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Microbial Pb(II) Precipitation: The Influence of Elevated Pb(II) Concentrations

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The objective of the study was to determine the influence of elevated lead concentrations on the rates of lead precipitation by a local industrially-obtained consortium. The consortium was sourced from a lead battery recycling plant located in Gauteng, South Africa. The experiments were performed under anaerobic conditions using commercial Luria Bertani (LB) broth and simulated LB broth (reduced NaCl concentration) as the growth media. The respective media were spiked with various concentrations of lead, ranging from 80 ppm to 1000 ppm.
All concentrations of lead resulted in a black precipitate with a final medium pH of between 6 and 8, indicating the presence of lead(0). A 99 % removal of lead occurred with 500 ppm lead after 11 days. A lead concentration of 1000 ppm was reduced by 87 % after 22 days. The higher NaCl concentration in the commercial LB broth formed a white precipitate ($PbCl_2$) upon initiation of the high lead(II) concentration experiments. These experiments also presented with a black precipitate at a later stage, indicating that the lead in the $PbCl_2$ precipitate remained available for reduction to elemental lead. No white precipitate was observed during experimentation where the simulated LB broth was used with a lower concentration of NaCl.
The results indicate that the consortium precipitates lead concentrations up to 1000 ppm. This provides support that biological precipitation has potential application in industry for the hydrometallurgical processing of lead as well as industrial lead bioremediation.

1. Introduction

A projected 17 years' supply of global lead (Pb) reserves remains; there is a total estimated global Pb reserve of 84 million tonnes (Statista, 2017), with Pb ores being mined at a rate of 5 million tonnes per annum (International Lead Association, 2017). Pb is a heavy metal, toxic to living organisms, which induces acute and chronic effects on human health (Mudipalli, 2007). Pb pollution is almost entirely due to anthropogenic releases from industrial processes and products containing Pb additives (Sharma et al., 2016: 9).
Currently a range of physico-chemical processes are being used to treat water containing heavy metals such as adsorption, chemical precipitation, membrane filtration, electrodialysis, and photocatalysis (Barakat, 2011). But these methods are expensive and produce harmful waste products and sludge that requires additional processing (Barakat, 2011). Also, hydrometallurgical processing of Pb requires expensive electrodes and high process energy inputs during the electrowinning step (Lee et al., 1986). An alternative solution is therefore required which is cost effective as well as environmentally suited for the bioremediation and recovery of Pb from contaminated water environments together with the industrial processing of Pb.
Much research has proposed using microbes in the bioremediation of heavy metals. Kang et al. (2016) reported that bacterial mixtures obtained from an abandoned mine site performed better in a mixture of Pb, Cd, and Cu than the isolated single cultures, exhibiting higher growth rate, urease activity and resistance to heavy metals.
Remediation of heavy metals from contaminated environments can be accomplished by a range of technologies. These include methods such as isolation, immobilisation, toxicity and/or mobility reduction, physical separation, and extraction (Wuana and Okieimen, 2011). Reducing the toxicity and/or mobility of a heavy metal can be achieved by biological treatment of the contaminated site (Wuana and Okieimen, 2011).

Immobilisation is defined as decreasing the mobility of a contaminant by changing the chemical or physical properties of the contaminant (Akhtar et al., 2013). The remediation method proposed in this research falls in this category of toxicity and/or mobility reduction, because microbes could theoretically reduce Pb(II) to Pb(0) and in its elemental form Pb is no longer mobile or bio-available (Hayes, 2010: 25).

Microbes used in the bioremediation of inorganic toxic compounds such as heavy metals should be exploited according to their specialisation of the specific contaminant as the bioremediation strategy is governed by the active metabolising capabilities of microorganisms (Dixit et al., 2015). It was based on this criterion that the consortium of microbes used in this research was sourced from a local Pb battery recycling plant.

