Bak and Widdel 1996) of highly halogenated contaminants (Sulflita, Loundry, and Ulrich 1997; Shannon and Unterman 1993). These contaminants can undergo reductive dehalogenation, and the resulting dehalogenated products can then undergo aerobic degradation.

- **Radiorespirometry** -  $^{14}\text{C}$ -labeled contaminants can be added to microcosms and used as convenient tracers of the fate of the contaminant in the sediment (Marinucci and Bartha 1979). Radiochemical analyses provide rates of contaminant mineralization (from rates of  $^{14}\text{CO}_2$  production), removal of the contaminant, and formation of intermediate degradation products.

In the example shown,  $^{14}\text{C}$ -acetate was added to sediment soil slurries to determine a basal rate on microbial metabolism (Figure 8). The two dredged materials showed different patterns of microbial activity. The New York Harbor material showed a slight lag period in activity, which was not apparent in the Bartram Island material. The New York Harbor material also showed a slight enrichment rate of acetate mineralization because of phosphate addition, whereas the Bartram Island material was stimulated by nitrogen addition. Neither nutrient amendment resulted in a substantial increase in microbial activity (beyond that seen in the controls). Slurries were comprised of 2 g of soil and 2 ml total volume of additives.

Radiorespirometry is well suited to the third phase of the proposed dredged material evaluations in that fate of specific contaminants in specific biotreatment regimes can be evaluated. It can be used concurrently with polar lipid fatty acid analyses and instrumental measures of the abatement of contaminants. Working with radionuclides does however require a license from the U.S. Nuclear Regulatory Commission.

- **Genetic Potential** - The genetic makeup of a microbial population defines how that population will react to and utilize contaminants and nutrients under certain environmental conditions. By determining the presence and quantity of genes (Chamberlain and Chamberlain 1994; Knaebel and Crawford 1995) encoding enzymes involved in contaminant reduction (Table 2), transformation or degradation, exposure effects, and the biodegradative potential of the in situ community can be assessed. This is necessary to relate laboratory-derived data (respirometry, oxygen utilization, plate counts) to the in situ community. Cultivation techniques are simple and inexpensive, but examine only the 1-10 percent of bacteria that are cultivatable under current techniques. Direct extraction of DNA from environmental samples bypasses this barrier to assess the in situ community.

Analysis of the New York Harbor and Bartram Island dredged material indicates that both have significant populations of obligate anaerobes (Figures 9 and 10). Bartram Island had

