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Lead Removal Using Industrially Sourced Consortia: Influence of Lead and Glucose Concentrations

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The objective of the current study was to explore the lead removal capabilities of two locally sourced industrial consortia using batch fermenters. The consortia were obtained from lead contaminated soil at a) a lead mine and b) an automotive battery recycling plant. The experiments were performed under anaerobic conditions using a rich growth medium (Luria Bertani Broth) with two different background lead concentrations (80 mg/L and 160 mg/L). The influence of added glucose to the medium was also studied. The residual aqueous lead was used as a measure of the lead removal capacity.
Findings attest to the effectiveness of the industrially obtained consortia at removing lead from solution for both lead concentrations. Lead removal of between 91% and 93% in 168 hours and between 63% and 76% in 240 hours were achieved for the 80 mg/L and 160 mg/L experiments respectively. Minimal change was measured in the absorbances of these experiments, indicating a non-growth related lead removal mechanism.
A glucose concentration of 60 g/L caused severe substrate inhibition which reduced the lead removal effectiveness to between 5% and 30% over a 214-hour period.
The results for both consortia displayed significant similarities, indicating that analogous strains of organisms were present and active under the specific conditions.

1. Introduction

Lead (Pb) is used extensively in various industrial applications, including battery plates, electrical cable sheathing, ammunition, radiation shielding, type and bearing metal, and solder. In contrast to various other metals, the occurrence of Pb in the earth's crust is limited with an estimated 15 years' global supply of workable raw Pb at the current usage rate (www.statista.com).
The prevalence of Pb in the environment is almost entirely due to anthropogenic activities. These activities include industrial processing of silver, platinum and iron, the use of Pb-containing paints, the combustion of Pb petroleum products (Gioia et al., 2006), and the combustion of coal for power generation (Block and Dams, 1975).
The increase in environmental Pb concentrations are of concern due to the potential harm to human biological systems and the biosphere. It is known that Pb concentrations below 10 mg/L can cause endocrine disruption (Supanopas et al., 2005), while entire ecosystems can be eradicated at concentrations greater than 1000 mg/L (Eisler, 1988). Pb pollution has a disproportionate tendency to affect vulnerable groups such as children (Taylor et al., 2013) and marginalised groups (Barbosa et al., 2009). In the South African context, Pb pollution affects mostly poorer communities as a result of peeling Pb-containing paint which subsequently contaminates the food and water supply (Mathee, 2014), recycling of Pb from batteries for use in sinkers by subsistence fishermen (Mathee et al., 2013), and the unregulated use of Pb in traditional medicines (Mathee et al., 2015).
Orthodox technologies used for the treatment of toxic heavy metals (Cd, Pb, Fe, Mn, Cr, and As) include sand filtration, GAC adsorption, precipitation sedimentation, flotation, ion-exchange, and electrochemical deposition (Aziz et al., 2008). The major limitations of the adsorption methods are a lack of selectivity to specific metals, while the presence of minerals such as Na, Ca, K, and Mg inhibits the adsorption processes due to competition for adsorption sites (Ngwenya and Chirwa, 2010). In the case of precipitation processes, the pH and redox requirements for the precipitation of metals such as Pb, Al, and Fe are outside the natural

