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Microbial Pb(II) Precipitation: The Effects of Aeration Conditions and Glucose Presence on a Lead-Mine Consortium

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Increasing non-localised outputs of lead to the environment, in conjunction with limited raw lead supplies, have generated interest in the recovery of lead from polluted sources and areas. This study aimed to quantify the effects of aerobic and anaerobic conditions, as well as the presence and absence of glucose, on the effectiveness of an industrially obtained consortium at precipitating aqueous lead(II) under batch conditions. The consortium was obtained from an operational lead mine in the Northern Cape, South Africa. The experiments were performed aerobically and anaerobically, using 80 ppm lead(II) in a rich growth media in the presence and absence of 33 g/L glucose. The residual aqueous lead(II) was used as a measure of the lead(II) precipitation, and the metabolic activity were used as a measure of the active biomass (biocatalyst) present in the system. Without glucose, it was observed that 61.74±1.91 % and 80.74±2.53 % of lead(II) was removed within 2 d under aerobic and anaerobic conditions respectively. Whereas with 33 g/L glucose, the lead(II) removal was 42.91±0.35 % and 39.38±0.90 % within 9 d under aerobic and anaerobic conditions respectively. Biological activity was promoted under aerobic conditions in the presence of glucose with an almost tenfold difference in metabolic activity, however this activity did not translate into a significant improvement in lead(II) removal. The results indicate a dissimilatory lead(II) reduction mechanism in which lead(II) is used as an electron acceptor and consequently led to anaerobic respiration. The presence of glucose introduced a fermentation mechanism which led to a marginal decrease in pH of the system, under anaerobic conditions. The fermentation mechanism provided an alternative energy producing metabolism preventing dissimilatory reduction as an energy generating process leading to an absence in lead-precipitation.

1. Introduction

Lead is an extremely toxic heavy metal, it is recognized as one of the most nefarious chemicals that has caused serious health and environmental problems around the world; small children are the most vulnerable with the effects of Pb poisoning evident even at relatively low concentrations (Mathee et al., 2013).
The global production of new Pb for 2018 was estimated at 4.4 Mt/y, while Pb reserves are estimated at 8.3 Mt, i.e. less than 19 year's supply (Bernhardt and Reilly, 2019). Lead is most commonly found in the form of Galena, also known as lead sulfide (PbS) (Zhang et al., 2015). When exposed to air, lead oxidizes quickly, forming a dull grey coating, which is known to be a mixture of elemental lead and lead monoxide (PbO) (Merian, 1981). Lead's popular use is largely due to its low melting point and excellent corrosion resistance. A prominent use of lead is for rechargeable batteries. Other uses include antiknock agents in gasoline and for soldering, wire and cable insulation, jacketing and radiation shields (Zhang et al., 2015).
There are several sources of lead pollution, but one of the major contributors are the emission of lead from cars using lead-containing fuel as well as the mining of lead ores (Brinkmann, 1994). Other sources include that of paint, mining operations, smelting and industrial emissions, drinking water from lead pipes, and soldering from food cans (Harrison and Smith, 2007). Studies have shown that a lead concentration of more than 100 mg/L in a person's blood can lead to decreased cognitive functions and an increase in behavioral problems. Acute exposure to elevated concentrations of lead can lead to coma, convulsions, and even death (Vallero, 2014).

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The maximum amount of allowable lead in drinking water is 0.01 ppm and the maximum allowable amount to sustain aquatic life is about 0.0058 ppm (Duruibe et al., 2007). The bioremediation and biorecovery of lead from polluted wastewater could provide a feasible mechanism to achieve economic removal of lead from the biosphere.

