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BIOREMEDIATION

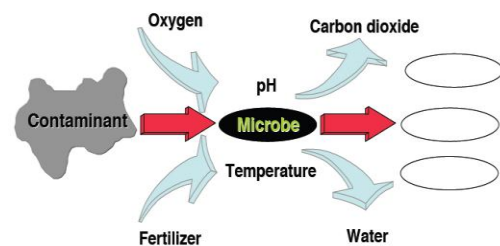
SMALL SOLUTIONS TO BIG PROBLEMS

Prof Esta van Heerden | 051 401 2472 | vheerde@ufs.ac.za
Dr Peter Williams | 051 401 9039 | williamspj@ufs.ac.za
Mrs Elizabeth Ojo | 051 401 9897 | ojoao@ufs.ac.za
Mr Kay Kuloyo | 051 401 3721 | kuloyooo@ufs.ac.za
Mr Rohan Posthumus | 051 4013391 | posthumusjj@ufs.ac.za

BACKGROUND

Bioremediation is the use of living organisms, primarily microorganisms, to degrade environmental contaminants into less toxic forms. Research has demonstrated that there are very few environments where microbes have not been able to survive, adapt, and indeed, thrive. Microbes are able to utilize a near infinite combination of electron donors and electron acceptors to drive their metabolism. In addition to these redox (oxidation / reduction) reactions, they have also developed a myriad of other strategies enabling them to detoxify their environment. Bioremediation applies these principles to select a suitable combination of microbial community activity, electron donor / acceptor / contaminant concentrations and other physical and practical parameters to remediate / recover a targeted pollutant.

Bioremediation strategies are often more beneficial than traditional strategies because it can be implemented *in situ* (directly at the site of the contaminant with no need to transport the contaminated material). Innovative *in situ* technologies permit biological treatment of contaminated water by means of reactive molecules produced by microbes. This provides a simpler, less intrusive, and cheaper method than conventional 'pump and treat' systems that often employ hazardous chemicals that create an additional environmental risk.



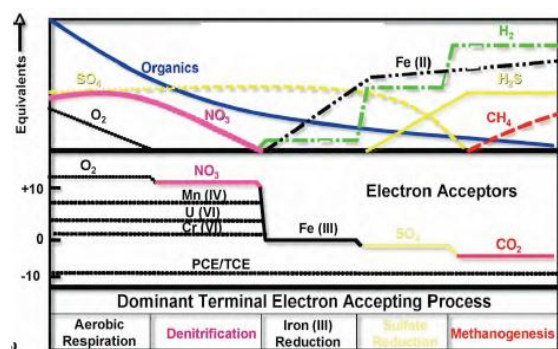
The microbes may be indigenous to a contaminated area or they may be isolated from elsewhere and brought to the contaminated site. In the latter case, this is referred to as bio-augmentation, whereas, if the naturally occurring population is encouraged to proliferate by the addition of extraneous electron donors or electron acceptors, this is called biostimulation. The advantage microorganisms already living in contaminated environments have, is that they are often well adapted to environmental conditions and are able to utilize available nutrients and electron pairs ultimately lowering costs.

Biostimulation - The addition of organic or inorganic compounds to cause indigenous organisms to effect remediation of the environment, e.g. fertilizer, surfactants.

Bioaugmentation - The addition of organisms to effect remediation of the environment, e.g. contaminant degrading bacteria injection into a contaminant zone

In South Africa, remediation strategies have focussed heavily on physical and chemical processes. Even though these technologies are generally effective, the facilities are typically expensive to construct, operation requires a high level of training, involves the use of hazardous chemicals and result in high waste volumes. Although these methods have historically been applied with success, the scope for improvement using actively growing bacteria is vast, and this has been successfully demonstrated worldwide. Our approach is to integrate and extend all types of technologies by enhancing bioremedial and other efforts.

Bioremediation techniques are more economical than traditional methods and pollutants can be treated on site, thus reducing exposure risks for personnel. When compared to standard practices, effluent volumes generated by bioremediation are substantially smaller reducing the problem of sludge disposal. Additionally, since bioremediation is based on natural processes, the public considers it more acceptable and 'green' than other technologies.