environmental range, while overlapping precipitation ranges produce sludge consisting of a mixture of metals (Aziz et al., 2008). The disadvantages of ion-exchange methods are that they cannot handle concentrated metal solutions as the matrix is easily fouled. Additionally, ion-exchange is indiscriminate and highly sensitive to the pH of the solution (Barbaro and Liguori, 2009).
The removal of Pb using dead or reconditioned biomass as biosorbents has been studied by Chatterjee et al. (2012). A study by Teekayuttasakul and Annachhatre (2008) used biological methods to convert mobile Pb complexes (Pb sulphate or Pb acetate) to less mobile Pb carbonates. Rhee et al. (2012) converted mobile Pb ions to a leadphosphate multinuclear pyromorphite ($Pb_5(PO_4)_3Cl$) which was shown to be stable in sediments. The stability of pyromorphite is a result of its insolubility which inhibits Pb bioavailability. However, certain fungi and plants can use pyromorphite as a phosphorous source which leads to a bioaccumulation of Pb and P and a subsequent release back into the environment during the plants' biodegradation (Sayer et al., 1999).
The reduction of metal species to a lower oxidation state is thermo-dynamically feasible for a wide redox range; it usually requires a significant activation energy to initiate the reaction. In order to reduce this activation energy, it is customary to introduce a catalyst to the system. In biological systems enzymes act as catalysts by allowing reactions normally requiring elevated temperatures to proceed under ambient conditions (Karp, 2009). This capability of biological systems has been utilised successfully in previous studies on the reduction of various toxic heavy metals: Cr(VI) to Cr(III) (Molokwane & Chirwa, 2009), U(VI) to U(IV) (Chabalala and Chirwa, 2012), and Se(VI) to Se(0) (Li et al., 2014). These cases revealed organisms facilitating the biotransformation of the metals despite the thermodynamic barriers. It was found that the transformations were either a defensive mechanism which converted the metal to a less mobile phase thereby preventing entrance to the cells (Kessi et al., 1999), or as result of the metal species being used as an electron acceptor which aided the energy metabolism (Wade and DiChristina, 2000).
There has been only one study, reported by Saiz and Barton (1992), in which the feasibility of the biological reduction of Pb(II) to Pb(0) was investigated using an anaerobic culture of *Moraxella bovis*. The quantitative results from the study were not reported; the only documentation available is a conference abstract. According to the abstract a dark grey precipitate, an indicator of Pb(0), was reportedly formed during the investigation. In an abiotic, enzyme-catalysed study, the reduction of Pb(II) to the metallic state was achieved using a purified cytochrome 3 from *Desulfomicrobium baculatum* [strain 9974] (Abdelouas et al., 1999), indicating that in principle the biological reduction of Pb(II) to Pb(0) is possible.
Due to the inherent restrictions confronted by contemporary technologies used for Pb removal, its extraction from the environment for direct reuse has thus far not been achieved. It was hypothesised that Pb-contaminated industrial sites would harbour microbial consortia that a) would be able to survive in environments with elevated background Pb concentrations and b) have the capability to decrease the background Pb concentration by precipitation. This research studied the removal of Pb in batch biological reactors operated with industrially sourced consortia. The reactors were cultured anaerobically using a rich medium (Luria Bertani Broth) with two different concentrations of background Pb as well as in the presence and absence of additionally added glucose, under mesophilic conditions.

2. Materials and Methods

2.1 Pb resistant consortium screening

Pb contaminated soil samples were obtained from an operational Pb mine in the Northern Cape Province of South Africa as well as from an automotive-battery recycling plant in Gauteng, South Africa. The samples were initially screened for Pb resistant microbes by suspending 1 g of soil in sealed serum bottles (Sigma-Aldrich, St. Louis, MO) containing sterile screening medium: Luria Burtani (LB) broth (Merck, Darmstadt, Germany) with a background Pb concentration of 80 mg/L. No attempt was made to purify or identify the consortia. The screening cultures were grown anaerobically for 24 hours at 32 °C ensuring facultative anaerobic consortia. After culturing, sterile glycerol was added to the solution (20 % v/v) and 1 mL stock cultures were extracted and cryogenically stored at -77 °C.
The fermentation and screening media were prepared by blending individually sterilised LB broth and a 1000 mg/L Pb (Merck, Darmstadt, Germany) stock solution in the required ratios.

2.2 Preparation of microbial cultures

All fermentations were inoculated from LB agar plates. Prior to fermentations, the cryogenically stored stock cultures were revived by thawing in a 5 °C cold room for 45 minutes after which the cultures were streaked on LB agar plates and anaerobically cultured for 24 hours at 32 °C in an AnaeroGen™ pouch (Oxoid Ltd, Basingstoke, UK). After 24 hours' growth, the plates were stored at 5 °C until required for fermentations.
The fermentation media, which consisted of LB broth with different Pb and glucose concentrations (Table 1), were inoculated by suspending two loops of the plated cultures in the media and purging the bottles with

nitrogen for 3 minutes prior to sealing with a rubber stopper to ensure anaerobic conditions. Experiments were performed in triplicate.