Naik and Dubey (2013) reported that the diverse mechanisms that Pb resistant bacteria utilise include extracellular sequestration, efflux mechanism, biosorption, intracellular Pb bioaccumulation, enhanced siderophore production, alteration in cell morphology, and bioprecipitation. Other reports on the bioprecipitation of Pb mainly covers Pb precipitation in a compound form, such as $PbHPO_4$ that precipitates on the cell surface of *Citrobacter* sp. (Aickin et al., 1979). *Bacillus iodinium* GP13 and *Bacillus pumilus* S3 are Pb resistant and precipitates Pb as Pb sulfide (PbS) (De et al., 2008). Only one study investigated the viability of the biological reduction of Pb(II) to Pb(0) using *Moraxella bovis* under anaerobic conditions (Saiz and Barton, 1992). The only available documentation of the research is the conference abstract, which excludes any quantitative results, but it was reported that a dark grey precipitate formed, which is an indicator of Pb(0). Research that proves that the biological reduction of Pb(II) to Pb(0) is in principle feasible was done by Abdelouas et al. (1999) by using cytochrome 3 from *Desulfomicrobium baculatum* [strain 9974] in an enzyme catalysed, abiotic study where Pb(II) was reduced to insoluble Pb(0).

Previous research done by this research group showed that this specific locally sourced consortium can reduce 80 ppm Pb in water under anaerobic conditions by more than 90 % in 7 days (Brink et al., 2017a). Supplementary research showed that adding glucose to the LB broth and Pb mixture causes substrate inhibition which reduces the Pb removal effectiveness, and therefore LB broth was used as the main food source in this study (Brink et al., 2017b).

A dark grey precipitate in Pb bioremediation studies combined with a solution pH that ranges from 6 to 8 can be used as an indicator that the precipitate that formed is Pb(0) (Stumm and Morgan, 1970). This is concluded using the Pb Pourbaix diagram for Pb(II), Pb(0), PbO, PbO_2, $Pb(OH)_2$, and H_2O (Stumm and Morgan, 1970) which shows how the oxidation-reduction behaviour of these compounds of Pb depends on pH (Daintith and Martin, 2010: 657).

The purpose of the current study was to determine the impact elevated Pb concentrations has on microbial precipitation in the endeavour of advancing microbial Pb precipitation as a method for Pb bioremediation, recovery, and processing. Luria Bertani (LB) broth was used as a food source for the microbes and the removal rate of Pb at elevated concentrations were studied. The microbial consortium was obtained from a local Pb battery recycling plant. The influence of concentrations ranging from 80 ppm to 1000 ppm was investigated under anaerobic conditions in serum bottles using commercial LB broth, and simulated LB broth with a lower NaCl concentration. Atomic absorption spectroscopy was used to quantify the remaining Pb(II) concentration in the solution at several time intervals after a black precipitate formed. There was no attempt to isolate the bacteria or determine the kinetics associated with the Pb reduction achieved by the bacteria. The Pb precipitate was also not characterised, but pH measurements, the Pourbaix diagram of Pb and visual observations in precipitate colour was used as indications for Pb(0) in the precipitate.

2. Materials and methods

2.1 Materials

Standard LB broth (Miller) from Sigma-Aldrich was used as the main food source for the microbes, which was also used in another Pb bioremediation study using microbial cultures sourced from a mine (Kafilzadeh et al., 2012). LB broth consists of yeast extract, tryptone, and NaCl; all of these separate compounds, which were used in the preparation of the simulated broth, were obtained from Merck. $Pb(NO_3)_2$, also acquired from Merck, was chosen as the source for Pb(II) as it is highly soluble and has also been used in a previous Pb bioremediation study (Levinson et al., 1996).

2.2 Microbial culture preparation

Preparation of microbial cultures and all experiments were performed under sterile conditions, autoclaving the appropriate medium, Pb stock solutions and glassware as well as ensuring sterile conditions on the experimental surface. The inoculum was prepared by adding 1 g of the Pb contaminated soil sample to a mixture of sterile LB broth and 80 ppm Pb in a 100 ml serum bottle (Sigma-Aldrich), incubating it for 24 hours at 32 °C under anaerobic conditions on a shaker plate. Anaerobic conditions in the serum bottles were achieved by purging each serum bottle with nitrogen for 3 minutes before sealing it with a rubber stopper and

cap. Glycerol was added to a final ratio of 20 % v/v and 1 ml samples were stored cryogenically at -77 °C as inoculum. A pre-culture was prepared in a 100 ml serum bottle, containing LB broth, 80 ppm Pb and 1 ml of the inoculum. The culture was prepared anaerobically and used to inoculate all the experimental runs.

