

# VERIFICATION OF A SEMI-PASSIVE MICROBIALLY-ASSISTED BIOTECHNOLOGY FOR LARGE-SCALE TREATMENT OF ACID MINE DRAINAGE

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## ABSTRACT

The Loskop Dam is a valuable resource for recreational fishing and as a wildlife preserve. It is also at the headwaters of the Olifants River, which is an important source of water for farming activities in South Africa and Mozambique.

The ecological integrity of the Loskop Dam and thus the Olifants River is threatened by input of contaminants. A number of highly publicised fish kills have drawn attention to the deleterious impacts on the dam of upstream activities. Significant contributions to contaminant load into the dam include acid mine drainage (AMD) from coal mines – active, or abandoned and derelict. These activities pose a threat currently, and future impacts are expected to increase from decant from abandoned and derelict mines. The custodians of the mines are the mining community and the Department of Minerals and Energy.

The scale of the problem of AMD is such (current and future) that remediation of this input into the Loskop Dam needs to be low-cost, low-tech and able to treat large volumes of water.

The Permeable Reactive Barrier (PRB) technology is investigated in this study as a means for ameliorating AMD inputs to the Loskop Dam. While the technology of bioremediation of AMD with fermenting natural organic material and sulphate reducing bacteria (SRBs) is well known and developed, the focus of the current study is the use of engineered microbes (EM) to facilitate the biochemical reactions of fermentation, with a view to increasing the effectiveness of decontamination of AMD streams.

Column experiments were used to determine the volume of AMD contaminated water that can be passed through reducing organic material prior to breakthrough of the AMD. Column tests simulated concentrations and flow-rates expected in the field application.

Laboratory-simulated AMD representative of the site water was pumped up at a flow-through velocity of  $9.8 \times 10^{-4}$  cm/s through vertically-mounted columns. Porosity of the column material was 60%, which ensured a high hydraulic conductivity of the medium.

Engineered microbial communities specifically developed to enhance fermentation reactions were obtained from a South African company for the specific purpose of testing their efficacy in reduction of AMD.

The effect of EM on performance of column experiments is presented in the following table.

Column parameter	Effect of EM	Extent of effect
Effluent pH	Increased	52%*
Effluent Fe	Decreased	80%
Effluent EC	Decreased	9%
Effluent COD	Increased	+5 mg/ℓ O <sub>2</sub>
Column longevity	Decreased	5%

\* - measured as decrease of titratable acidity

EM facilitates more efficient breakdown of complex cellulosic components of the Casuarina mulch into smaller, water soluble organic molecules which are used as electron donors by the sulphate reducing bacteria (SRBs). The greater rate of production of more water soluble organic molecules results in greater efficiency of reduction of sulphate concentrations and acidity (thus increasing pH and decreasing EC) of effluent solution.

Sulphate is more effectively converted to sulphide by the SRBs, which, reacting with iron to form insoluble sulphides, drastically reduces the iron concentrations in the effluents.

The greater rate of consumption of labile cellulosic material decreases the longevity of the Casuarina mulch, which is the thermodynamic cost of increased efficiency of sulphate reduction.

This study has shown that the introduction of engineered microbes (EM) into organic mulch (Casuarina leaves in this instance) enhances bioremediation of AMD.

## 1. INTRODUCTION

### Background

The zone of interest in this study is the Loskop Dam in Mpumalanga, South Africa (25°26'40.01"S 29°19'35.36"E).

Loskop dam is currently showing signs of stress from the highly contaminated inflow. Significant fish kills and deaths of other aquatic animals such as terrapins and crocodiles have been reported. The Bed and Breakfasts (B&Bs) and guest lodges (such as Aventura Loskop) in the area are being severely impacted by the toxic effects of the inflowing water, as is the fishing industry that uses the dam for a number of competitions, notably the Three Species competition. The effect of the pollution in the dam has received much media interest and the public outcry over these issues has been detailed in a number of newspapers. The public reaction is potentially detrimental to the good standing of the mining companies in the area as custodians of the "downstream" environment.

A multi-organisational study is underway to determine the chemical and microbiological causes of the animal mortalities in the Loskop Dam. Participants include the Environmental Geoscience Unit of the Council for Geoscience, the Mpumalanga Parks Board (MPB), the Veterinary Science Department of the University of Pretoria (Onderstepoort), the Zoology department of the University of Johannesburg (UJ) and the UP Microbiology Department. A workshop specifically convened by the MPB to address the pollution of the dam included members of the EGU, Anglocoal, Anglo-American, Xstrata-Coal, Sasol Mining and Golder Associates.

