Control of Biologically Active Degradation Zones By Vertical Heterogeneity: Applications In Fractured Media

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Abstract
The objective of this research is to determine the relationship between of biologically active contaminant degradation zones in a fractured, subsurface medium and vertical geological heterogeneities. The research is being performed on samples collected from the Test Area North (TAN) site at the Idaho National Engineering and Environmental Laboratory (INEEL) where a dissolved trichloroethylene (TCE) plume is migrating in the basalts and interbed sediments of the Eastern Snake River Plain (ESRP) aquifer. Research results are leading to an enhanced understanding of the constraints that the geochemical and hydrological environment place on the activities and distribution of TCE-degrading organisms in this fractured subsurface medium. Enhanced understanding allows better decisions to be made regarding the use of remedial technologies such as natural attenuation and in situ bioremediation at geologically complex waste sites. Through this research, investigations conducted by the Subsurface Science Program (SSP) at TAN are being extended in order to develop a mechanistic understanding of the coupled geomicrobial and hydrogeochemical processes that are necessary to predict the field-scale intrinsic degradation rates of TCE. The research objective is being accomplished by characterizing paired cores and water samples from boreholes located in differing geochemical and flow environments within the plume. Analysis of these samples will allow the determination of the spatial correlation

and microbial characterization. The results presented in this report consist primarily of TAN-33 data as many of those ‘analyses have been completed. Nearly all of the TAN-37 data has yet to be acquired. It should be noted that most of the cores were collected from zones that consist of relatively competent, massive basalt. This was because we were doubtful about the quality of samples obtained from rubble zones due to potential alteration by the drilling fluids. Thus, microbiological results on the core samples likely represent a conservative estimate of the types, activities and numbers of the microorganisms present in the borehole.

Geochemical analysis of water from distinct vertical locations in the aquifer at TAN will be studied in FY-1998 using the MLS. Preliminary experiments using bromide diffusion from the MLS in TAN-33 indicated that downhole incubation times should be on order of at least l-2 months rather than the initial estimates of approximately a week. Bromide diffuses slowly from the MLS cells indicating that although the aquifer may be quite permeable, groundwater flow is slow. Future MLS studies will take this into account and the MLS will remain downhole for periods long enough to allow colonization and chemical equilibration.

Collecting reliable samples for microbiological characterization requires that cores are acquired under conditions that minimize core contamination from drilling fluids and other sources of microorganisms within the borehole (e.g., more superficial strata, drilling equipment). In order to evaluate the quality of the core nontoxic, microbial-sized indicators were introduced during coring to allow subsequent evaluation of the degree of potential microbial contamination (see for example, Colwell et al. 1992). Microsphere indicators were used during the collection of all cores from TAN-33 as previously described (Colwell et al. 1992). The quality assurance/quality control tracer data acquired for TAN-33 indicates that most of the samples demonstrate a marked decrease in the concentrations of fluorescent microspheres (Fig. 4). Subcore samples, which were obtained by paring away the outside of the core under anaerobic conditions, had concentrations of microspheres that were approximately two orders of magnitude lower than in the parings. Perfluorocarbon tracers (PFT), a non-toxic chemical added to the drilling fluids, also showed a marked decrease between the parings and the subcores in the core intervals where the PFT was successfully deployed (Fig. 4). Samples obtained from 95.7 m (ca. 314 ft) and shallower were not exposed to the PFT due to a malfunction in the tracer pump. However, below 95.7 m, this extremely conservative tracer was often at the limits of detection in the subcores. The tracer data indicates that the paring and careful handling of these samples minimized the potential contamination during the coring process to the inside of the core and that the results of the microbiological analyses on the core materials should be representative of the microbial communities that exist in the fractured TAN basalts.

TCE concentrations were measured in the TAN-33 core samples by A Fryar (U Kentucky) and were found to be low µg/kg range) in all of the samples (Fig. 5). A single sample from 103.2 m (339 ft) contained 6 µg/kg, but all other samples showed lower concentrations of TCE with two of the samples yielding undetectable concentrations (< 0.25 µg/kg). These data are in agreement with the findings of J Ingram (INEEL) who is studying the interactions of TCE with minerals in basalts (personal communication) and indicate that TCE is weakly partioned from the dissolved phase to the basalt. Determinations of the concentrations of TCE in the groundwater are part of the first MLS study at TAN-33 which will be completed in FY-1998.

Our data indicate that relatively low concentrations of potential TCE-degrading microorganisms appear to be present in TAN-33 cores. These microorganisms include phenol oxidizers which ranged in concentration from below our limits of detection to levels somewhat above 100 cells/g. These organisms were highest in the deeper sections of the borehole at depths greater than 91 m (300 ft) (Fig. 6). Methanotrophic bacteria (determined by T Phelps, ORNL), showed a similar pattern of distribution with the highest concentrations of cells ($\leq$ 25 cells/g) at depths greater than 91 m (300 ft). Our own methanotrophic enrichments failed to detect any cells at higher concentrations. In general, aerobic heterotrophic bacteria were found at higher concentrations than

other bacteria (up to 10^5 cells/g) and followed a vertical distribution pattern similar to that of the other organisms (Fig. 6).