Although a number of companies active in South Africa purport to be applying bioremediation, these 'bioremediation' strategies generally involve passive chemical mechanisms such as absorption of contaminants onto non-living biological material with bacterial action taking place fortuitously. Only very few examples exist where classic efficient bioremediation principles have been applied.

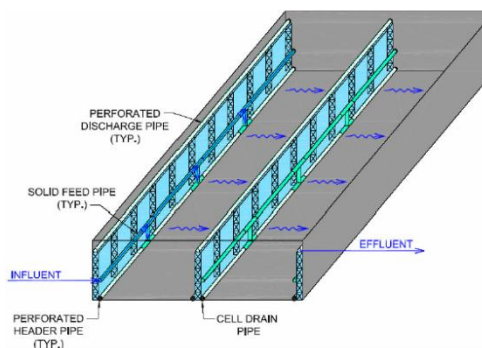
This is in stark contrast where it has been estimated that hazardous waste remediation in the US could exceed \$1.7 trillion and bioremediation is a rapidly expanding field worldwide. In a country heavily reliant on mining and manufacturing, with limited water resources and skills shortages, it is essential to learn from the rest of the world and harvest expertise from successful projects abroad. As such, we have a skills transfer agreement with Geosyntec Consultants, a leading bioremediation firm based in the USA (www.geosyntec.com) under the Technology Innovation Agency Platform.

OUTLINE

Every site is unique in its composition of intrinsic parameters (such as contaminants and microbial community) and physical parameters (ambient temperature and pH). In concert with this, practical considerations such as availability of materials and choice of electron donor or acceptor have to be balanced. Each site, therefore, is unique and any bioreactor needs to be optimised and tailor made with these specific constraints and needs in mind.



In its simplest form, our bioreactors are encapsulated systems based on a simple flow-through cell with bacteria immobilized on an inert aggregate ('reactor matrix') with electron donor / acceptor dosing adjusted to reach target ORP (oxidation reduction potential) for contaminant removal. Key design features of the treatment cell are simplicity, robustness and versatility to treat almost any type of contaminant.



A project would typically be developed in 5 stages:

- A Preliminary Investigation aims to compile a comprehensive picture of the problem – where is the source of contamination, how is it exacerbated by water movement, are there potential remediative bacteria present and what materials and expertise are available.
- A Site Evaluation is performed by a scientific and engineering team to assess: potential locations for the pilot scale treatment cell, sources for supplies, evaluation of local materials for use, identifying potential engineering, design and regulatory constraints, assessing the working environment and establishing communications with local personnel who may be responsible for day-to-day system operations. This also involves adhering and understanding the client's legal and environmental restrictions.



- Laboratory Testing of small scale bioreactors is performed to evaluate potential matrix materials and electron donors and to empirically determine the balanced state of electron donors and acceptors. These simulations provide key design parameters such as hydraulic retention time and the redox state necessary to achieve the desired level of contaminant removal. Many up-scaled processes have failed because the groundwork for reactors are not set in controlled and upset conditions. Detailed construction drawings are prepared and actual construction is outsourced.
- Start-up of the reactor involves several steps to confirm vital parameters such as integrity and flow characteristics of the media bed and establishing steady state operational conditions. Responses to system upsets such as unplanned shutdown and electron donor or acceptor interruptions are also determined.
- Operational support comprises a 12 to 24 month period for personnel training, ongoing data analysis, progress reports and on-demand troubleshooting after which the system should be able to operate autonomously. Our focus is on the application of “green” (solar) technology thereby accelerating bio-processes to enhance time-to-outcome and the use of “smart” remote management systems to control bio-solution processes.