The lead removal capabilities of two consortia using batch fermenters were investigated in a study done in 2017 by the authors of this investigation (Brink et al., 2017a). The consortia were obtained from lead-contaminated soil at the northern shaft of a lead mine and a borehole at an automotive battery recycling plant. In the 2017 study batch experiments were conducted under anaerobic conditions at 32 °C using a rich growth medium (Luria Bertani Broth) with two different lead concentrations namely 80 ppm and 160 ppm, in the presence and absence of glucose. The study reported removal of lead from the aqueous phase of between 91 % and 93 % of the 80 ppm Pb(II) in 7 d, compared to removals of between 63 % and 76 % in 10 d for the 160 ppm initial Pb(II) concentration. In both cases, a dark grey precipitate formed of unknown identity. The batch reactors containing glucose concentrations of 60 g/L only removed between 5 % and 30 % of 80 ppm Pb over a 9 d period, while no significant precipitation was observed. It was concluded that the results for both consortia displayed similarities, indicating that analogous strains of micro-organisms were present and active under these specific conditions.

Another study by the authors on the effectiveness of an industrially obtained consortium at precipitating aqueous Pb(II) under aerobic and anaerobic batch conditions were performed in 2017 (Brink et al., 2017b). The same consortium obtained from a borehole at an automotive battery recycling plant (Brink et al., 2017a) were used. The experiments were again done using a rich growth medium (Luria Bertani Broth), spiked with Pb(II) concentrations of 80 ppm. In this study it was found that the precipitation of Pb(II) was encouraged by anaerobic conditions; lead concentrations were lowered by between 91 % and 93 % within 7 d under anaerobic conditions, while nearly constant biomass growth was observed. Aerobic conditions limited the lead precipitation to between 8 % and 60 % over a 9 d period. The biomass growth increased by between 6 and 7.5 fold. It was concluded that the anaerobic precipitation mechanism involved an anaerobic respiration mechanism in which Pb(II) acted as an oxidizing agent, in turn being reduced to elemental Pb. This hypothesis was supported by the production of a dark precipitate under anaerobic conditions as opposed to a light precipitate under aerobic conditions. It is stated that the slow decline of Pb(II) concentrations in the aerobic runs were likely due to sorption of the lead on to the biomass. From both the aforementioned studies it is illustrated that the consortia previously investigated did not perform well in the presence of glucose.

Due to the cost and complexity involved in the industrial application of anaerobic conditions using a complex media (LB-broth), the current study proposed to investigate a second consortium, in this case obtained from the southern shaft of an operation lead mine. This investigation focused on the removal of lead aerobically and anaerobically, in the presence and absence glucose.

2. Materials and methods

2.1 Materials

Batch reactors were set up with the use of 100 mL serum bottles for the anaerobic batch reactors and 250 mL Erlenmeyer flasks for the aerobic runs. Both types of reactors contained exactly 100 mL sample each. A rich growth media, standard Miller Luria Bertani Broth (LB broth) (Sigma Aldrich, St Louis, MO) was used as directed. For the runs that contained glucose, a concentration of 33 g/L was used (D[+]Glucose, Glassworld, Johannesburg). The lead stock solution was prepared using $Pb(NO_3)_2$ (Merck, Kennelworth, NJ).

2.2 Microbial culture

The lead resistant consortium was obtained from the southern shaft of an operational lead mine in the North-Cape, South Africa. The inoculum was prepared by adding 1 g of lead-contaminated soil to a mixture of sterile LB (Luria Bertani) broth and 80 ppm Pb in a 100 mL serum bottle, incubating it for 24 h at 32 °C and at 120 rpm under anaerobic conditions. Glycerol was added to a final ratio of 20 % v/v and 1 mL samples were stored cryogenically at −77 °C.

A preculture was prepared from the cryogenically stored stock cultures. The preculture, which was used in all the experiments was prepared by adding one loop of the stock culture to a mixture of LB broth and 80 ppm Pb in a 100 mL serum bottle. The serum bottle was purged with nitrogen gas for 3 min and sealed with a rubber stopper and clamp to ensure anaerobic conditions, then incubated at 32 °C and 120 rpm for three days before inoculation of the anaerobic and aerobic experiments.