2.3 Experiments

First a control experiment was conducted to prove that the black precipitate and therefore Pb reduction is due to the microbial activity. The standard LB broth concentration of 25 g/L was used for all the serum bottles, with a Pb concentration variation as follow: 80 ppm, 200 ppm, 400 ppm, 600 ppm, 800 ppm, 900 ppm, 1000 ppm.
In total four experiments were performed with the microbial culture and different Pb(II) concentrations. The first set of experiments was carried out using the commercial LB broth, containing 10 g/L NaCl. A low LB broth concentration (25 g/L) was used and spiked with 500 ppm, 750 ppm and 1000 ppm Pb(II). Similarly a high broth concentration (100 g/L) was used and spiked with 500 ppm, 750 ppm and 1000 ppm Pb(II).
The second set of experiments was conducted with the prepared simulated LB broth. The low concentration of simulated broth (25 g/L) consisted of 12 g/L tryptone, 12 g/L yeast extract, and 1 g/L NaCl. The Pb concentrations that were investigated were 500 ppm, 750 ppm, and 1000 ppm Pb(II). The high concentration of simulated broth (50 g/L) showed the most promise for success and were tested on 80 ppm, 250 ppm, 500 ppm, 750 ppm and 1000 ppm Pb(II). The broth consisted of 24.5 g/L tryptone, 24.5 g/L yeast extract, and 1 g/L NaCl. It was determined from the solubility product of $PbCl_2$ that 1 g/L of NaCl will ensure that Pb will not precipitate as $PbCl_2$ within this concentration range (Mullin, 2001: 478).
During the experiment of the low concentrated broth, no growth occurred in the 1000 ppm experiment and it was subsequently spiked with 10 ml of the high concentration simulated LB broth on day 7 to investigate if adding nutritional sources to the serum bottle will initiate Pb reduction. The 500 ppm and 750 ppm experiments were spiked on day 7 as well with the expectation that it would increase Pb reduction. For the same reason the 750 ppm and 1000 ppm experiments of the high concentration simulated broth study were spiked on day 16. The dilution that took place as a result of the spiking was taken into account in the reported results.
Samples were taken on the reported days by extracting 2 ml of the solution from the serum bottle. This was accomplished by piercing the rubber stopper with a sterile syringe under aseptic conditions and placing it in a cryogenic vial. The vials were centrifuged at 6000 rpm for 10 minutes (Eppendorf® Minispin Z606235, Hamburg, Germany), followed by placing the supernatant in separate cryogenic vials and storing it at 5 °C for analysis.

2.4 Analysis

The Pb(II) concentration in the samples was determined by atomic absorption spectroscopy (Perkin Elmer AAnalyst 400, Waltham, Massachusetts). The analyses were executed with a Pb Lumina hollow cathode lamp and the results were used to quantify the Pb removal in each experiment. As soon as the experiments were terminated, the pH of each solution was measured with an HQ11d Portable pH/ORP Meter (Hach®, Loveland, Colorado).

3. Results and discussion

3.1 Control

After 14 days the control experiment was terminated with conclusive results that the microbial culture is the facilitator of the Pb reduction, which causes the grey/black precipitate. A summary of the results are presented in Table 1. No growth occurred in any of the serum bottles and the final pH reading ranged from 4.72 to 6.06. The white precipitate that formed in the 600 ppm Pb experiment and up is due to the presence of chloride ions from NaCl in the broth that caused the formation of $PbCl_2$, which has a very low solubility product (Mullin, 2001: 478).

Table 1: Summary of the results of the control experiment

Pb concentration	pH	Visual observation
80 ppm	4.72	No growth.
200 ppm	4.92	No growth.
400 ppm	5.17	No growth.
600 ppm	5.38	No growth, white precipitate formed.
800 ppm	5.90	No growth, white precipitate formed.
900 ppm	6.06	No growth, white precipitate formed.
1000 ppm	5.46	No growth, white precipitate formed.