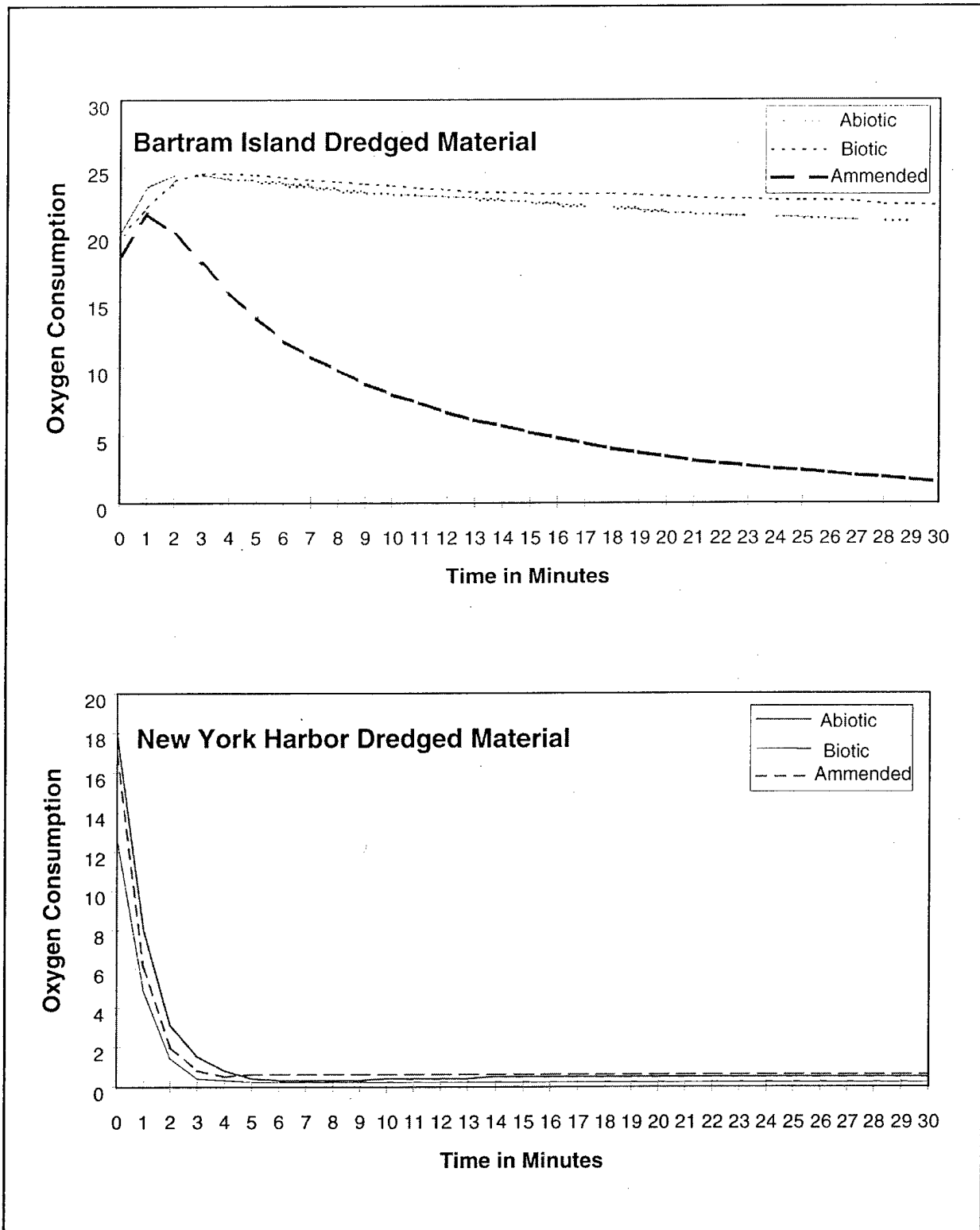


Figure 7. Removal of dissolved oxygen from 20 percent slurries of dredged material (Abiotic controls were killed with formaldehyde. Nitrogen and phosphorus were the amendments)