2.3 Experimental

The lead stock solution and LB broth growth media with or without glucose were prepared and then autoclaved separately. When cooled to room temperature, the Pb stock solution was added to the growth media in a

biological safety cabinet, in the presence of an open flame to ensure sterile conditions. The serum bottles and flasks were then inoculated with a loop from the prepared preculture. The anaerobic batches were purged with N_2 gas for 3 min, sealed with a rubber stopper and clamped with a metal cap to ensure anaerobic conditions. The aerobic Erlenmeyer flasks were plugged with sterile cotton wool to ensure movement of air. The batch reactors were placed in a shaker at 120 rpm and 32 °C for a period of 9 d. To assess and elucidate the effects of aeration conditions and presence of glucose on the Pb(II) reducing capabilities of the consortium, the study focused on the lead removal and metabolic activity of the consortium in the presence of 33 g/L of glucose. The Pb(II) reducing capabilities without glucose were measured to provide a baseline comparison, therefore these experiments were conducted for two days and only the residual Pb(II) was measured. The experiments were performed in triplicate to ensure repeatability.
Anaerobic and aerobic controls were set up in parallel to the batch reactors to investigate the abiotic influences on the batch reactor performances. The controls were prepared identically to the batch reactors, with the exception that no inoculation was performed.

2.4 Sampling

Samples were taken from time zero under sterile conditions for 9 d. The batch reactors were shaken well before sampling. A hypodermic needle and sterile syringe were used to pierce the rubber stopper and remove sample of the anaerobic reactors. The needle and syringe were only used for extraction in the aerobic reactors, after removing the cotton wool, simultaneously working in the vicinity of an open flame to ensure a sterile environment for both cases. Three 1 mL samples were extracted from each reactor and stored in sterile cryogenic vails. One of the 1 mL samples, which was used for metal content analysis, were centrifuged for 10 min at 9,000 rpm and 20 °C. The supernatant was decanted from the solid precipitate (pellet) and stored in another cryogenic vail. The remaining two 1 mL samples were used for the optical density at 600 nm and metabolic activity analysis at 550 nm. One of the two samples were filtered with 25 mm nylon syringe filters with 0.45 µm pores (Anatech) representing the samples without biomass and the other was used as biomass samples.

2.5 Analysis

The metabolic activity measurements were all performed immediately after sampling. All the dilutions made, were considered when calculating final data. The metabolic activity of bacterial cells was quantified using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (Wang et al., 2010). MTT is a water-soluble yellow dye that can be reduced to water-insoluble purple formazan crystals by the dehydrogenase system of active cells. The formazan concentration can be quantified spectrophotometrically after dissolution in an organic solvent dimethyl sulfoxide (DMSO). It is assumed that the formazan concentration is directly related to the number of metabolically active cells in the sample. The MTT solution contained 5 g/L MTT in ultra-purified water, which was filtered with sterile filters and stored at -40 ºC. Two sets of analyses were performed, one with biomass and the other without. One of the two 1 mL samples were filtered with 25 mm nylon syringe filters with 0.45 µm pores (Anatech) before analysis was done on it, to represent the sample without biomass. The analysis was done by diluting the 1 mL sample (with or without biomass) to a total of 4 mL with ultra-purified water. The MTT solution (0.2 mL) was then added to 1.8 mL of the diluted sample and incubated for one hour at 35 °C. The samples were then dissolved in 2 ml DMSO after incubation. The absorbance at 550 nm was measured and recorded as an indication of metabolic activity.
The residual aqueous Pb(II) was measured using an atomic absorption spectrometer (Perkin Elmer AAnalyst 400, Waltham, Massachusetts), with a Pb Lumina hollow cathode lamp. The supernatants of the 1 mL samples stored at -70 °C were used, as to avoid subjecting the AA to any solids and so avoiding any blockages.
The pH measurements were taken before sterilization and at the end of the experiment with a HQ11d Portable pH/ORP Meter (Hach®, Loveland, Colorado).

3. Results and discussion

The control experiments were terminated after 72 h, showing the microbial culture to be the enabler of any Pb-removal. The results from the control experiment are visually and quantitively presented in Figure 1a and Table 1 below, respectively. The visual results over a 9 d period, for both the anaerobic and aerobic experiments that contained 33 g/L glucose are presented in Figure 1b. There was an initial change in colour observed in the anaerobic batch reactors within the first 24 h, followed by limited observed changes thereafter. No precipitation was observed in the anaerobic batch reactors. A similar change as observed in the anaerobic batch reactors was observed in the aerobic reactors within the first day. The colour changed to milky yellow and no precipitate was observed. A dark precipitate was however produced between 4 d and 7 d in the aerobic reactors, and the overall colour changed to dark grey-brown.