3.2 Commercial LB broth

At the beginning of the experiments done with the low concentration commercial LB broth, the serum bottles containing 750 ppm and 1000 ppm Pb presented with a white precipitate, which is an indicator of $PbCl_2$ formation. On day 3 the only serum bottle that showed growth and a grey precipitate was the one containing 500 ppm Pb. The experiments with 750 ppm and 1000 ppm Pb still presented with a white precipitate due to the high Pb concentrations. The Pb removal in these experiments, in the absence of a grey precipitate, is therefore exclusively due to the formation of $PbCl_2$. After 6 days the experiment was terminated and it was determined that 90 % of the Pb(II) was removed in the 500 ppm experiment (Table 2).
The experiments with the high concentration of commercial LB broth showed growth and a grey precipitate on day 3 in all three Pb concentration experiments (500 ppm, 750 ppm, 1000 ppm), even though upon initiation the 750 ppm and 1000 ppm serum bottles also exhibited a white precipitate. It is concluded that growth occurred in the 750 ppm and 1000 ppm experiments due to the higher broth concentration, and initial lower Pb concentration due to the formation of $PbCl_2$. The Pb in the $PbCl_2$ precipitate still remain available for reduction to elemental Pb, as the white precipitate dissolves again after a decrease in the concentration of Pb(II). It is interesting to notice that the 750 ppm experiment resulted in the highest final removal percentage of 88 % between the 3 concentration ranges, with a 1000 ppm being the lowest at 61 % removal of Pb(II).
The pH range of all the serum bottles in this set of experiments was between 6 and 7, which indicated that the grey precipitate is likely elemental Pb. The results are summarised in Table 2.

Table 2: Commercial LB broth

Low LB broth concentration				High LB broth concentration			
Concentration	Day	pH	% Pb removal	Concentration	Day	pH	% Pb removal
500 ppm	6	6.63	90.0 %	500 ppm	6	6.26	83.5 %
750 ppm	6	6.34	49.4 %	750 ppm	6	6.43	88.3 %
1000 ppm	6	6.21	56.0 %	1000 ppm	6	6.27	60.8 %

3.3 Simulated LB broth

The low concentration simulated LB broth experiment was performed over a period of 13 days. On day 2, the 500 ppm and 750 ppm serum bottles presented with a grey precipitate, with the serum bottle of 1000 ppm not showing any indications of Pb reduction. The absence of an observable white precipitate is an indication of the success that resulted from adding less NaCl to the solution. All the serum bottles were spiked with LB broth on day 7 with the expectation of increasing the Pb reduction process by providing additional nutrition to the microbes. On the final day, the 1000 ppm experiment still did not present with a grey precipitate and with only 0.8 % Pb removal, it can be concluded negligible. The serum bottle of 500 ppm Pb had a final removal percentage of 55 % and the 750 ppm experiment 56 %, performing nearly at the same average rate. In Figure 1a the change in Pb(II) concentration in each serum bottle is presented. Again the final pH measurements were between 6 and 7, indicating that the grey precipitate is Pb(0). The results are summarised in Table 3.
The high concentration simulated LB broth experiment was performed for a total duration of 22 days. Once again no white precipitate formed, indicating an absence of $PbCl_2$. On day 2 all the serum bottles presented with a grey precipitate. The serum bottles got increasingly darker in colour with time. The results from the atomic absorption spectrometer of the samples taken on day 11 showed almost complete removal of Pb(II) in the 500 ppm serum bottle (99 %). On day 12 the 750 ppm and 1000 ppm experiments already showed higher removal percentages than the same concentration range on day 13 of the low concentration simulated broth experiments. The 750 ppm and 1000 ppm experiments were continued and spiked on day 16 with LB broth to investigate how much Pb could be removed using the high LB broth concentration if enough time is allowed. On day 22 the 750 ppm serum bottle succeeded in removing 83 % of Pb(II) and the 1000 ppm serum bottle 87 %. It can be observed in Figure 1b how the concentration of Pb(II) changed in each serum bottle. The final pH measurements once again ranged from 6 to 7. The results are summarised in Table 3.
Pb removal by the current bacterial consortium compares well to results presented in literature. *Escherichia coli* sourced from Pb contaminated soil has previously been reported to remove 60 % of Pb(II) in 11 hours from a solution initially containing 450 ppm Pb(II) (Kafilzadeh et al., 2012). *Rhodopseudomonas palustris*, sourced from a heavy metal contaminated site near a river, removed 92 % Pb(II) from a 100 ppm solution in 4 days (Sinha and Biswas, 2014). Pb adsorption onto activated *Cassia grandis* seed gum removed 60 % Pb in 11 hours from a solution with an initial concentration of 200 ppm (Singh et al., 2007). The high Pb tolerance and Pb removal capacity of the bacterial consortium indicates the potential for industrial application. Examples of Pb concentrations in industry include 2 g/L Pb in battery wastewater (Singh et al., 2007) and 100 g/L Pb in