LABORATORY-SCALE BIOREMEDIATION OF CONTAMINATED ENVIRONMENTS

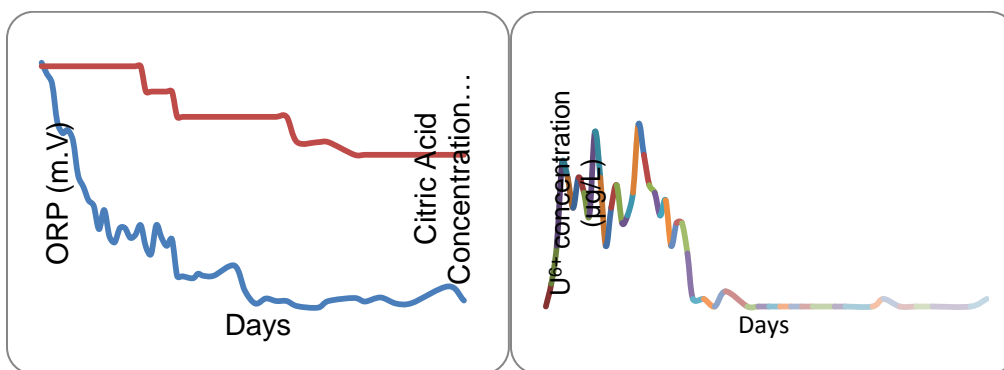
Biological metal decontamination or recovery is possible through a process of *in situ* immobilization of certain soluble metal species [such as uranium(VI) and chrome(VI)] via microbial reduction to insoluble metal species. Several microorganisms are known to mediate this process, including the Fe(III)-reducing *Geobacter* sp., the Fe(III)- and sulfate-reducing *Desulfotomaculum* sp., the sulfate-reducing *Desulfovibrio* sp. and the fermentative anaerobic *Clostridium* sp. The UFS has extended knowledge that microbial metal reduction by bacteria in groundwater and aquifer sediments and these studies have concluded that indigenous microorganisms capable of metal reduction are present in natural aquifers, . It is now known that these novel site specific micro-organisms can successfully manipulated to control eefctive bioremediation. Therefore, to stimulate the bioreduction of certain metal species, electron donors are typically provided in the form of simple carbon substrates, for e.g acetate, lactate or glycerol.

The UFS has published on their fundamental research about metal interactions with micro-orgaimss and to extend upscale treatment approaches to remove certain metal species from surface- and groundwater is based on the La Cienega Filtration Project to treat selenium in San Diego Creek, Orange County, California developed by Geosyntec Consultants, USA. The system employs natural indigenous biological processes to create anoxic conditions by controlling the oxidation-reduction (redox) state. This will be accomplished by encouraging bacterial growth on the substrate matrix by supplying a suitable food source (electron donor) for the microorganisms and controlling the retention time of water processed through the system.

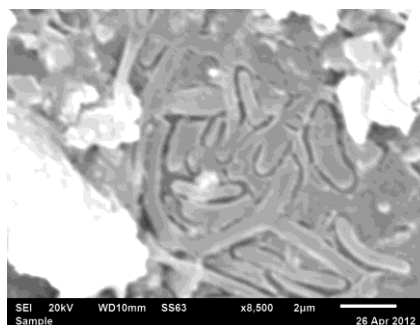
Continuous up-flow bioreactors have been constructed from PVC pipe with threaded end caps, containing taps and appropriate rubber and silicon tubing. The reactor is filled with matrix material to act as a suitable surface for biofilm formation within the reactor. Influent water, containing the appropriate electron donor, is pumped in at

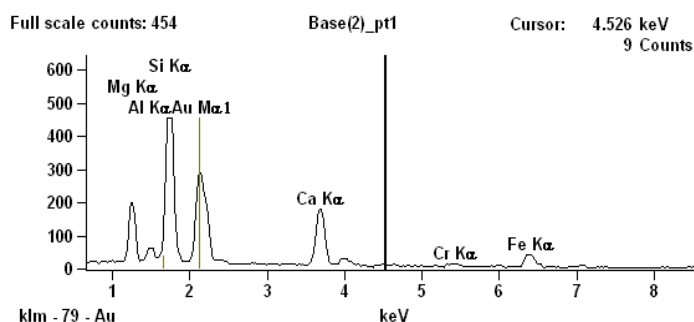
the base of the reactor and collected in a sampling container at the top after passing through the reactor. The effluent is analyzed for ORP, pH, temperature, as well as metal contaminant concentrations, allowing for the comparison with the influent data collected.