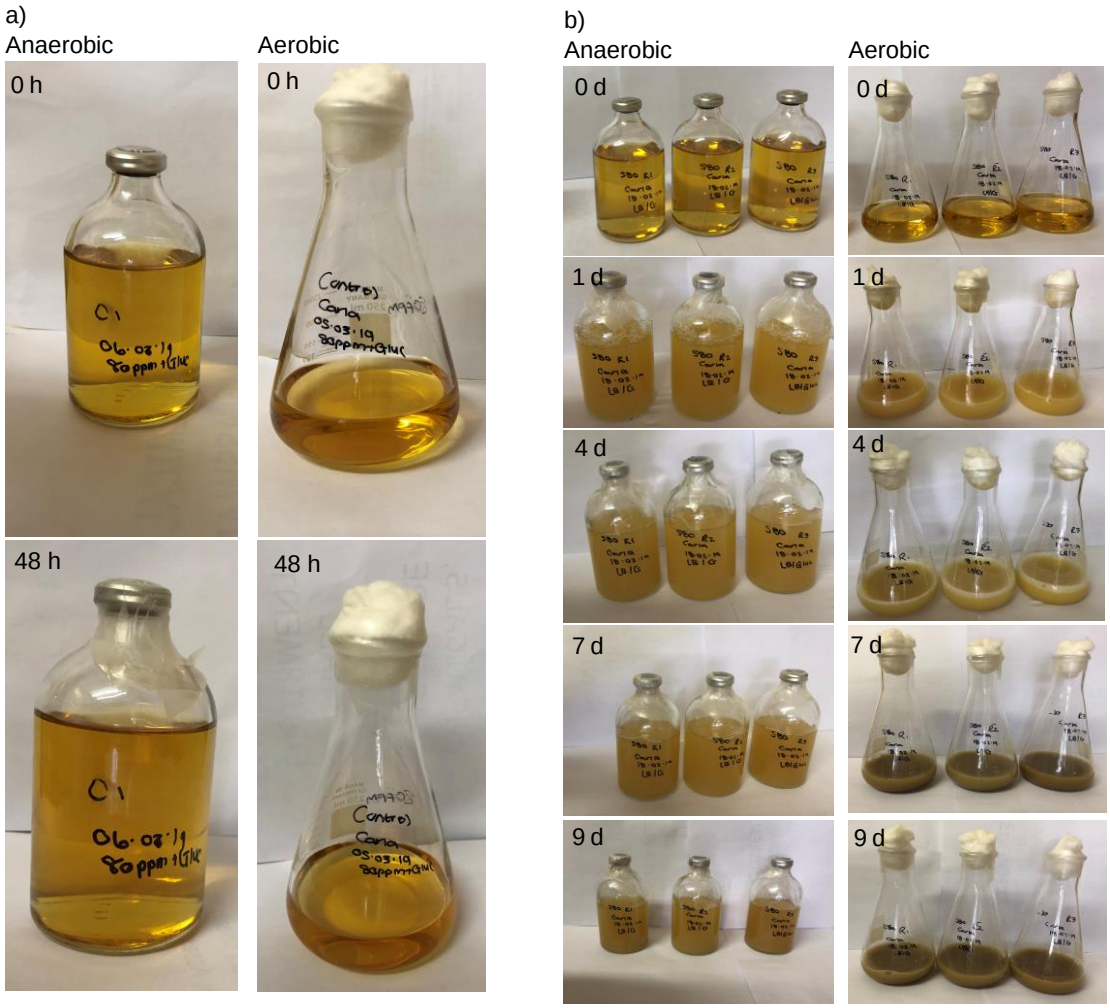

Figure 1: a) Control experiments with 33 g/L glucose. b) Anaerobic and aerobic batch reactor changes over the period of 9 d with 33 g/L glucose.

Table 1: Control experiment Pb(II) readings with 33 g/L glucose.