Parallel to the above study is the study discussed in this paper. The object of this study is to research methods of reducing the concentrations of contaminants closer to the source. Thus this project aims to determine appropriate, low cost, large-scale water treatment options for the inputs into Loskop Dam.

The contribution of coal mines to the impacts on the Loskop Dam are in the form of Acid Mine Drainage (AMD). AMD is generated by active mining operations and decant from abandoned and ownerless mines.

The mining companies which are perceived to be active in polluting the Olifants River system are hard-pressed to find affordable large-scale water treatment technologies. Current efforts of some of the active mines are restricted to basic and erratic liming of the waters of some of the rivers, a practice which is considered to have very detrimental effects on downstream users of the river waters.

The DME is responsible for abandoned and ownerless mines in the Loskop Dam region. These mines are expected to decant a significant plume of pollution within the next five years. The scale of the anticipated pollution of the Loskop Dam by high salinity, sulphate and iron and low pH water necessitates a low-cost treatment solution that may not necessarily yield potable water, but which will significantly ameliorate impacts on the Loskop Dam.

### Permeable Reactive Barriers

The current study aims to test technologies which will significantly ameliorate contamination of the rivers flowing into the Loskop Dam. Criteria for these technologies based on above constraints are that they are to be low-cost, low-tech (not requiring high levels of specialists' input) and able to treat large volumes of water.

Permeable Reactive Barriers (PRBs) have successfully been used for passive water treatment. PRBs allow contaminated water to diffuse through a chosen medium and emerge 'clean'. Contaminants are treated and/or removed by the reactive barrier medium.

Typical media used in a PRB are granular iron, activated carbon, engineered bacteria, chemicals, or special clays and/or combinations of these ingredients. Media considered for trial in a pilot PRB in this study include woodchips, Casuarina mulch, mushroom compost, cow dung, horse dung and/or combinations of these with lime, limestone and dolomite.

The overall objective of this project is to perform a pilot study using PRBs aimed at treating mine water pollution before it enters the Loskop Dam, thereby allowing the natural ecosystem to return to normal. The project is in the initial exploratory phase of kinetic testing using columns.

### Bioremediation of AMD

The geochemistry of AMD is well known, so it suffices here to simply state that major chemical signatures of AMD are low pH, high salinity (measured as electrical conductivity (EC)) and high sulphate concentrations.

Studies to date on the remediation of AMD by organic matter have revealed that the process is significantly influenced by the presence of microbes. Many studies have been performed on the bioremediation of AMD. The contribution that this study makes to the field is the inclusion in testing of locally engineered microbial populations. These microbial populations are optimised for composting of complex carbohydrates and are expected to facilitate the primary step of remediation by decomposing the cellulosic fraction of the carbon source into smaller molecules that act as food for the sulphate reducing bacteria (SRB's).

## **Aim and Hypothesis**

This study aims to test the efficacy of engineered microbes in combination with native sulphate-reducing bacteria (SRB's) in enhancing quality of water effluent from a PRB filled with sand and organic mulch.

Thus the working hypothesis of this study can be stated "The locally engineered microbes (EM) augment native SRB populations in the amelioration of AMD". Thus, we expect to see an enhancement of performance of carbon-based bioreactors with the addition of EM.

Measures to assess the relative performance of the EM-augmented systems include pH levels, acidity levels, EC levels and longevity of column material in the process of ameliorating AMD. An additional parameter to be noted is the emission of organic material in the elutriate, measured as chemical oxygen demand (COD).

## **2. METHODS**

### **Column Experiments**

Column experiments were used to determine the volume of AMD contaminated water that can be passed through reducing organic material prior to breakthrough of the AMD. Column tests simulated concentrations and flow-rates expected in the field application.

Glass columns of 3-centimeter (cm) inside diameter fitted with 20- $\mu$ m end-cap screens were used. The columns were 92 cm long and had a volume of 650 cm<sup>3</sup>.

The column material consisted of swimming pool filter sand mixed in various proportions with fermented (1 day) Casuarina mulch. The percentage of Casuarina mulch in the mixture ranged from 5% to 20% by mass. The choice of Casuarina mulch for this study was based on the great familiarity of the project team with the properties of Casuarina. Nineteen flow-through experiments were performed.