Microbial acetate mineralization under aerobic and anaerobic conditions was used as an overall indicator of microbial activities in the TAN-33 core samples. The results suggest that the upper portion of the borehole (< ca. 80 m or 260 ft) has relatively higher aerobic acetate mineralization rates than deeper portions of the borehole (Fig. 7). Most of the anaerobic acetate mineralization experiments yielded negative results on samples from < 105 m (344 ft). At greater depths, anaerobic acetate mineralization appeared to be higher, overall, except for samples obtained from the QR interbed. The preliminary results from TAN-37 indicate that the levels of aerobic and anaerobic acetate mineralization at this location (close to the injection well) are much higher than in the dissolved plume location (TAN-33) at some distance from the injection well.

DNA extractions from eight samples obtained from TAN-33 did not yield polymerase chain reaction (PCR) bands. If the same samples were spiked with as few as 10^4 cells, PCR bands were evident, so nothing in the samples themselves prevented the success of this procedure. In an effort to assess whether non-visible bands could be detected, invisible PCR bands from two of the samples along with a full procedural blank (processed without basalt) were cloned and a subset of the clones was submitted to Amplified Ribosomal Dna Restriction Analysis (ARDRA). Six ARDRA types (all eubacterial) were idendified from the 113.3 m sample and three ARDRA types (all eubacterial) were idendified from the 125.4 m sample. Basic Local Alignment Search Tool (BLAST) was used to compare the sequences of these eubacterial clones to that of known microorganisms. Most of the sequences that resulted from these clones were approximately 90% similar to known eubacterial sequences. Preliminary evaluation of the DNA extractions from ten samples from TAN-37 indicate much higher levels of DNA than in the samples from TAN-33. Eubacterial PCR bands were recovered from all of the samples that were amplified except the combusted basalt sample. Only one of the ten samples yielded an archaea PCR band. These PCR bands are currently being cloned.

One aspect of the research is to attempt to detect the presence of messenger RNA (mRNA) for soluble methane monooxygenase (sMMO) in the core materials. mRNA is the molecule that carries the information held in DNA to the ribosome for enzyme synthesis and sMMO is the enzyme that methanotrophic bacteria use to obtain energy from methane. sMMO is also capable of oxidation of TCE. Thus, the presence of mRNA specific for sMMO in the TAN core materials would indicate that methanotrophs in the subsurface at TAN are actively making sMMO that could be degrading TCE in situ. Our initial approach was to establish a method to extract RNA from a laboratory culture of a methanotroph (Methylosinus trichosporium OB3b) that was added to basalt samples. Subsequently, we would attempt to amplify a portion of the sMMO mRNA using reverse transcriptase polymerase chain reaction (RT PCR). RNA isolation from OB3b was successful without core using pure cultures of 10^9 cells. However, when the organism was added to cores, RNA could not be extracted. Coincident with this finding was the determination that only few (< 25) methanotrophs could be cultured per g of core material from TAN-33. This finding suggests that the level of mRNA for sMMO in a gram of basalt would be too low to be detected. Although the biomass in cores from TAN-37 appear to be considerably higher than the biomass measured in cores from TAN-33, these levels are still probably too low to yield measurable mRNA in any reasonably sized sample. Prior studies have indicated the presence of methanotrophs in the Snake River Plain aquifer at TAN and elsewhere (J Barnes, personal communication). As a result, our initial studies to detect mRNA will be conducted on large volumes of water that can be pumped and filtered from the Snake River Plain aquifer.

Future Plans

Early in FY-1998 the MLS will be placed in TAN-33 for long term incubation. The depth interval in TAN-33 where the MLS will be placed is from 94.5 m (310 ft) to 114.8 m (376.5 ft). This

corresponds to a 20 m interval of the aquifer which traverses differences in fracture distribution and densities and also coincides with a zone that was intensively sampled during the coring effort. The MLS will be set up with repeating, 36 cm long intervals that contain seven cells of 30 ml each. Over the 20 m distance, 55 discrete zones will be sampled. Four of the seven cells in each interval will be dedicated to microbiological analyses (10 µm pore size membrane filters): one of these will contain water and three will contain ground basalt. The three remaining cells will be used to determine water chemistry characteristics (0.2 µm pore size filters) (see Fig. 8). Subsequent MLS studies will be conducted in TAN-33 at different vertical intervals and also in TAN-37 when other (EM-40) studies are completed in that borehole.