As an example, steady-state conditions within the continuous up-flow bioreactor for the reduction of uranium(VI) were reached after approximately 35 days of operation, and levels of uranium decreased below detectable levels (>99.95%), which could be maintained until bioreactor termination after 82 days, thereby showcasing the sustainability of the system. Thus, the results indicate that developing a fixed-film bioreactor may be an effective and sustainable treatment strategy for the remediation of uranium-contaminated water containing low levels of uranium.



Bioreactor termination entails cutting open the PVC column and sampling the matrix material for SEM/EDX analyses to determine whether biofilm formation occurred within the reactor, as well as whether the metal contaminant precipitated on the surface of the matrix.





PILOT-SCALE BIOREMEDIATION OF CONTAMINATED ENVIRONMENTS

Laboratory studies have demonstrated that indigenous bacteria from contaminated sites could colonize a reactor matrix and remediate contaminated water using a suitable electron donor. A fixed-film bioreactor has been constructed and operated to treat 30 – 120 KL of water daily containing on average 6 - 15 mg/L Cr⁶⁺.

The reactor consists of a lined concrete containment structure filled with a dolomite stone matrix. The chromate contaminated influent water is gravity fed to the reactor from an elevated distribution dam to an engineered influent system allowing the controlled influent into the reactor through an inlet manifold. The manifold allows for the even distribution of influent across the width of the reactor. The reactor is controlled remotely through a Programmable Logic Controller (PLC). The PLC allows for the remote setting of water influent flow rates, electron donor flow rates and flushing of the influent manifolds. In addition, the PLC is connected to a web interface that allows remote monitoring of the reactor. Parameters that are monitored include ORP, pH and temperature through probes installed in the reactor that are connected to the PLC.

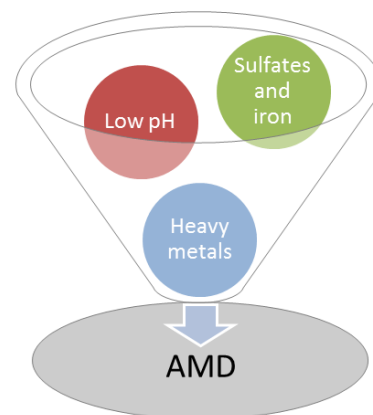


This process is the subject of National Patent # 2009/02378.32: A PROCESS FOR THE REMOVAL OF CHROMIUM (VI).