Table 1: Initial Pb and glucose concentrations used for the three respective experiments

Experiment number	Initial background Pb concentration (mg/L)	Added glucose concentration (g/L)
1	80	0
2	160	0
3	80	60

2.3 Experiments

All experiments were performed under anaerobic mesophilic (32 °C) batch conditions, using standard LB broth and the initial Pb and glucose concentrations described in Table 1.
Experiment 1 was performed for a total of 168 hours, with the first three samples taken 24 hours apart, followed by a final sample at the end of the run.
Experiment 2 was performed for an initial 168 hours, with the first four samples taken at 24 hour intervals. At 168 hours a sample was taken and an additional “spike” of sterile LB broth (10 mL) was injected into reactors to determine if more Pb removal could be achieved. This was followed by another three samples at 24 hour intervals.
Experiment 3 was performed for a total of 216 hours, with the first three samples taken at 6, 24 and 48 hours and the final sample at the termination of the experiment.
The pH of each batch reactor was taken at the termination of the respective experiments.

2.4 Sampling and analysis

The biological reactors were sampled by aseptically extracting approximately 1 mL sample through the rubber stopper using a sterilised hypodermic needle. After extraction the samples were centrifuged for 10 minutes at 6000 rpm (Eppendorf® Minispin Z606235, Hamburg, Germany), and the effluent was decanted and stored at 5 °C until analysis. The pellet was resuspended in distilled water and the absorbance measured at 540 nm (Biochrom WPA Lightwave II, Harvard Bioscience, Inc., Holliston, MA).
The dissolved Pb concentrations were quantified by an atomic absorption spectrometer (Perkin Elmer AAnalyst 400, Waltham, Massachusetts) equipped with a Pb Lumina hollow cathode lamp (Perkin Elmer AAnalyst 400, Waltham, Massachusetts).
The pH of the samples was measured using a HQ11d Portable pH/ORP Meter (Hach®, Loveland, Colorado).

3. Results and Discussion

The results from experiment 1 are shown in Figure 1. Figure 1a presents the measured Pb concentrations in the sample effluents and Figure 1b displays the measured absorbances of the respective samples. From Figure 1a, it is clear that a significant decrease in dissolved Pb was observed (between 91% and 93% removal in 168 hours), while Figure 1b indicates that the absorbance of the sample only exhibited a small change in the absorbance measured over time; this did not mirror the significant reduction in Pb.
Figure 2 shows the results obtained from experiment 2. Figure 2a shows the measured Pb in the samples and Figure 2b indicates the sample absorbances. These results correspond with those presented in Figure 1, with a significant decrease in measured Pb concentrations over the first 96 hours. Between 96 hours and 168 hours the Pb removal rates decreased significantly. By 168 hours total Pb removal values of between 48% and 57% were measured. In order to determine if more Pb removal was possible it was decided to “spike” the reactors with an additional 10 mL of LB broth. This resulted in significant additional removal of Pb indicating that substrate limitations were experienced between 96 hours and 168 hours, resulting in the decrease in Pb removal rates observed. Final lead removal of between 63% and 76% were measured after a total run time of 240 hours.
As was the case in Figure 1b, Figure 2b only shows a marginal change in absorbance measurements for the duration of the experiments. In comparison, unpublished aerobic absorbance results from the same consortia showed a 16-time increase in absorbance over a 200-hour period. These results hint at a non-growth related Pb precipitation mechanism which might be a result of a defensive mechanism or non-growth related energy requirements; significant non-growth related bacterial metabolism has been observed due to cellular maintenance energy requirements of microbial organisms (Brink & Nicol, 2014).
Interestingly, there does not seem to be a distinct variation between the results for the respective consortia. This is likely an indication that similar strains of organisms were active under the specific conditions, i.e. an

anaerobic environment with a high background concentration of Pb might favour a limited population distribution capable of removing Pb from the solution.