Coring of TAN-48 is scheduled to occur in early FY-1998. This borehole will be drilled in the dissolved plume portion of the aquifer where the nominal TCE concentration is 100-300 µg/L. Only the upper ca. 15 m of this borehole will be cored for microbiological samples. This zone has been targeted because it should traverse the PQ sedimentary interbed within the aquifer. Subsequent development of the well with screened zones in the basalts and across the interbed will allow MLS studies that provide samples over these zones of contrasting geology.

Specific Relevance to Environmental Management and TAN groundwater cleanup at the INEEL

EM-40 cleanup of the waste plume in the groundwater at TAN has technical needs and this EMSP research is helping to address these needs by:

- determining the specific vertical distribution of contaminants in the Snake River Plain aquifer,
- establishing the presence and location of naturally occurring microbial communities that are capable of contaminant degradation,
- determining the abiotic conditions (chemical and physical) under which these microorganisms are active in the degradation of the TCE, and
- acquiring data to allow estimates of the natural rates of waste remediation.

Other Progress

- In collaboration with EM-40 scientists charged with characterization and cleanup of the contaminants at TAN, the schedule and locations for EMSP core collection and MLS studies in the Snake River Plain aquifer have been determined. The proposed boreholes were selected based on EM-40 needs; however, on a case-by-case basis these locations were considered for their relevance to addressing questions outlined in this EMSP research. The selected boreholes include TAN-37 (cored in June, 1997; just downgradient from the “hotspot” near the TAN injection well), TAN-48 (to be cored in early FY-1998; ca. 2500 ft downgradient from the “hotspot” on the primary axis of the TCE plume where TCE concentrations are nominally 100-300 µg/L), and TAN-32 (already drilled, open borehole which can be developed for long-term MLS studies; just south of the main axis of the TCE plume).
- Coring methods, sample processing technologies, MLS strategies and analytical techniques for microorganisms and geochemistry that were developed by the Subsurface Science Program (SSP) have been adapted for the field and laboratory investigations required by this EMSP research at a DOE cleanup site.
- Significant progress was made regarding issues related to acquiring, shipping, and analyzing samples obtained from a CERCLA waste site. The resolution of these problems through the development of sampling plans which are direct adaptations from SSP sampling plans ensures that future research-related coring efforts at TAN for EMSP are likely to proceed more rapidly. Coordination of EMSP investigators Colwell and Smith with INEEL EM-40 scientists has enabled the collection of samples from a site that is tightly controlled and regulated by the State of Idaho’s Department of Environmental Quality, the U.S. Environmental Protection Agency and elements of DOE-ID and Lockheed Martin Idaho Co. Environmental Safety and Health offices.

- Since funding was started in late FY-1996, co-principal investigators have met on four occasions to plan research.
- Coordination of sampling efforts continues with other EMSP investigators including E Majer (LBNL), F Brockman (PNNL), J Geller (LBNL), M Conrad (LBNL) and EM/ER investigator J Ingram (INEEL). Future collaborations with NABIR researchers are possible as a result of the meeting planned for January 1998 in which investigators from these different programs consider cross-cutting issues. These collaborations will provide access to boreholes at TAN for microbiobgical and geochemical samples or geophysical studies. The joint efforts by various EMSP projects will provide a more detailed understanding of the complex geology of the TAN site.
- Training is complete for scientific participants on the project so that field activities at the TAN-37 core site and other boreholes can proceed. EMSP researchers are fully compliant with the Environmental Safety and Health requirements needed to work at this CERCLA site.
- Presentations have been given at Fractured Rock Science Team meetings at the INEEL (Jan. and March 1997), at the 1997 Conference on Hazardous Wastes and Materials (invited symposium speaker, April, 1997), at the INEEL Citizen's Advisory Board Meeting in Post Falls, ID (Sept. 1997) and at the national Geological Society of America in Salt Lake City, UT (two papers, Oct. 1997).

References Cited

Colwell, F., G. Stormberg, T. Phelps, S. Birnbaum, J. McKinley, S. Rawson, C. Veverka, S. Goodwin, P. Long, B. Russell, T. Garland, D. Thompson, P. Skinner and S. Grover. 1992. Innovative techniques for collection of saturated and unsaturated subsurface basalts and sediments for microbiological characterization. J. Microbial. Meth. 15: 279-292.

Budgets

This project had $166.973 K carryover at the change of the fiscal year. However, $141 K of that amount represented outstanding commitments to: 1) PNNL for research that was ongoing but had started late due to late receipt of samples from TAN-37 and 2) Parsons Environmental and PC Exploration for coring the borehole TAN-48, an effort that was planned for late FY-1997 but was delayed due to ES&H concerns until the early part of FY-1998. The balance ($26 K) should be used to support the A-L Reysenbach effort at Rutgers which was also delayed due to the late acquisition of samples from TAN-37 and the subsequent delay of analyses.

Figure 1. TAN TCE contamination plume (from 1994 analytical data) and well locations.