Laboratory-simulated AMD representative of the site water was pumped up through vertically-mounted columns. This arrangement was chosen in order to maintain a constant flow-through velocity of  $9.8 \times 10^{-4}$  cm/s, representative of the planned field application. Porosity of the column material was 60%, which ensured a high hydraulic conductivity of the medium.

The column elutriate was monitored continuously (one reading per minute) for pH and EC. The midpoint of column breakthrough was determined by fitting a sigmoidal curve to the data around the breakthrough. The time to breakthrough was taken as the period from emergence of elutriate at the top of the column to the midpoint of column breakthrough.

### **Engineered Microbes**

Engineered microbial communities specifically developed to enhance fermentation reactions were obtained from a South African company for the specific purpose of testing their efficacy in reduction of AMD. The identity of the company is at the moment confidential, due to the highly preliminary nature of the current research.

### **Data Analysis**

Samples of the column elutriates before, during and after breakthrough were taken and submitted to Waterlab (Pty) Ltd for analysis of pH, EC, Fe and COD. There were insufficient sample volumes for the simultaneous determination of COD and accurate SO<sub>4</sub> measurements.

Data from the nineteen column experiments normalised to 20% mulch, and the averages were calculated before and after column breakthrough.

## **3. RESULTS**

### **Efficacy of EM on Degradation of Cellulose**

When EM was added to the Casuarina mulch during fermentation of the mulch, the breakthrough period of the columns was dramatically and consistently reduced (data not represented here).

Thus the EM were efficiently degrading the labile organic material in the Casuarina mulch and were thus shown to be exerting a significant influence on the mineralisation of labile cellulose.

## Effect of EM on PH

The effect of EM on the pH of columns before and after breakthrough are presented in Figure 1.

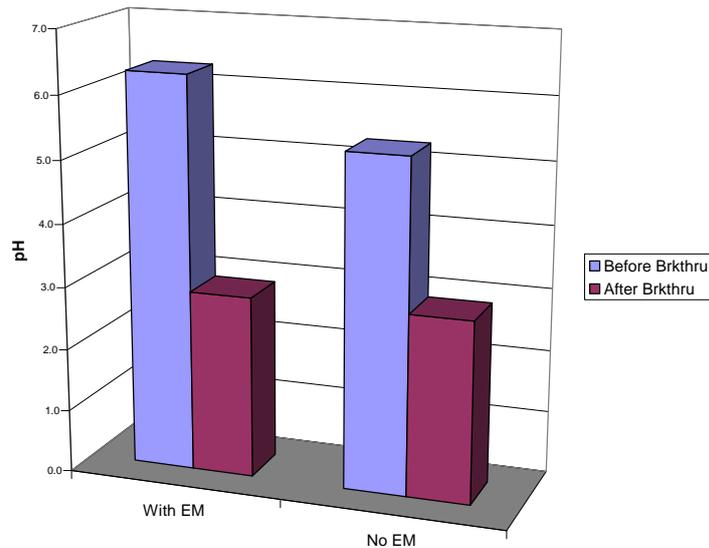


Figure 1. Influence of engineered microbes on pH before and after breakthrough

The pH values of column elutriate before breakthrough are higher in the columns that had EM added (pH = 6.3) to those without EM (pH = 5.3). These values represent a reduction in titratable acidity of 52%.

This suggests that the engineered microorganism communities were influential in maintaining pH in the columns closer to neutral.

The pH values of the column elutriates after breakthrough are identical (pH = 2.9), indicating that the microbes were no longer functioning in their pH modification after the source of labile organic material was exhausted.

## Effect of EM on Iron Concentrations

The effect of EM on the iron concentrations of column effluents are presented in Figure 1.

