MICROBIAL DIVERSITY

FOR SITE MONITORING

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BACKGROUND

Microbes have been found in all environments on earth explored to date. They make up more than 60% of all biomass and contain more than 10 times the amount of nitrogen (N) and phosphorous (P) captured in all plant biomass on earth. It has been shown that 1 g of soil typically contains 1 million to 10 billion microbial cells representing about 4,000-10,000 species. As such, it is clear that the contribution bacteria make to the ecosystem at large is vastly underrated.

A specific group of microbes called extremophiles (lovers of extremes) are able to proliferate in environments that were once considered too extreme to sustain life. They prefer pH values equivalent to concentrated acid or bases, temperatures exceeding 70°C, pressures of 1400 times of that on the surface of the earth and near saturated salt and metal concentrations. These extremophiles are highly specialized and have established adapted microbial communities depending on the particular parameters of the environment.

One can identify the microbes involved in environmental processes (e.g. polluted environments or soil quality) and provide valuable information for the development, monitoring or remediation of any site. As these microbes have adapted to a particular site (or pollutant), they can be used as biomarker for specific pollutants or as an indicator of soil or water quality.

Services

Microbial baseline establishment prior to operations

Identification of biomarkers

Monitoring of microbial populations as indicator of impacts due to operations

Continuous monitoring for soil and water quality (chemical & microbial)

Development of site specific value-added bioproducts

ASSESSING AND MONITORING MICROBIAL DIVERSITY

Current legislation requires a Biodiversity Assessment as an integral part of any Environmental Impact Assessment. It is commonplace to include water quality and a variety of higher organisms, ranging from plants to insects and animals such as fish, in routine Environmental Impact Assessments. Although these organisms are all much more complex than the microorganisms (can only be visualised with magnification), the microorganisms are a much more sensitive indicator of any environmental shift, e.g contamination, temperature, etc.

Microbial populations are a quick and effective indication of changing environmental parameters in the form of shifts in the microbial diversity. Changes in the relative abundance of particular groups of microorganisms indicate that the environment has been altered. Microorganisms are able to do this because they are much more sensitive to selective pressures (in the form of a changing environment), and are able to adapt quickly. In as little as 20 minutes some microbes are capable of 4 simultaneous mutations in every gene and doubling in cell count. Consequently, a microbial population can produce a next generation in only a few hours as compared to weeks, months or years for higher organisms. Therefore, if a change in Microbial Diversity is detected due to an environmental impact, steps can be taken to remedy the situation before plants, animals and humans are affected or crops are planted.

Microbial Diversity does not currently form part of South African legislation. However, if international trends are taken as an indicator, this aspect will soon become compulsory in all EIA's.

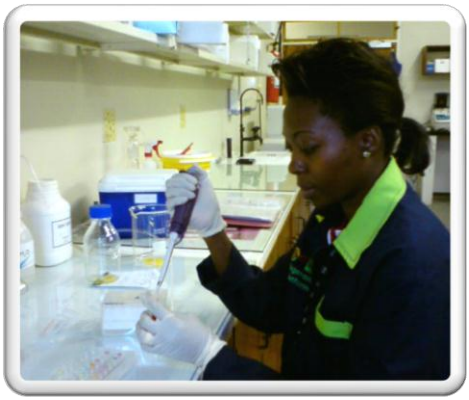
Current Legislation and Trends

The Minerals and Petroleum Resources Development Act (MPRDA Act 28 of 2002) prohibits Reconnaissance, Prospecting and Mining without an approved Environmental Management Program.

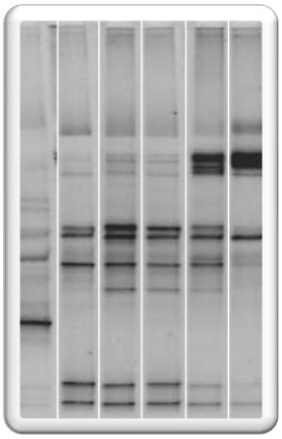
The US EPA includes microbial diversity in their guidelines since it can be the quickest, most efficient biomarker for soil and water quality assessments.