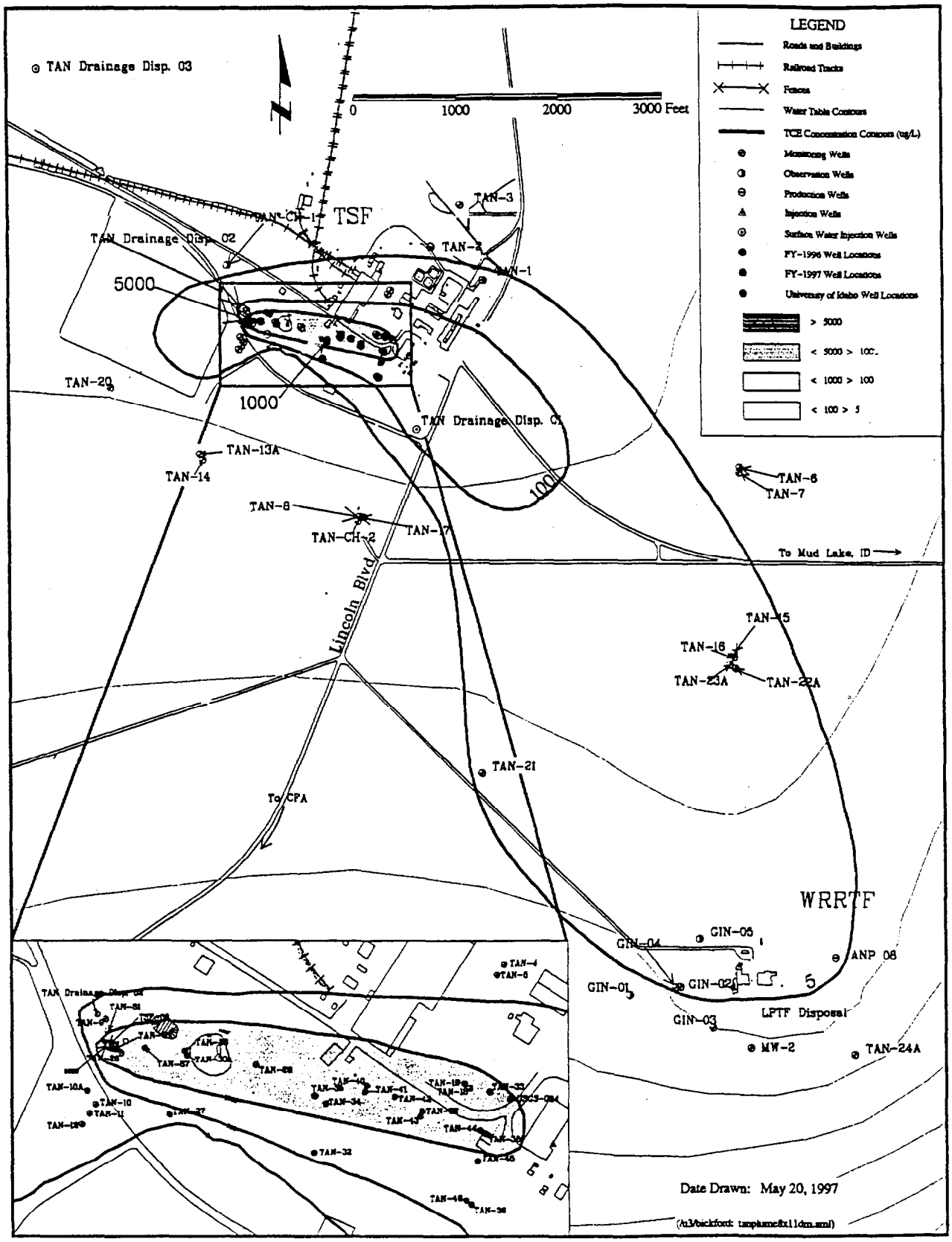

Figure 2. TAN-33 well construction, stratigraphic information and sample locations.

