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The WRC operates in terms of the Water Research Act (Act 34 of 1971) and its mandate is to support water research and development as well as the building of a sustainable water research capacity in South Africa.

TECHNICAL BRIEF

Acid mine water

Towards the success of mine-water pollution reducing systems

A completed Water Research Commission (WRC) study developed a toolkit to enable quantitative microbial ecology studies of sulphate reducing and sulphide oxidising systems.

Background

Acid rock drainage (ARD), particularly that arising from diffuse sources such as waste rock dumps, tailings impoundments and coal spoils, represents one of the most significant threats to the sustainability of water resources in parts of South Africa. Large areas are likely to be impacted by the ARD, which can persist for decades or even centuries.

There is a need for sustainable remediation systems to address the challenge.

Biological treatment systems, based on biological sulphate reduction, potentially offer a sustainable alternative to conventional physical and chemical processes. Stable performance of sulphate reducing systems depends on the maintenance of a stable, robust microbial community.

Currently, there is a lack of techniques available for the quantitative evaluation of microbial communities in sulphate reducing systems. The development of tools, with a relatively rapid turnaround, to assess the structure of sulphate reducing communities would be valuable in the management of remediation systems based on sulphate reduction.

Methodology

The first part of the WRC project involved generating comprehensive clone libraries of the sulphate reducing and sulphide oxidising communities. The total genomic DNA was extracted from community samples and the 165 rRNA gene from all component species was amplified using universal PCR primers.

The amplified gene fragments were cloned into vectors and used to transform competent ***E. coli*** cells. The transformed cells were plated out and over 150 cloned colonies were selected for amplified ribosomal DNA restriction analysis to identify unique sequences, which were then sent for DNA sequences.

The resulting sequence data were compared to known sequences in the National Centre for Biotechnology Information database to identify closest known relatives.

The 16S sequence information was used to design qPCR primer sets and fluorescent in situ hybridisation probes for the sulphate reducing bacteria group and individual species. These were evaluated for specificity and cross-reactivity.

In parallel, a series of sulphate reducing and sulphide oxidising reactors were operated, under conditions likely to induce changes in the community structure. Samples were taken for DNA extraction at regular intervals and these were used as templates to test the molecular tools.

Results

The clone library constructed from mixed sulphate reducing community consisted of 48 unique species, of which 17 grouped closely with known sulphate reducing species. Of these, members of the genus ***Desulfomicrobium*** were most common.

Three separate clone libraries were constructed for the sulphide oxidising community. The first, using samples taken from carbon deficient reactors, showed low diversity and was dominated by autotrophic sulphur oxides, such as ***Chromatium*** and ***Chlorobium***.

By contrast, under carbon replete conditions the diversity was much greater and the dominant sulphur oxidisers were ***Thiobacilli*** and ***Halothiobacilli.*** In addition, a number of heterotrophic bacteria were detected.

The third library was generated using samples obtained from an experimental sewer site, investigating accelerated corrosion of concrete pipes due to sulphur cycling. The data generated in this study showed clear evidence of microbial succession and evolution of the microbial community in response to increasingly acidic conditions.

This represents one of the first studies to characterise a complete community in that environment and, coupled with the performance data being generated in that study, could provide a significant breakthrough.

The development of species specific qPCR primer sets was less successful. A number of primer sets were designed, based on the 16S sequences of known species.

These were evaluated using software packages to assess specificity and the likelihood of dimerization. While the ***in silico*** evaluation suggested the primers would work well, in practice this was not the case.

Primer sets and fluorescence ***in situ*** hybridisation (FISH) probes were developed for the sulphate reducing bacterial group and ***Desulfomicrobium***.

These were successfully used to assess the impact of reduced hydraulic retention time on the relative proportion of sulphate reducers to non-sulphate reducing bacteria.

Conclusions

The most significant conclusions are that comprehensive clone libraries have been constructed for the sulphate reducing and sulphide oxidising communities. Furthermore, the microbial community associated with accelerated concrete corrosion has been characterised and clear evidence of microbial succession provided.

Fluorescence in situ hybridisation probes and qPCR primer sets to quantify total sulphur reducing bacteria and the ***Desulfomicrobium*** group have been designed and tested.

The qPCR primer sets have been used to illustrate changes in the relative proportion of sulphate reducing to non-sulphate reducing bacteria in stirred tank reactors, as a function of reducing hydraulic retention time.

The final report recommends that the information generated to date be used to continue designing and testing primers and probes to allow for quantification of a more complete set of species within the mixed community. The potential of utilising next generation sequencing facilities in South Africa should be investigated.

Further reading:
To order the report***, Development of a toolkit to enable quantitative microbial ecology studies of sulphate reducing and sulphide oxidising systems (*****Report No. 2109/1/14**) contact Publications at Tel: (012) 330-0340, Email: orders@wrc.org.za or Visit: www.wrc.org.za to download a free copy.