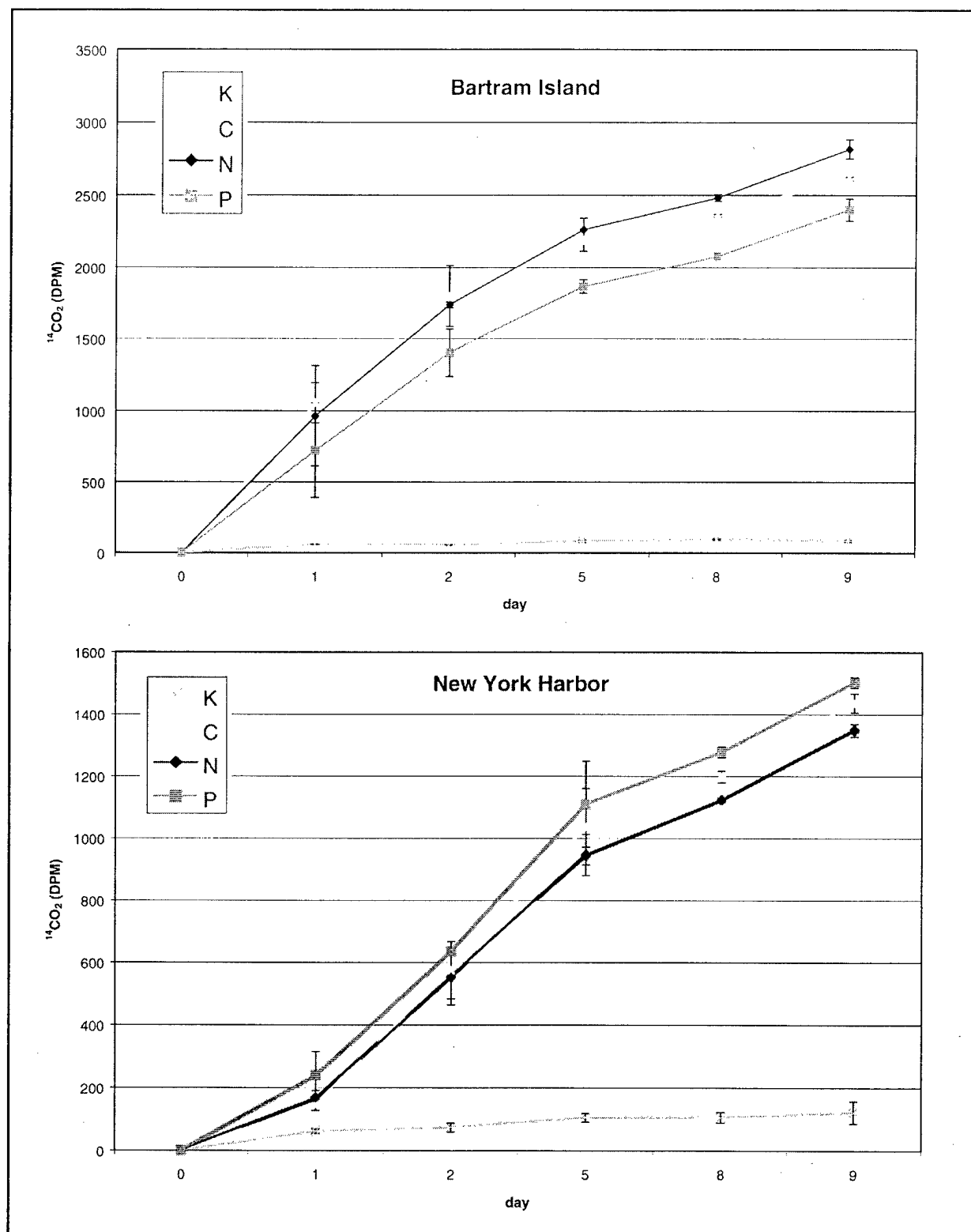


Figure 8. Production of  $^{14}\text{CO}_2$  from  $^{14}\text{C}$ -acetate by microbial respiration in slurries of New York Harbor and Bartram Island dredged material (Triplicate microcosms (2 g dredged material) were incubated per treatment: K (killed control, 3 percent formalin), C (control, no additives), N (nitrogen addition, 12.5 mM  $\text{NH}_4\text{NO}_3$ ), P (phosphate addition, 10 mM  $\text{K}_2\text{HPO}_4$ )

higher populations of *Desulfovibrio* sp., *Archeoglobulus* sp., and atrazine-reducing bacteria. In general, New York Harbor had a lower diversity of different genes, probably because of stress from higher contaminant levels. These results also indicate that the in situ microbial populations are in a very reduced environment as judged by the high density of reductase-type enzymes. A population adapted to a reducing environment would facilitate reductive dehalogenation of PCBs as a remediation option.

**Table 2**  
**Analysis of Replicate Samples of Dredged Material by Multiplex PCR**

	Boston Harbor minimum gene copy g <sup>-1</sup> sediment	New York Harbor minimum gene copy g <sup>-1</sup> sediment
Mercury reductase	7.3E+04	2.0E+05
Nitroreductase	2.0E+05	2.0E+05
Dissimilatory sulfite reductase 1*	1.6E+03	1.3E+03
Dissimilatory sulfite reductase 2*	6.8E+03	nd
Catechol 2,3 dioxygenase	1.4E+03	2.0E+05
Atrazine reductase	8.0E+02	nd
Note: nd, none detected.		
* <i>Desulfovibrio</i> sp., <i>Archeoglobulus</i> sp.		

**SUMMARY:** Bioreclamation potentially affords practical means of handling large volumes of lightly to moderately contaminated dredged material. If physical and chemical characterizations (i.e., Winfield and Lee 1999) of dredged materials raise concerns about contamination, procedures outlined in this technical note can be used to determine if the dredged material is or is not suitable for bioreclamation. As discussed, a number of screening assays can be used to produce useful information. Each assay has its shortcomings, and no one assay can be used to produce information for judging bioreclamation potential unequivocally. It is preferable to base a judgment on a weight of evidence. If the material is judged potentially suitable for bioreclamation, a second phase of evaluations should be performed to identify which bioreclamation system is appropriate for the particular dredged material.