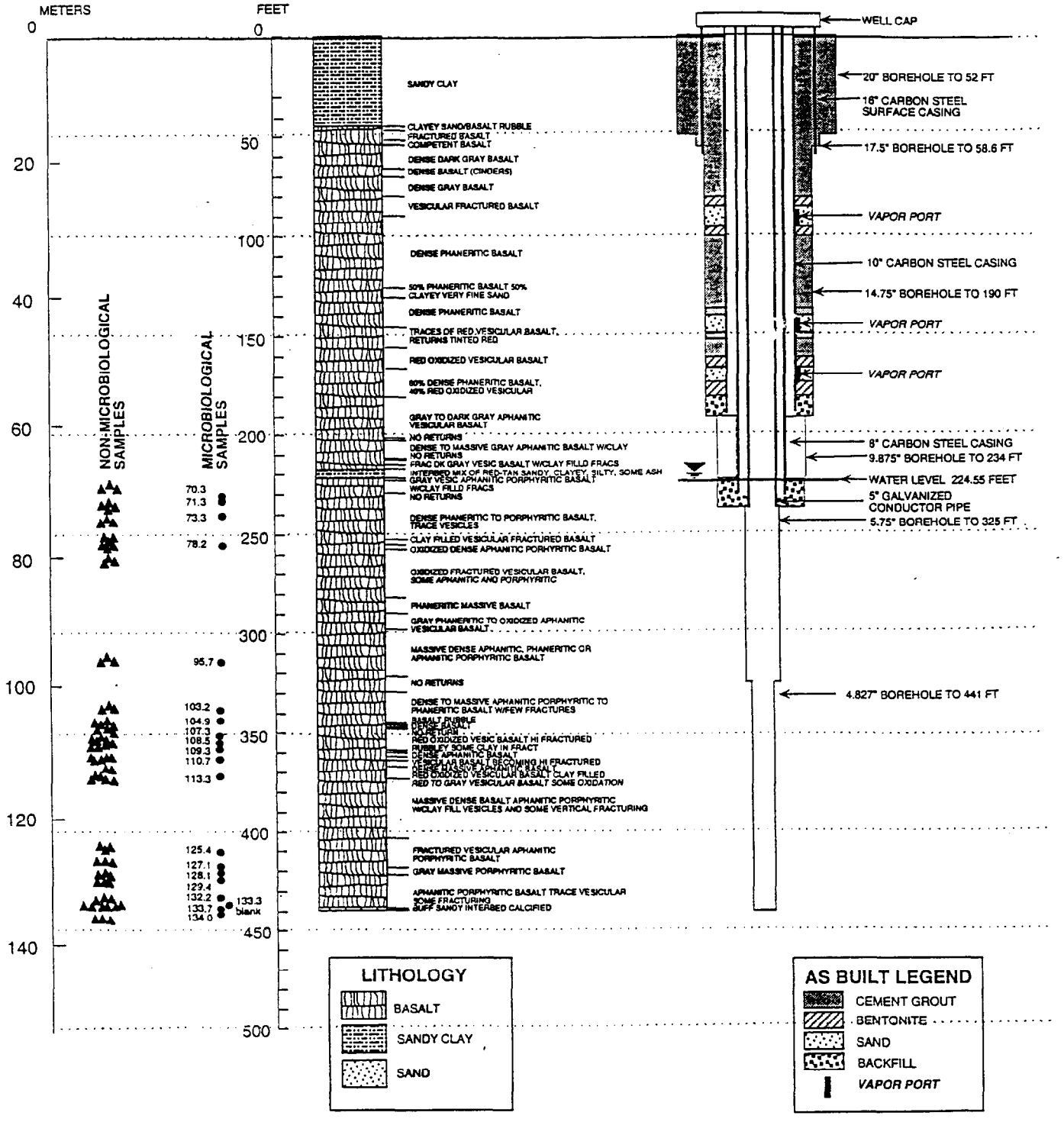

Figure 3. TAN-37 well construction, stratigraphic information and sample locations.