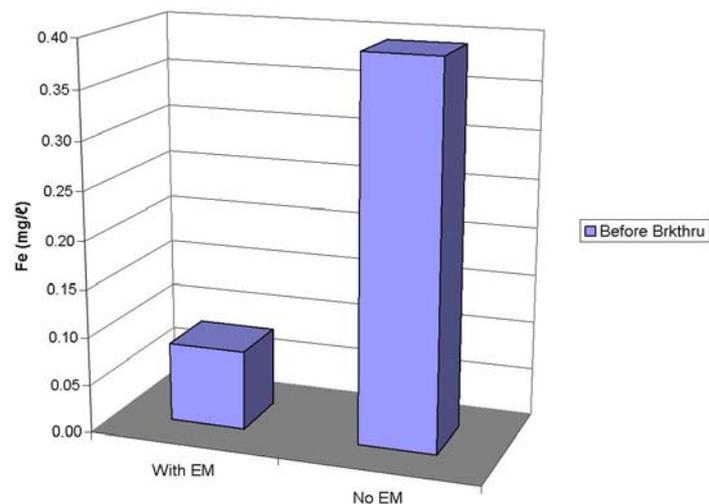


Figure 2. Influence of engineered microbes on Fe concentrations

Iron concentrations are reduced by the columns from the influent concentrations of 24.9 mg/l to about 0.2 mg/l, a reduction of 99%.

The iron concentrations in the column elutriates are much lower in the columns that had EM added ( $\text{Fe} = 0.08 \text{ mg}/\ell$ ) to those without EM ( $\text{Fe} = 0.39 \text{ mg}/\ell$ ), a reduction in concentration of 80%.

This suggests that the engineered microorganism communities were influential in reduction of sulphate to sulphide, which would immobilise Fe as FeS, reducing iron concentrations in the effluent.

### Effect of EM on EC

The effect of EM on the pH of columns before and after breakthrough are presented in Figure 3.

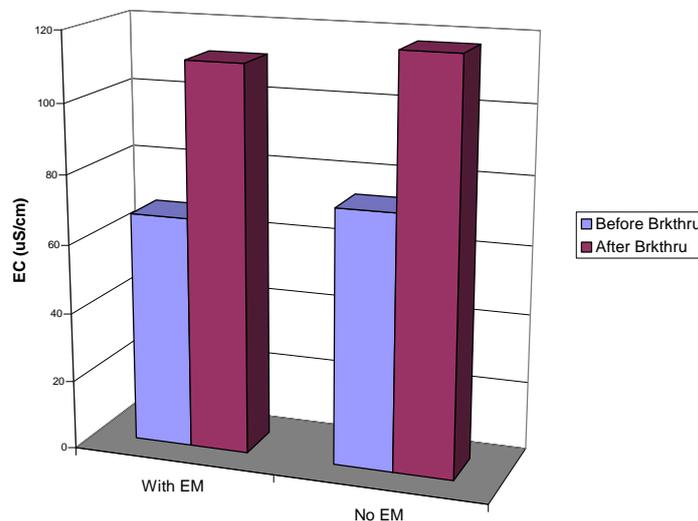


Figure 3. Influence of engineered microbes on EC before and after breakthrough

The solutions influent to the columns had EC values of  $2,720 \mu\text{S}/\text{cm}$ . The effluent EC values were of the order of  $70 \mu\text{S}/\text{cm}$  and were thus reduced by 97%.

Average EC values for columns with EM ( $67 \mu\text{S}/\text{cm}$ ) were lower than for columns without EM ( $74 \mu\text{S}/\text{cm}$ ), a 9% reduction in EC resulting from the activities of the EM. The EC values of the columns after breakthrough are less different (a 5% reduction) for those with EM to those without EM.

Thus EM are revealed to moderate EC values closer to desired low values.

### Effect of EM on COD

The effect of EM on the COD values of columns before and after breakthrough are presented in Figure 4.

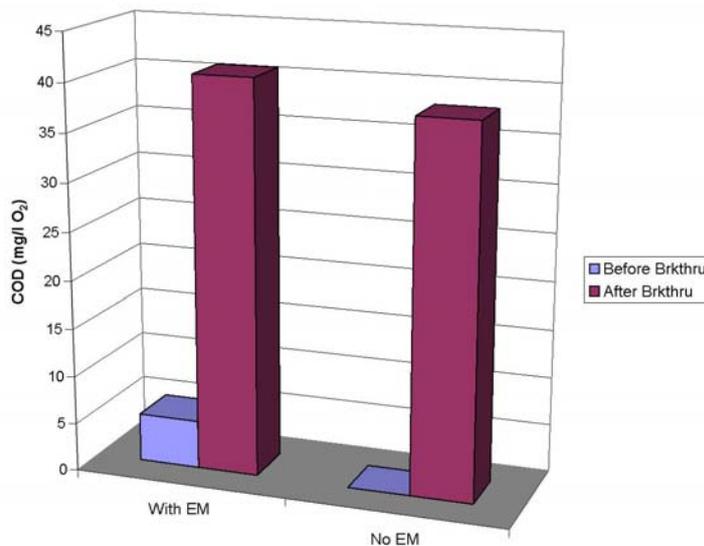


Figure 4. Influence of engineered microbes on COD before and after breakthrough

The COD values of the effluents from the columns before breakthrough are markedly different to those after breakthrough.