ACID MINE DRAINAGE

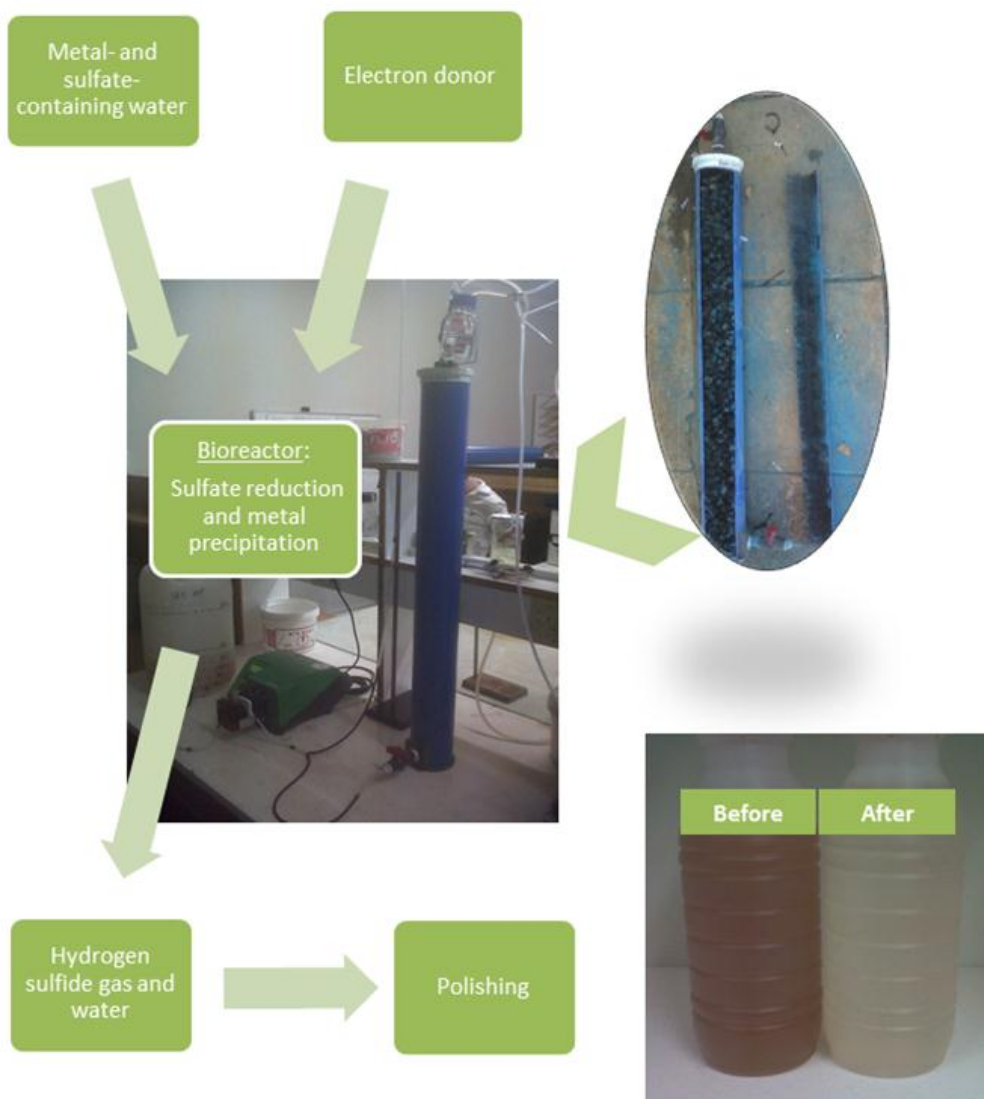
Acid mine drainage (AMD) is a worldwide problem commonly associated with coal mining and is characterized by yellow tailings typically of low pH (<3) and containing high metal and sulfate concentrations ($\pm 3000\text{ppm}$). Approximately 80% of South African electricity demand is met by burning coal and understandably, coal mining is a massive industry with the resultant problem of AMD.

The treatment approach relies on either an active treatment (e.g. anaerobic filter bioreactors), passive treatment (e.g. anoxic ponds) or a combination of the two. These sulfate-reducing bioreactors increase the alkalinity, pH and reduce the heavy metals concentrations. Influent conditioning and effluent polishing treatment processes may be required to optimize the bioreactor performance and/or achieve the desired water and environmental quality objectives. The bioreactor employs natural



biological processes to create anoxic conditions by controlling the oxidation reduction (redox) state.