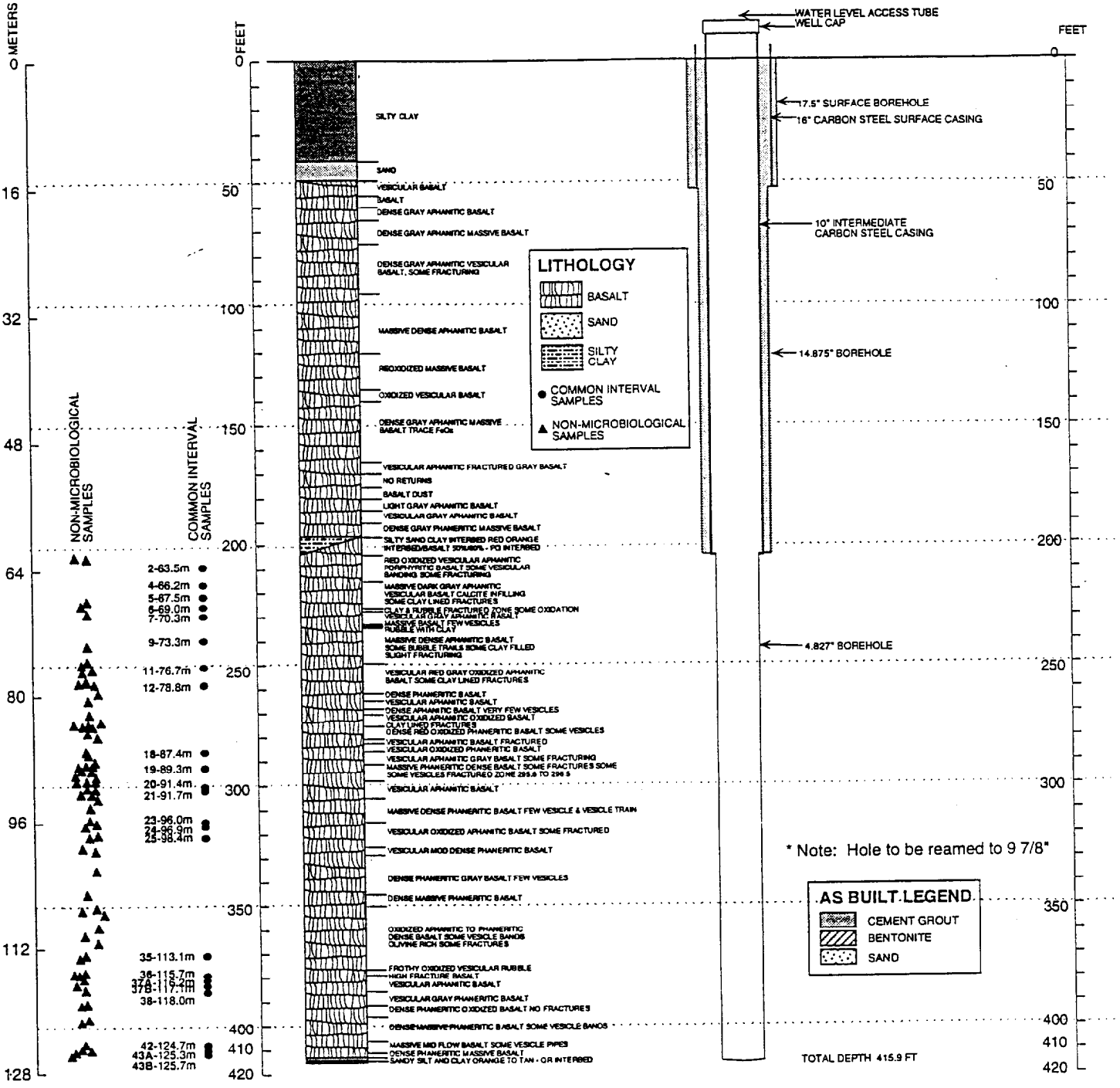

Figure 4. QA/QC tracer data for samples acquired from TAN-33. Microspheres refers to fluorescent microspheres added to the core barrel as surrogate contaminating microorganisms and PFT refers to perfluorocarbon tracers added to the drilling fluid during coring. Parings are the outer portion of the core which is removed during sample processing and subcore is the inner (higher quality) material that is distributed to investigators.