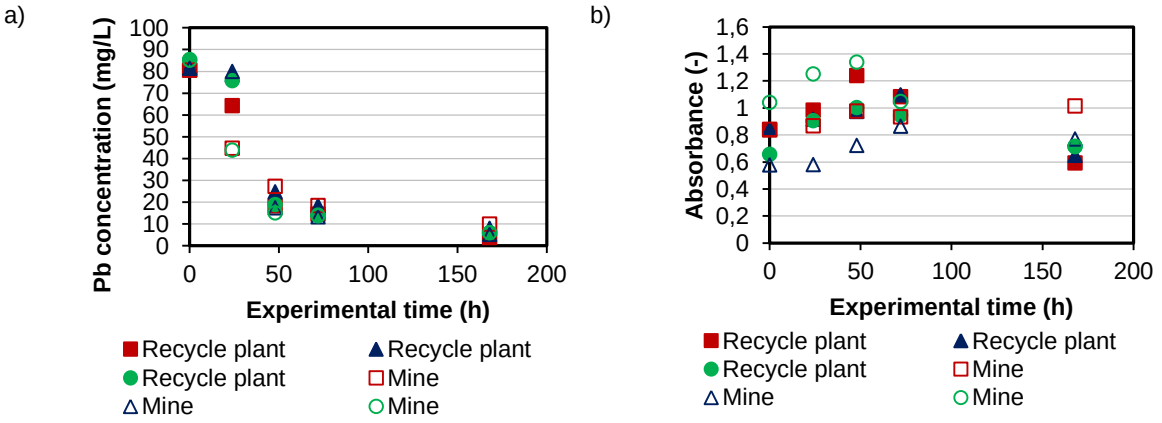

Figure 1: Results obtained using an initial Pb concentration of 80 mg/L: a) The measured Pb concentrations b) the measured absorbance values for the respective batch reactors.

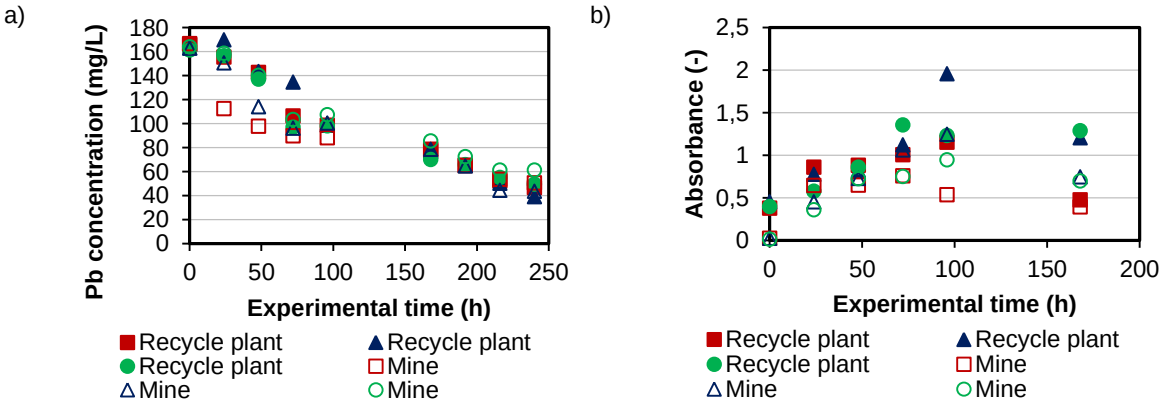

Figure 2: Results obtained using an initial Pb concentration of 160 mg/L: a) The measured Pb concentrations b) the measured absorbance values for the respective batch reactors. A "spike" of LB broth was introduced at 168 hours.

In response to the observation in experiment 2 that the consortia experienced substrate limitation leading to a decline in the Pb precipitation rates, experiment 3 was performed with the addition of 60 g/L of glucose as additional substrate. The results obtained from experiment 3 are shown in Figure 3. Figure 3a shows the measured Pb concentrations and Figure 3b shows the measured absorbance measurements. Figure 3a demonstrates that the Pb removal was limited when compared to the results from experiments 1 and 2 (Figure 1a and Figure 2a). Overall lead removals of between 5% and 30% were observed, with a mean removal of only 16% over all the reactors. From Figure 3b it can be seen that after an initial increase in absorbance in the first 6 hours, corresponding to most of the observed Pb removed, limited microbial growth was achieved for this experiment. These results indicate that the consortia experienced severe substrate inhibition which limited that ability of the organisms to remove Pb. This experiment hints at a small contribution of growth to the removal of Pb as observed in the first 6 hours, which warrants further investigation.