TECHNIQUES UTILIZED

The Metagenomics Platform utilises DNA-based tools to monitor Microbial Diversity in complex communities. Because the environments created by mining / industrial /agricultural activity and associated waste disposal are so unique, culturing the bacteria can be extremely challenging. An inability to culture all of the microbes within that complex environment necessitates the use of culture independent methods. The Metagenomics Platform has developed and standardized methods and procedures specifically for soil and groundwater samples from impacted environments. They have established themselves as the leader in the field of Microbial Diversity in South Africa and are currently the only institute in South Africa to provide this comprehensive array of analysis.



Sampling techniques have been developed for an array of different samples ranging from soil to fissure water. The samples are transported to the laboratory under controlled conditions where Microbial Diversity Assessments are performed by exponentially increasing targeted areas (PCR amplification) of the genetic starting material (DNA) using probes that target all 3 domains of life (Eukaryotes – nematodes, yeast and fungi, etc. Prokaryotes – bacteria and Archaea). The generated fragments are then subjected to a specialised electrophoretic technique (DGGE) that is used to separate these fragments based on compositional differences. Statistical analysis provides a means of comparing and measuring shifts in Microbial Diversity.



This method is fast and useful to monitor changes in the microbial population. It gives a general assessment of Diversity by representing each sample (soil, water or other) as a vertical column and each microbe as a horizontal band / line. The intensity of a band is related to the concentration of that specific microbe.

In the figure on the left, the first column corresponds to a sample collected from a specific site at a certain time with discreet environmental conditions (temperature, contaminant concentration, etc.). A high number of bands present in a sample may be an indication of a high level of Microbial Diversity, and a change in banding pattern indicates a shift in the composition of the microbial population. Also, combining different methods for analysis of microbial community structure in a given environment could increase the amount of information and taxonomic resolution. DGGE combined with Terminal Restriction Length Polymorphism (T-RFLP) or Length heterogeneity PCR (LH PCR), provide comprehensive microbial information needed for the microbial assessment of a given environment since each technique can contribute to the total picture, we select these techniques o ensure you have a clear understanding of the questions addressed for your needs.

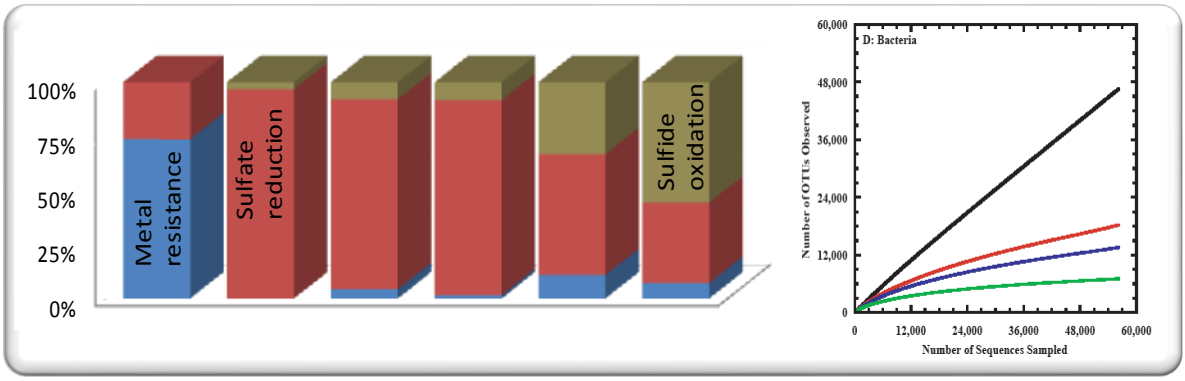
TRFLP is a method used to generate a fingerprint for profiling microbial communities based on the position of a restriction site closest to the labelled end of an amplified gene. The mixture of PCR amplified variants of a gene encoding ribosomal RNA is digested with one or more restriction enzymes and the size of each individual terminal fragment is detected by DNA sequencer. TRFLP gives highly reproducible results for repeated samples.

Length heterogeneity PCR (LH PCR) is similar to TRFLP. The difference between these two fingerprinting methods is that TRFLP identifies PCR fragment length variations based on the restriction site variability while LH-PCR analysis distinguishes organisms based on the variations in the length of 16S rRNA gene.