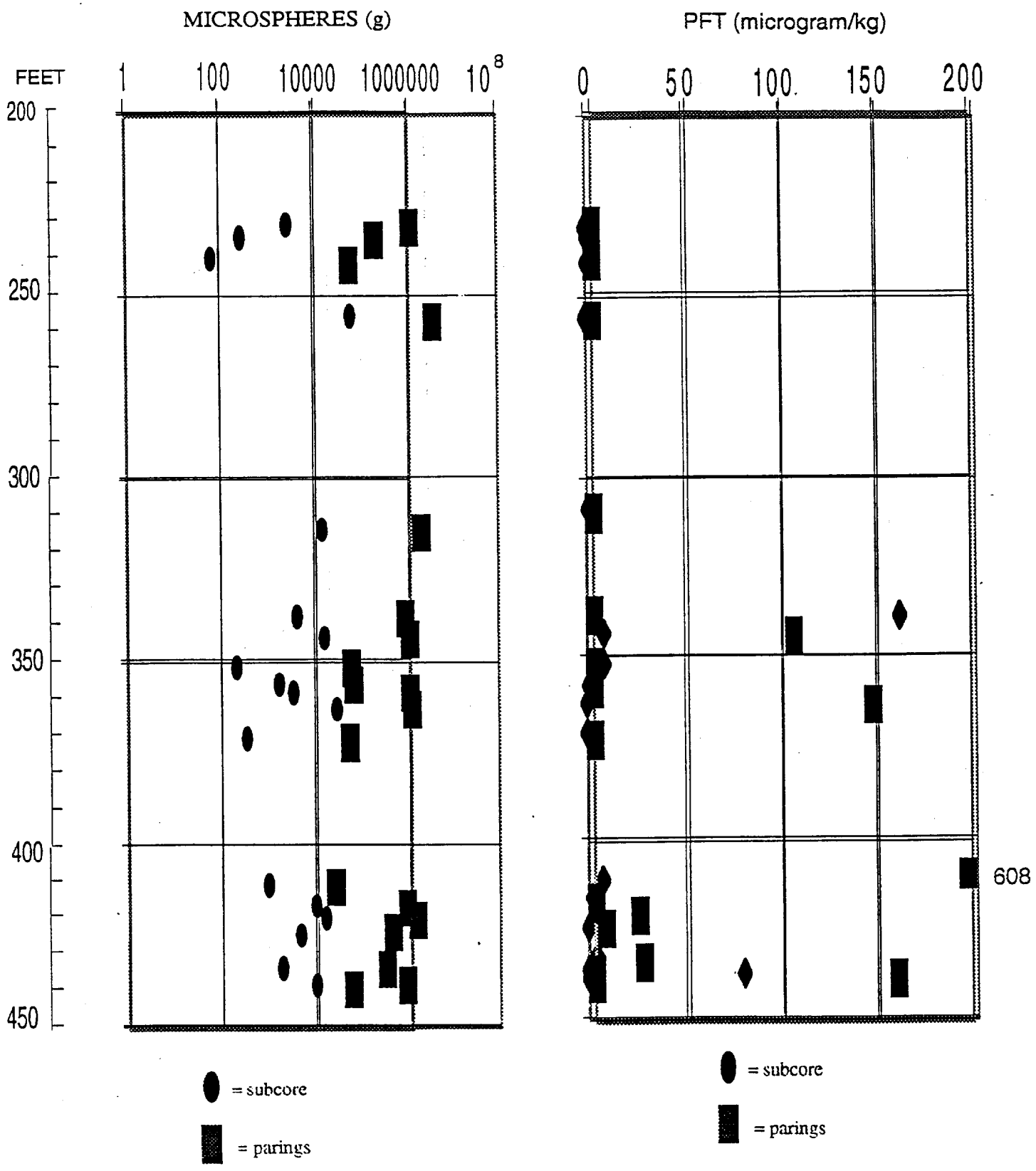

Figure 5. TCE and aerobic heterotrophs (Phelps) and methanotroph (Phelps) data obtained for core samples from TAN-33. Microbial enumerations were performed using the most probable number technique for these classes of organisms.