Figures 4a and b show representative pictures corresponding to experiment 1 and 3 respectively. The pictures were taken 24 hours after inoculation of the vials. These figures demonstrate the distinct differences observed between the vials with and without the addition of glucose. The identity of the dark grey precipitate observed in experiment 1 and 2 (Figure 4a) remains uncertain. However, the pH of all the runs (Table 2) were between 6.4 and 7.3, strongly suggesting the presence of elemental Pb. The Redox equilibrium for the Pb-H_2O system predicts only Pb(s), PbO, and PbO_2(s) at this pH, and only Pb(s) is a dark precipitate. The identity of the white precipitate observed in experiment 3 (Figure 4a) remains ambiguous. The light colour is an indicator of $PbCl_2$;

NaCl is an ingredient of LB broth. However, the limited removal of Pb in experiment 3, as shown in Figure 3a, suggests that this would be a less favourable Pb removal method than that employed in experiment 1 and 2.

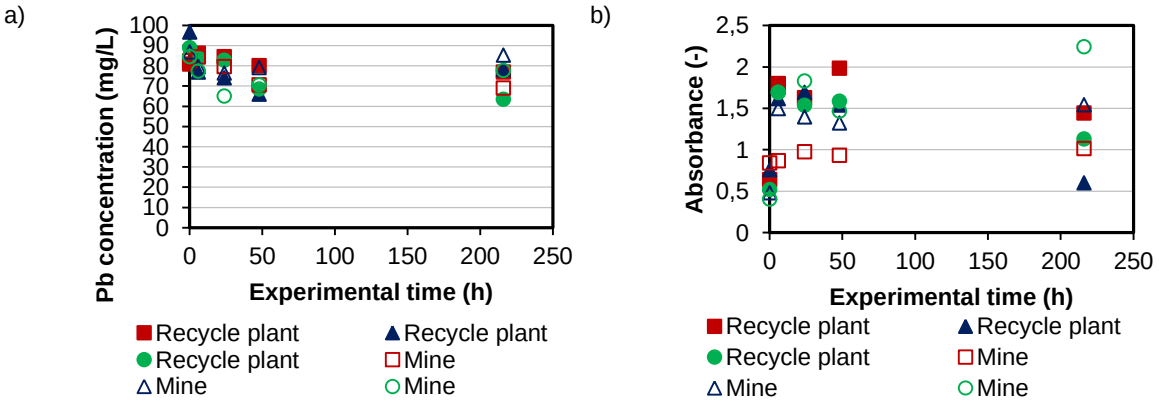

Figure 3: Results obtained using an initial glucose concentration of 60 g/L and a Pb concentration of 80 mg/L: a) The measured Pb concentrations b) the measured absorbance values for the respective batch reactors.

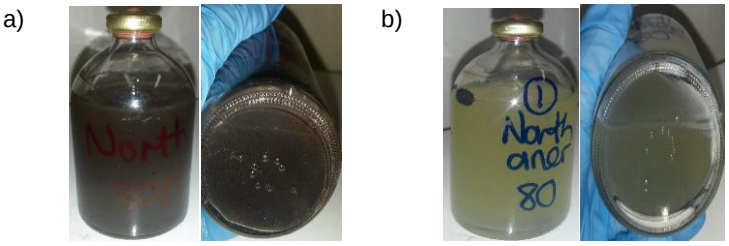

Figure 4: Representative picture corresponding a) experiment 1 and b) experiment 3 showing a distinct difference in the observed reactor and precipitate colours at 24 hours.

Table 2: Final pH-values measured at the termination of the respective experiments and runs

Experiment number	Recycle plant			Mine		
	Run 1	Run 2	Run 3	Run 1	Run 2	Run 3
1	7.02	6.95	7.29	6.94	6.70	6.59
2	6.46	6.84	7.32	6.47	6.67	6.84
3	6.36	6.89	7.24	6.54	6.70	7.16

4. Conclusions

The study showed that the industrially obtained consortia were highly effective at removing Pb from solution up to a background Pb concentration of 160 mg/L, with the main limitation being substrate availability. Pb removal of between 91% and 93% in 168 hours was achieved for the 80 mg/L experiment, while an overall Pb removal of between 63% and 76% was achieved after 240 hours' fermentation in the 160 mg/L experiment. The changes in absorbances measured for these experiments were minimal, indicating that a non-growth related Pb removal mechanism might have been active.

In contrast to these results, it was found that a glucose concentration of 60 g/L caused severe substrate inhibition which reduced the Pb removal effectiveness to between 5% and 30% over a 214-hour period.

The results for both consortia were similar, indicating that comparable strains of organisms were present and active under the specific conditions.

Findings from the study support the hypothesis that the industrially obtained consortia are lead resistant and able to remove lead from solution.

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