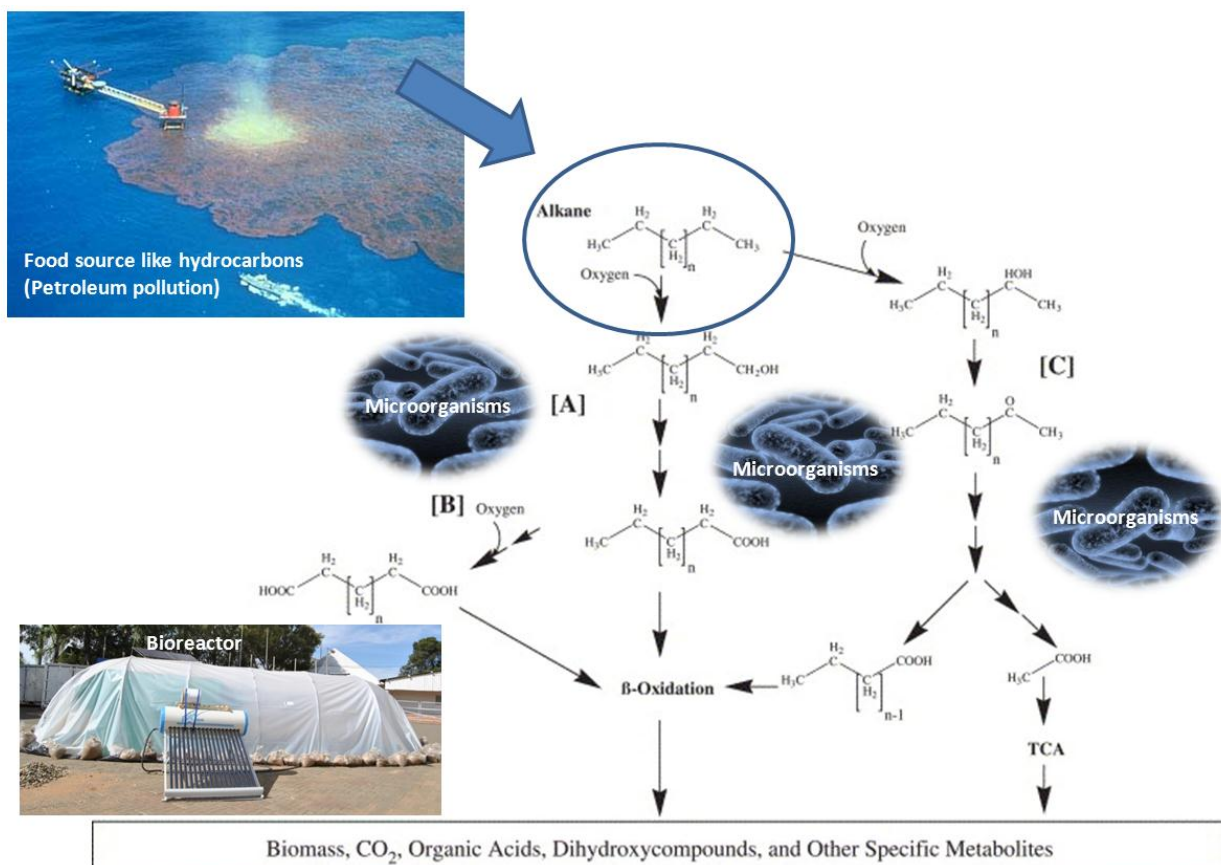
In order to adapt this process to the removal of high levels of sulfate by a sulfate-reducing microbial consortium, the microbial portion of the process, during which hydrogen sulfide is produced, must be separated from processes in which significant mineral phase precipitation occurs. This will prevent clogging of the bioreactor and minimize operation and maintenance issues. Both biological and abiotic conditioning and polishing processes will be investigated, with the most appropriate being incorporated into the final design.



PETROLEUM AND DIESEL (BIO)REMEDIATION

The basic requirements for bioremediation of petroleum to occur include a food source (hydrocarbons), oxygen (and even this is now being re-evaluated), and nutrients (phosphorous and nitrogen), in a compatible environment (proper pH, temperature and moisture.) Other nutrients such as potassium, calcium, iron, manganese, cobalt, copper, and zinc, are generally present in adequate concentrations in most soil systems, and usually do not need amendment in the design of a bioremediation process. The challenge in designing a bioremediative strategy is how to provide these essential requirements to the microorganisms in a practical way.

Populations of petroleum degrading microbes in most environments are limited by the availability of hydrocarbons. Following an oil spill, this limitation is overcome and the microbial population expands. In order for this to occur, the microbes need oxygen for respiration and other nutrients such as nitrogen or phosphorus that are not available in petroleum. Thus, the growth of oil-degrading microbes is generally limited by the availability of nitrogen and phosphorus.





Since petroleum is a complex mixture of many different classes of hydrocarbons, of which any particular microorganism has the potential to degrade only part, it follows that the microbial population will consist of a changing consortium that will adapt according to the substrates available to them. Remediation focuses on stimulating aerobic microbial activity through aeration, moisture and nutrient additions.

Bioremediation can be used to stabilize, extract, or reduce the toxicity of contaminated soil and groundwater.

It is a technology that will have huge implications for a country with very little water, a rapidly expanding population, extensive mineral resources compounded by water treatment and management challenges.

It is a simple, robust technology that can be applied to a broad range of pollutants.