Type	Initial Pb(II) ppm	Final Pb(II) ppm
Anaerobic control	85	86.54
Aerobic control	91.80	96

The residual Pb(II) concentrations in the anaerobic and aerobic batch reactors are shown in Figures 2a and b, respectively. The experiments were run in triplicate and the standard deviations are shown on each figure as error bars. For the reactors that contained glucose, a dramatic decrease in the anaerobic Pb(II) concentration was observed in the first 24 h, which is consistent with the visual observations presented in Figure 1b. The Pb(II) concentrations remained constant from 2 d onwards for the rest of the duration of the experiment; a total of 39.38±0.90 % of the Pb(II) was removed in the anaerobic batch reactors within two days. The Pb(II) concentrations for the aerobic batch reactors are presented in Figure 2b. A substantial drop in Pb(II) was observed in the first 24 h, as in the anaerobic runs. The reduction in Pb(II) concentration was again consistent with the colour change observed. The Pb(II) concentration elevated slightly between 1 d and 3 d, this could be a result of surface biosorption in the first 24 h followed by bioprecipitation (Naik and Dubey, 2013) of the adsorbed Pb. The late onset of bioprecipitation may explain the presence of a dark precipitate only at the end of the experiment (7 d). The change in colour is also consistent with Pb(II) removal from 4 d to 5 d. From 7 d to 9 d it was observed that 42.91±0.35 % of the Pb(II) had been removed. The amount of Pb(II) removed in both anaerobic and aerobic runs without glucose was considerably higher and faster compared to the runs containing glucose, also seen in Figures 2a and b below.

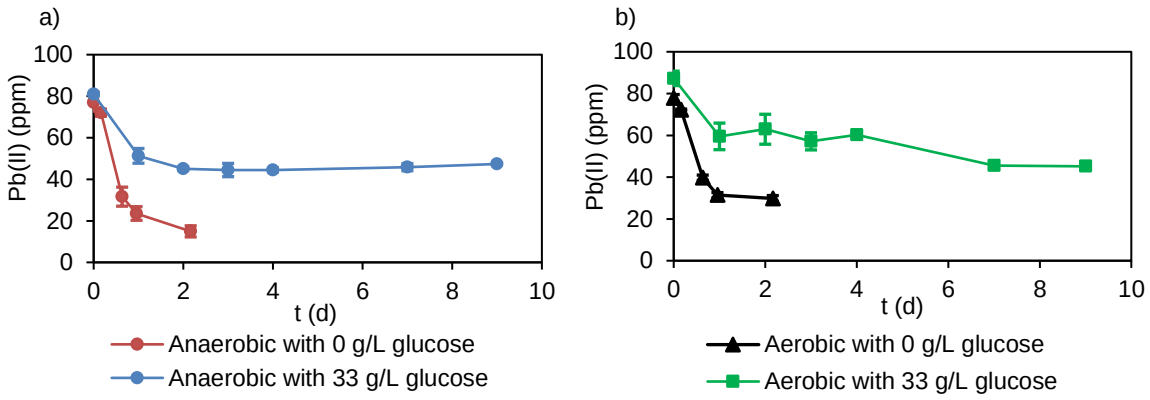

Figure 2: Residual aqueous Pb concentration in the a) anaerobic and b) aerobic batch reactors with 33 g/L glucose and without glucose. The data points indicate the average metabolic activity and the error bars the standard deviations of the triplicate experiments.

The metabolic activity for the anaerobic and aerobic batch reactors with glucose is shown below in Figure 3a and b. A spike in the metabolic activity (MA) was observed for the anaerobic runs on 1 d (Figure 3a), which was consistent with the drop in Pb(II) measurements and visual changes. The metabolic activity (MA) drops after 1 d and stabilizes from 2 d to a constant measurement of about 2.5 absorbance units. The stability in MA measurements can be reflected in the constant colour observed in the batch reactors. For the aerobic runs, a steady increase in MA measurements is observed from the start of the experiment up until 2 d, from which a brief plateau is reached from 2 d to 3 d, followed by a steady increase up to 7 d. The plateau corresponds with a small increase in Pb(II) (Figure 2b) concentration, possibly due to desorption after an initial biosorption. The slightly higher Pb(II) concentration may have had an inhibitory effect on the MA of the microbes. The plateau is followed by a steady increase in MA and so more Pb(II) removal took place and the formation of a dark precipitate. Metabolic activity greatly decreases from 7 d and a constant Pb(II) value is attained until the termination of the experiment. The aerobic metabolic activity readings were found to be considerably higher with a maximum of 250.16±45.88 absorbance units in comparison to anaerobic with a maximum of 2.48±0.11 absorbance units.