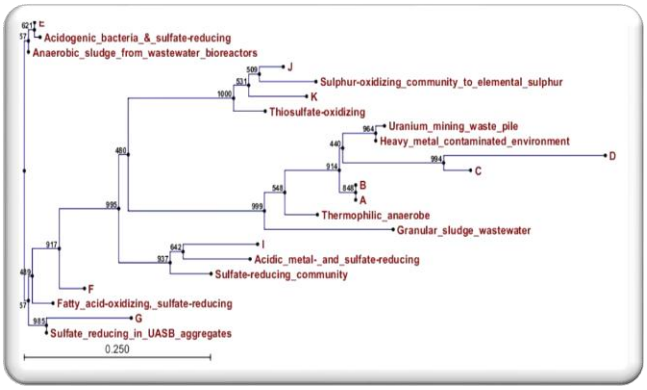
DNA sequencing is considered the Gold standard for microbial identification and Sanger's method also referred to as dideoxy sequencing or chain terminal has been used for this purpose. This is even extended to pyrosequencing, a new sequencing method which involves sequencing by synthesis offers accurate and quantitative analysis of DNA sequencing. It is very sensitive, accurate, flexible, parallel processing, and can be easily automated. Pyrosequencing helps in discriminating microbial species, types and strains which allows possible suggestions of specific metabolic activities for a particular organism. However this is still an expensive technique.

Differences between banding patterns in samples (e.g. collected at the same site over a time period) are evaluated by comparative analysis to generate a 'fingerprint' of the relative Microbial Diversity which can be used to gauge the stability and 'health' of any given site. This has been applied to the preceding figure and the results show a clear shift in microbial activity from metal resistance towards sulfate reduction and terminating in sulfide oxidation.

Software utilizing distance matrixes and specifically generated algorithms are used to determine if a complete assessment of the microbial diversity has been made. The relative predicted abundance is compared to the observed variation and the process repeated until redundancy is achieved.



Each band can be recovered and the identity of the microbe determined by comprehensive analysis and comparison to a genetic database. This is a necessary step in the initial stages of a Microbial Diversity Assessment to definitively establish baseline conditions against which subsequent samples can be compared. Identification allows for the development of strategies for isolating and growing targeted microbes or to identify a specific metabolism becoming dominant indicative of chemical imbalances.



Other available techniques include direct cell counts using different staining methods to distinguish between total numbers and live counts. This is a more accurate representation of the potential microbial activity and elemental cycling than that obtained by total microbial counts. Specific DNA probes targeting signature genes (FISH – fluorescent in situ hybridization) can also be used to detect microorganisms that perform specialized functions, e.g. sulfate reduction. FISH can be used as a monitoring tool to indicate not only the presence, but also the amount of target organism present in a sample. All this can be correlated with a full chemical analysis of water samples from accredited laboratories.

SYNOPSIS

Diversity analysis identifies the constituents of the microbial population and pinpoints the dominant groups in a consortium. Changes in the composition of the bacterial community are a clear biomarker of a change in the environment. A baseline can be established prior to operation commencement during the Environmental Impact Assessment. During operations, microbial diversity assessments can be applied as a monitoring tool to assess the status of the environment. In addition, this type of analysis can provide an early warning system for problems once a stable baseline of operations has been established.

Extended methods can also provide an assessment of the potential capabilities of the bacterial consortium in terms of value added bioproducts, as well as the type of amendments that might be needed in order to stimulate the desired activity.

Accredited Laboratories and UFS partners

Water Analysis → Institute for Ground Water Studies, Univ. Free State

Gas Composition → Stable Isotope Laboratory, Univ. Toronto

Isotopic Ratios (^{18}O, ^{13}C, ^{2}H & ^{15}N) → Stable Isotope Laboratory, Univ. Toronto

Noble Gas Isotopes → GFZ German Research Centre for Geosciences, Potsdam

Technologies Available

Pristine sampling of soil, water or gas

Diversity assessment through DNA extraction, PCR amplification, DGGE or equivalent, software analysis, DNA sequencing and microbial identification

Complimentary methods for direct targeting of microbes and correlation to microbial activity and cycling