The COD values of the effluents of the columns with EM are higher (5 mg/l O<sub>2</sub>) than those without EM (~0 mg/l O<sub>2</sub>), before breakthrough and similarly after breakthrough (41 mg/l O<sub>2</sub> vs 38 mg/l O<sub>2</sub>). This implies that the EM are functioning as expected – they are breaking down complex cellulosic components of the Casuarina mulch into smaller, more soluble organic molecules.

### Effect of EM on Column Lifetime

The effect of EM on the lifetimes of the columns is presented in Figure 5.

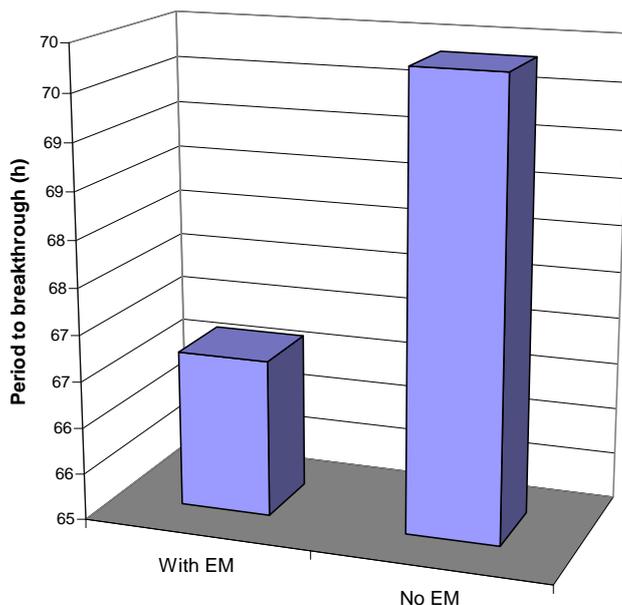


Figure 5. Influence of engineered microbes on column period before and after breakthrough

The periods to breakthrough of the columns with EM are reduced by 5% by comparison to those without the EM. Thus the longevity of the column material is reduced by the application of EM.

This observation concords with the hypothesis that the EM are functioning to rapidly break down the labile complex cellulosic components of the Casuarina mulch.

### Summary of Observations

The observations above are summarised in Table 1.

Table 1. Effect of EC on column parameters

Column parameter	Effect of EM	Extent of effect
Effluent pH	Increased	52%*
Effluent Fe	Decreased	80%
Effluent EC	Decreased	9%
Effluent COD	Increased	+5 mg/l O <sub>2</sub>
Column longevity	Decreased	5%

\* - measured as decrease of titratable acidity

The influence of the engineered microbes (EM) on the effectiveness of AMD amelioration by the Casuarina mulch is as hypothesised. EM facilitates more efficient breakdown of complex cellulosic components of the Casuarina mulch into smaller, water soluble organic molecules which are used as electron donors by the sulphate reducing bacteria (SRBs).

The greater rate of production of more water soluble organic molecules likely increase the biomass of SRBs, which in turn more efficiently reduce sulphate concentrations and acidity (thus increasing pH and decreasing EC) of effluent solution.

Sulphate would be converted to sulphide by the SRBs, which, reacting with iron to form insoluble sulphides, would drastically reduce the iron concentrations in the effluents.

The greater rate of consumption of labile cellulosic material decreases the longevity of the Casuarina mulch, which is the thermodynamic cost of increased efficiency of sulphate reduction.

#### **4. CONCLUSIONS**

This study has shown that the introduction of engineered microbes (EM) into organic mulch (Casuarina leaves in this instance) enhances bioremediation of AMD.

#### **5. ACKNOWLEDGEMENTS**

The author gratefully acknowledges the Council for Geoscience statutory grants for funding this investigation, and the tireless efforts of Jenny Glass of the EGU in the experimental component of the study.