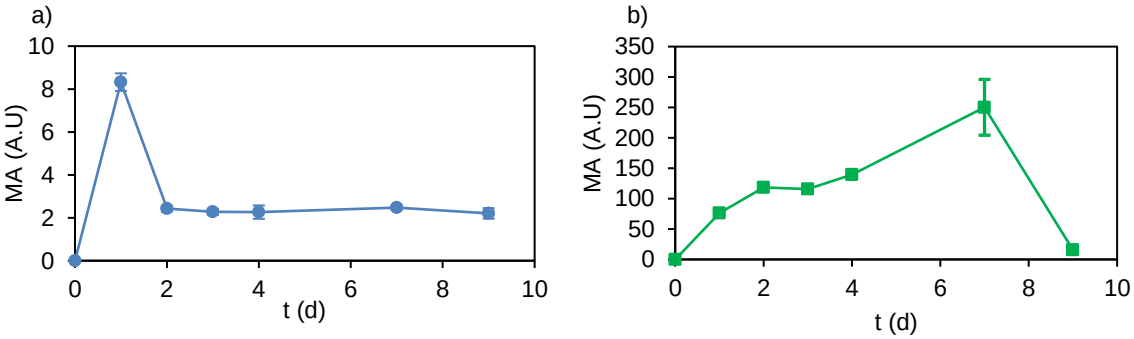

Figure 3: MA readings for the a) anaerobic and b) aerobic batch reactors with 33 g/L glucose. The data points indicate the average metabolic activity and the error bars the standard deviations of the triplicate experiments.

The initial pH measurement of the mixture of LB broth and glucose was found to be 6.67. The pH dropped significantly in the batches under anaerobic conditions, with a final pH of 4.96±0.03 measured, in contrast the pH in the aerobic batches increased from 6.67 to 8.96±0.04.

The results presented in the study indicated that bioprecipitation, facilitated by the industrial consortium, occurs under aerobic conditions in the presence of glucose. This contrasts with the observation that only biosorption was responsible for Pb(II) removal under anaerobic conditions in the presence of glucose. The drop in pH of the anaerobic runs indicated a fermentative mechanism which produces organic acids in the presence of glucose. The significant increase in metabolic activity in the aerobic runs containing glucose indicate a respiratory mechanism present which is known to provide a very large amount of ATP required for metabolic activity. The

observed dark-brown precipitate in the aerobic glucose containing run shows that an anaerobic respiration mechanism involving Pb(II)-bioreduction is present in the consortium during aerobic runs with glucose. However, the population fraction of the consortium involved in the anaerobic respiration is outcompeted for resources by the aerobic population fraction and consequently the precipitation of Pb(II) is delayed.

4. Conclusions

The results from the study demonstrate the potential for using the industrially obtained microbial consortia for the biological removal and recovery of Pb(II) from aqueous solution. Final Pb(II) removals of 42.91±0.35 % and 39.38±0.90 % within 9 d for the aerobic and anaerobic runs in the presence of glucose were measured,. This is significant as it shows that both aeration conditions remove roughly the same amount of Pb(II), but at different rates and by different mechanisms. However, the runs in the absence of glucose exhibited the best performance in terms of Pb(II) removal with 61.74±1.91 % and 80.74±2.53 % for aerobic and anaerobic conditions measured within 2 d.

The study shows that optimal dissimilatory reduction of Pb(II) requires anaerobic conditions in the absence of glucose due to competitive energy metabolisms present in the consortium. The study further highlights the requirement for more research on different anaerobic reactors configurations and economically viable substrates before industrial application of the consortium for lead bioremediation and biorecovery will be realised.

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