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Bartram Island | New York Harbor

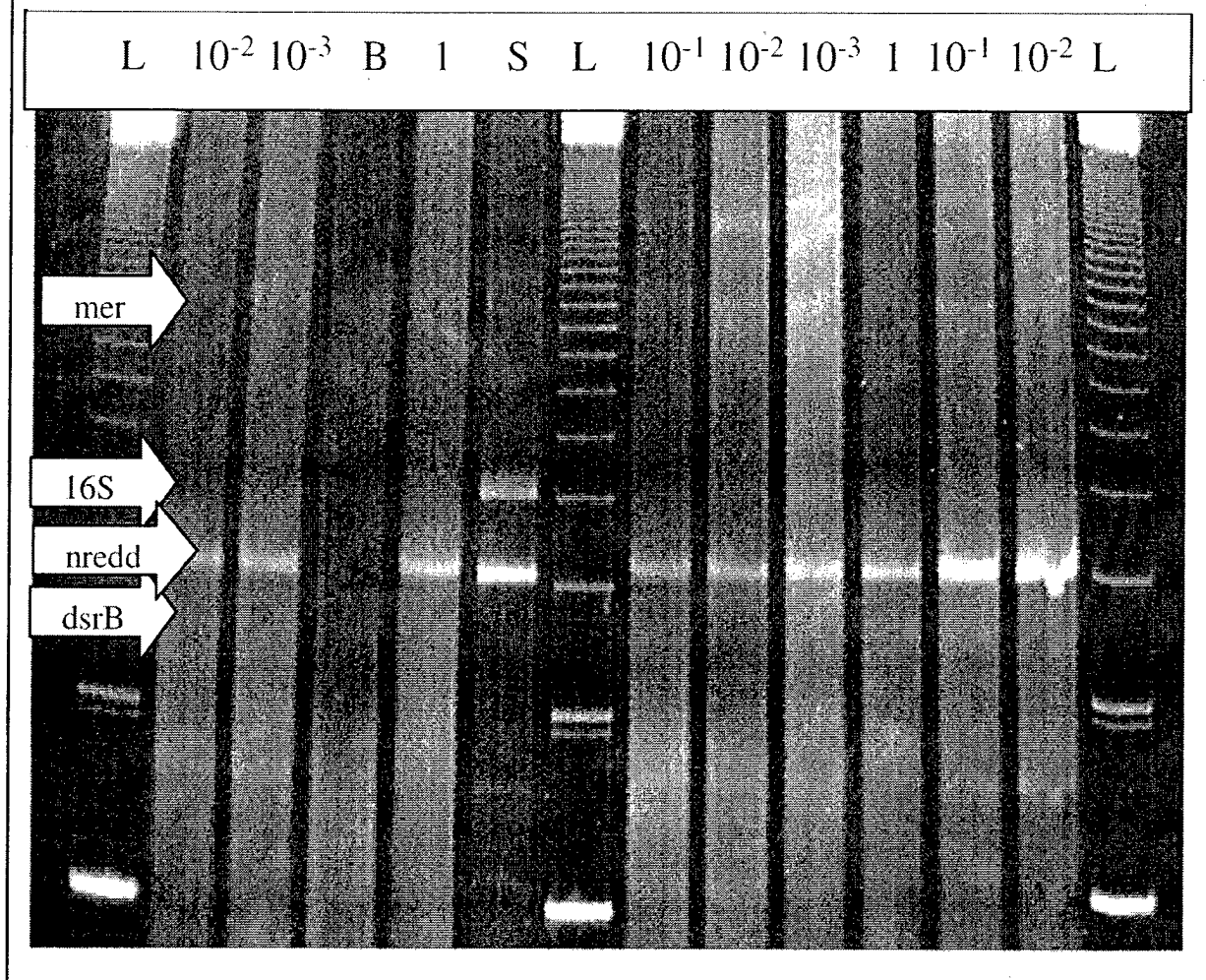


Figure 9. DNA extracted from dredged material, purified, and serially diluted (Selected genes were amplified using multiplex polymerase chain reaction. Dilution was used to estimate gene copy number. L is a DNA size ladder (123 bp ladder, Sigma). B is a water blank. S is a genomic standard with each of the target genes. The dilutions are indicated above the appropriate lanes. Mer indicates the mercury reduction target genes merRP. 16S indicates position of the target 16S ribosomal RNA gene. Nred indicates the target gene NAD(P)H nitroreductase)