hydrometallurgy streams (Lee et al., 1986). The removal of Pb in industrial processes such as hydrometallurgy (Strunnikov and Koz'min, 2005) and precipitation with lime (Aziz et al., 2008) is in the vicinity of 90 %.

Table 3: Simulated LB broth

Low LB broth concentration				High LB broth concentration			
Concentration	Day	pH	% Pb removal	Concentration	Day	pH	% Pb removal
				80 ppm	11	6.47	82.8 %
				250 ppm	11	6.82	90.2 %
500 ppm	13	6.58	55.0 %	500 ppm	11	6.42	99.2 %
750 ppm	13	6.77	56.3 %	750 ppm	12		61.0 %
1000 ppm	13	6.30	0.8 %	1000 ppm	12		55.3 %
				750 ppm	22	6.87	83.4 %
				1000 ppm	22	6.59	86.5 %

a) *b)*

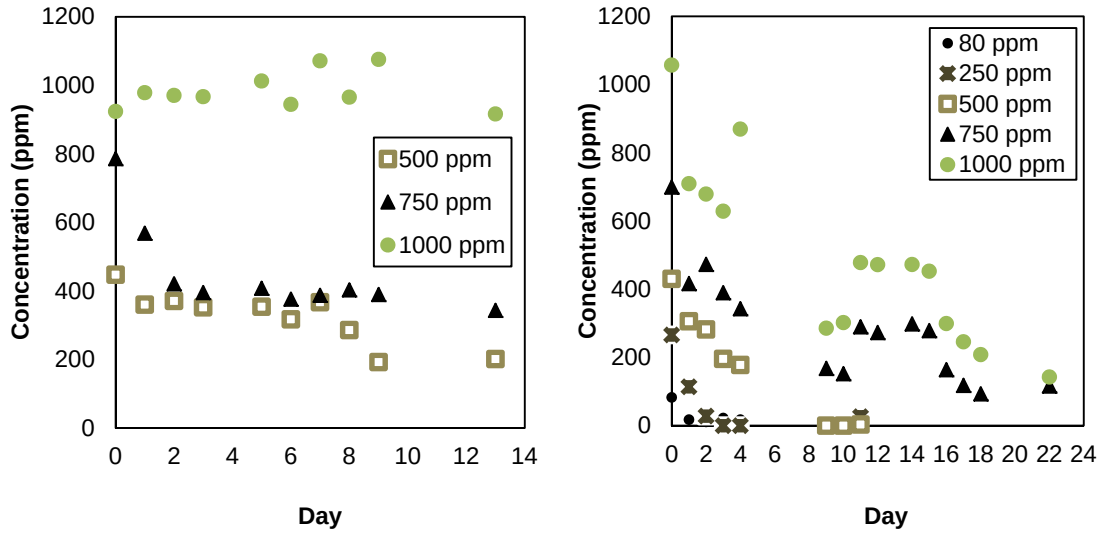

Figure 1: Change in Pb(II) concentration measurements of the a) low and b) high concentration simulated LB broth experiments.

4. Conclusions

Overall the high concentration simulated broth performed the best with 99 % Pb(II) removal after 11 days in the 500 ppm experiment, 83 % removal after 22 days in the 750 ppm experiment and 87 % removal after 22 days in the 1000 ppm experiment. The experiments that presented with a black precipitate all had final pH measurements that ranged between 6 and 7, indicating the presence of elemental Pb. The successful removal of Pb at these elevated concentrations with adjustment of the NaCl concentration in the broth mixture encourages the initiative of using this locally sourced consortium in bioremediation of Pb as well as the recovery and hydrometallurgical processing of Pb.

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