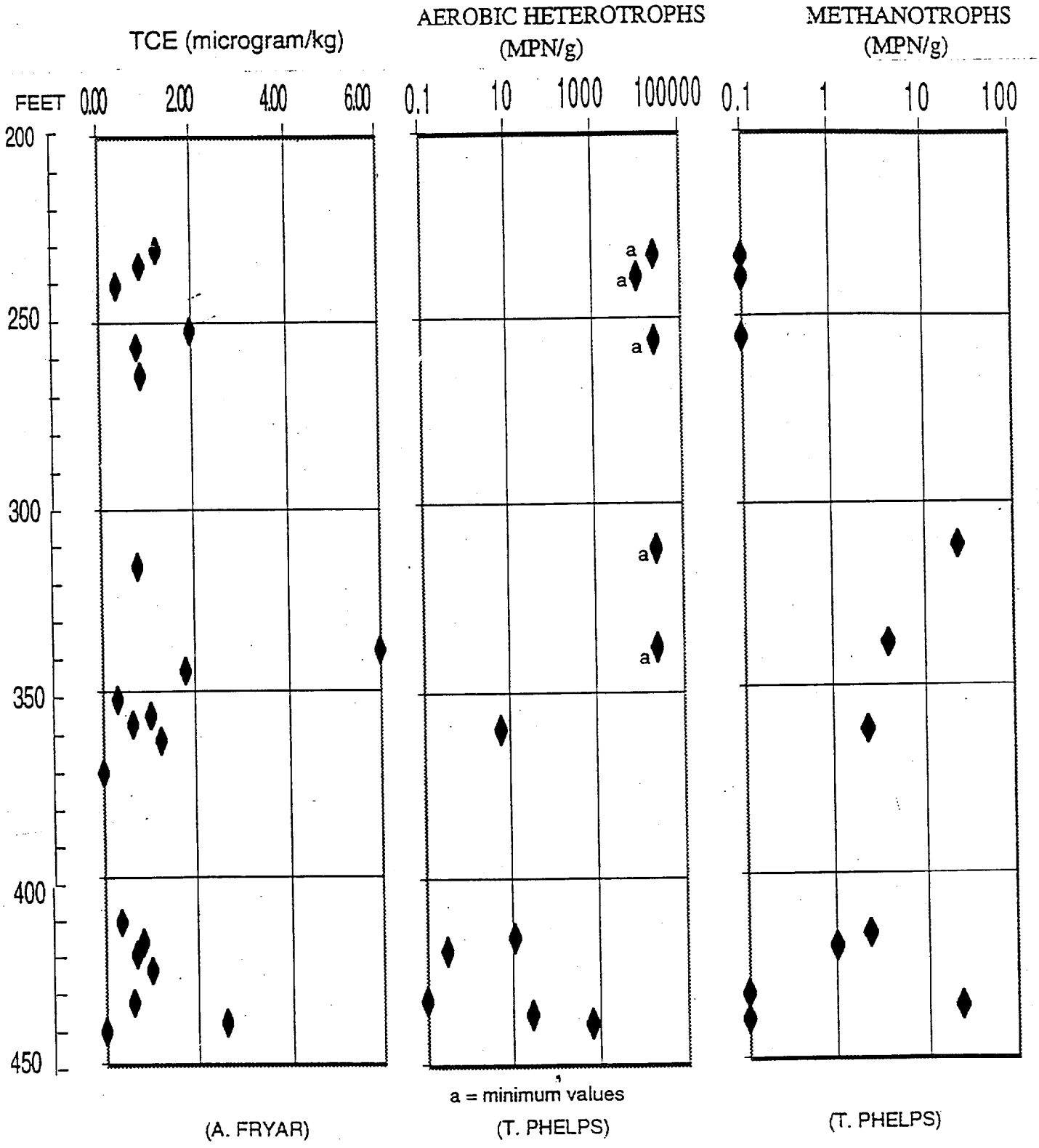
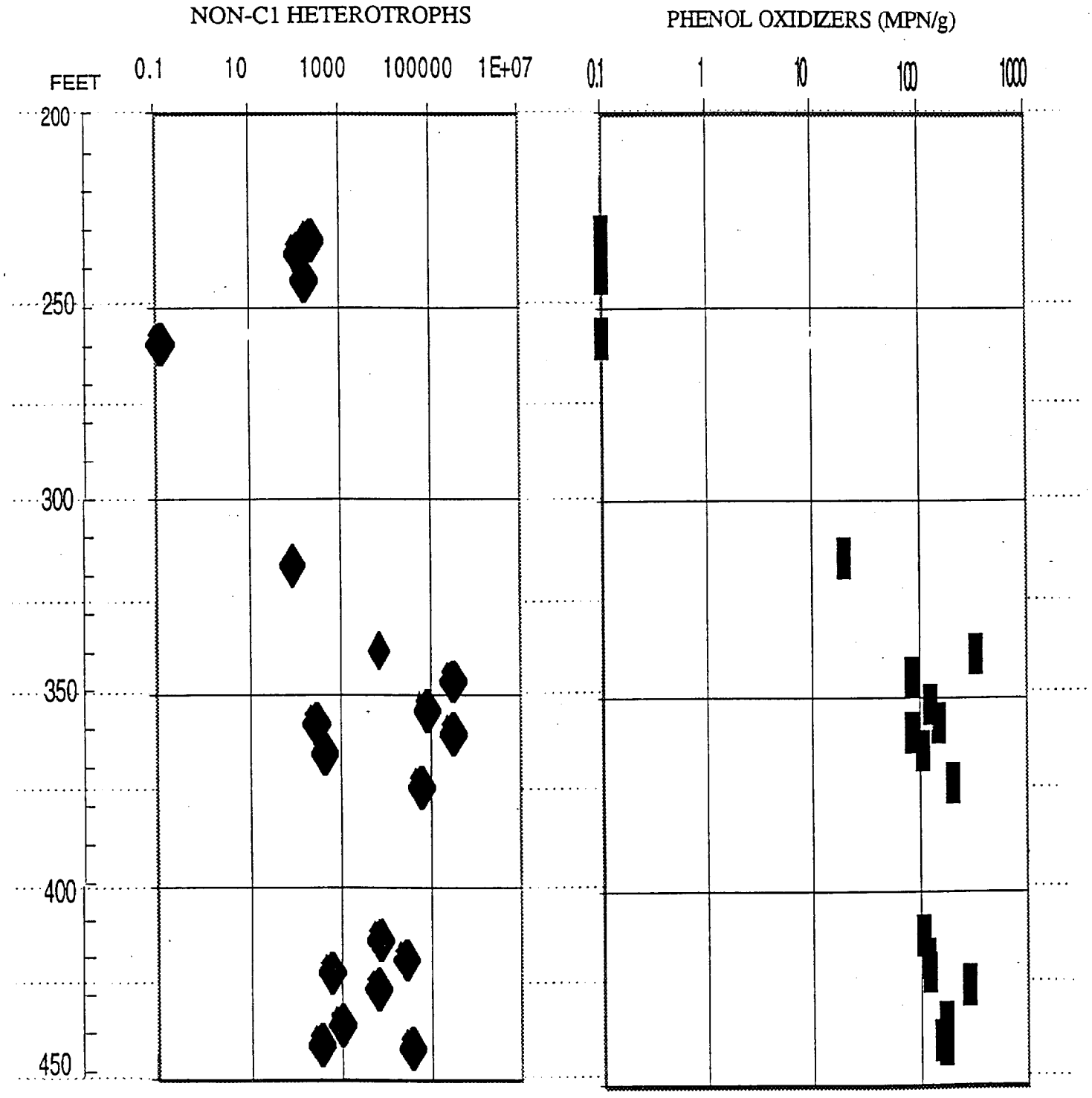
Figure 6. Non-C1 utilizing heterotrophs and phenol-oxidizers data obtained for core samples from TAN-33. Microbial enumerations were performed using the most probable number technique for these classes of organisms.