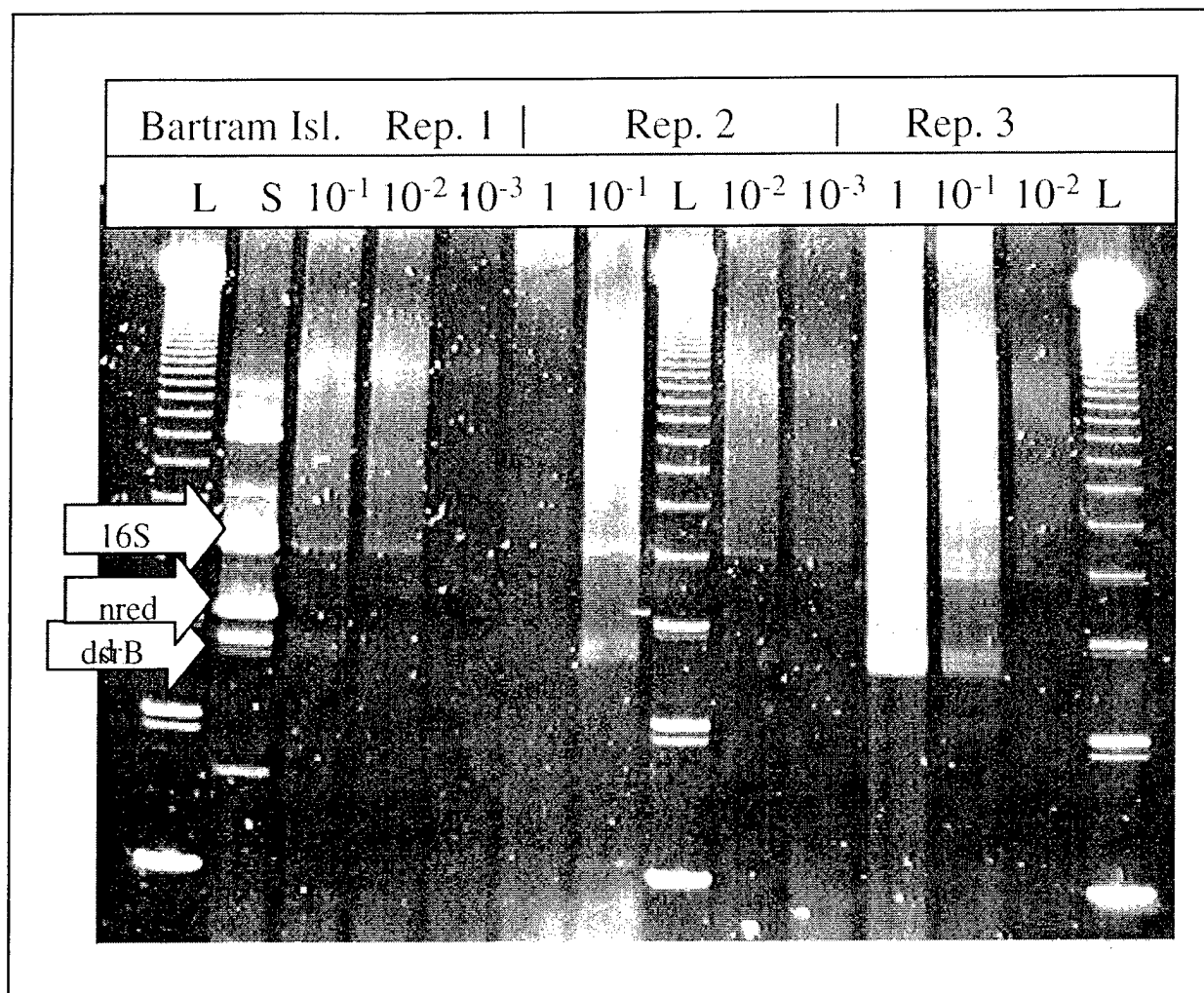


Figure 10. Multiplex PCR of Bartram Island dredged material samples (Analyses of three replicates are shown. A dilution to extinction is used to estimate gene copy number. L is a DNA size ladder (123 bp ladder, Sigma). S is a genomic standard with each of the target genes. The dilutions are indicated above the appropriate lanes. 16S indicates position of the target 16S ribosomal RNA gene. DsrB indicates position of the target gene dissimilatory sulfite reductase)

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