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Microbial Lead(II) Precipitation: the Influence of Growth Substrate

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The objective of the study was to explore the influence of various growth substrates on the removal of aqueous lead from solution. The fermentation media tested consisted of glucose- and xylose-supplemented LB-broth, with and without $CaCO_3$ as pH buffer. In addition, combinations of the various constituents of LB-broth, i.e. yeast extract, tryptone, and NaCl, were tested.
Results from the sugar-supplement runs showed that significant removal of lead, without observable precipitation, was measured both with and without pH buffering (75–90% in 48–72 hours). Significant gas build-up in all runs, and a drop in pH in the unbufferred runs, indicate an anaerobic digestion mechanism with either internal or external sequestration of lead.
The LB-broth constituent experiments indicate that even though the commercial growth medium LB-broth exhibited the best performance in terms of lead(II) removal (89%), yeast extract-NaCl complex medium performed nearly as well (80%). Results show that substituting the tryptone with corn steep liquor provided minimal gains in lead removal performance, compared to the yeast extract-NaCl medium. The aqueous lead removed by the yeast extract-only medium was limited to 56% in 144 hours. Dark grey precipitates and pH values greater than 5 indicate that the lead(II) were reduced to elemental lead. The observations also suggest that the growth factors in the LB broth, required by the consortium, can be largely replaced by the yeast extract-NaCl medium.

1. Introduction

Lead (Pb) is used ubiquitously in industry; applications include battery plates, electrical cable sheathing, radiation shielding, ammunition, and solder. The current rate of global Pb mining (5 million tonnes per annum) and the estimated global raw Pb reserves (84 million tonnes) imply depletion of Pb reserves in approximately 17 years (www.statista.com). The presence of Pb in the environment is mostly attributed to anthropogenic activities such as processing of silver, platinum, and iron; Pb-containing paints; combustion of leaded petroleum products (Gioia et al., 2006); and Pb-containing coal combustion products (Block and Dams, 1975). It is estimated that environmental Pb has increased 1000-fold over the past 300 years due to industrial activities and the propensity of Pb to bioaccumulate (Naik & Dubey, 2013). This, in addition to the toxic impact on vulnerable populations, specifically children (Taylor et al., 2013) and marginalised communities (Barbosa et al., 2009), has generated concern.
Traditional methods for heavy metal (Cd, Pb, Fe, Mn, Cr, and As) remediation include sand filtration, GAC adsorption, precipitation sedimentation, flotation, ion-exchange, and electrochemical deposition (Aziz et al., 2008). Adsorption methods lack sensitivity to specific metals, while competitive inhibition by cations, such as Na^+, Ca^{2+}, K^+, and Mg^{2+}, severely reduces the effectivity of the methods (Ngwenya and Chirwa, 2010). Precipitation methods tend to require pH and redox conditions outside of the natural environments, while overlapping precipitation ranges produce toxic sludges with mixed compositions (Aziz et al., 2008). Ion-exchange methods have a tendency to foul in concentrated solutions, while being indiscriminate and highly sensitive to the pH of the solution (Barbaro and Liguori, 2009).Bioremediation of Pb has been limited to biosorption (Chatterjee et al. 2012), precipitation as less mobile Pb species (Teekayuttasakul and

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Annachhatre, 2008), intracellular immobilization, and microbially produced exopolysaccharide sequestration (Naik and Dubey, 2013).

Although the reduction of metal species to lower oxidation states is thermodynamically favourable, the activation energy requirements tend to inhibit the spontaneous reduction in natural environments. The biological catalysis of metal reduction has been demonstrated for various 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 transformations have been shown to result from either a defensive mechanism due to the toxicity of the metal species (Kessi et al., 1999) or co-metabolism of the metal species as an electron acceptor in the energy metabolism (Wade and DiChristina, 2000).

The ongoing research project aims to identify a Pb remediation and recovery method possessing industrial potential. Previous work by this research group demonstrated significant anaerobic Pb(II) precipitation by an industrial consortium using the commercial microbial medium, Luria Bertani (LB) Broth (Brink et al. 2017a). In contrast it was found that glucose supplementation inhibits Pb(II) precipitation (Brink et al. 2017b). It was hypothesized that the identity of the precipitate is elemental Pb, due to the pH conditions and the colour of the precipitate. It is known that elemental Pb is stable in water at pH>5 and possesses a dark grey colour (Brink et al. 2017b). The objective of the current study was to determine the influence of complex growth substrates, with and without the addition of different concentrations of sugar substrates (glucose and xylose), on the ability of a local industrially obtained consortium to remove Pb(II) from solution. The experiments were performed under anaerobic batch conditions for 144 hours with an initial background Pb(II) concentration of 80 mg/L.

2. Materials and Methods

2.1 Pb resistant consortium screening

A Pb resistant consortium was obtained from a borehole at an automotive-battery recycling plant in Gauteng, South Africa, using the method described previously in Brink et al. (2017b). No attempt was made to purify or identify the consortium cultures; this will form part of a future project.

2.2 Preparation of microbial cultures

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 inoculated in a 100 mL solution of LB broth and 80 mg/L Pb. To ensure the cultures were anaerobically grown for 24 hours at 32 °C, the bottles were initially purged with nitrogen for 3 minutes prior to sealing with a rubber stopper.

2.3 Growth substrate preparation

The respective growth substrates investigated are summarized in Tables 1 and 2. The glucose, xylose, $CaCO_3$, yeast extract, tryptone, and NaCl were obtained from Merck KgaA (Darmstadt, Germany); the LB-broth and the corn steep liquor (CSL) were obtained from Sigma-Aldrich (St. Louis, MO). The CSL was clarified using the method described by Bradfield and Nicol (2014).

Table 1: Conditions in the sugar-supplemented experimental runs

Experiment number	Glucose (g/L)	Xylose (g/L)	$CaCO_3$	LB-broth (g/L)
1 (a)-(e)	0, 20, 40, 60, 80	0	0	25
2 (a)-(e)	0	0, 10, 20, 30, 40	0	25
3 (a)-(e)	0, 10, 20, 30, 40	0	1.6 g/g glucose	25
4 (a)-(e)	0	0, 10, 20, 30, 40	1.6 g/g xylose	25

Table 2: Experimental conditions in the LB-broth constituent experimental runs

Experiment number	Yeast extract (g/L)	Tryptone (g/L)	NaCl (g/L)	Clarified CSL (g/L)
5	5	10	10	0
6	5	0	10	0
7	5	0	0	0
8	0	10	10	0
9	5	0	10	10

2.4 Experiments

All experiments were performed under anaerobic mesophilic (32 °C) batch conditions. Due to excessive gas build-up and subsequent "popping" of the rubber stoppers (i.e. contamination of the reactors) experiment 1 and 2 could only be performed for 72 hours and experiment 3 and 4 for 48 hours each.
All other experiments, with the exception of experiment 8, were performed for 144 hours each. Samples were taken 24 hours apart. The pH of each batch reactor was taken at the termination of the respective experiments. Experiment 8 was terminated after 48 hours due to a lack of microbial growth in the tryptone-NaCl medium.

2.5 Sampling and analysis

The biological reactors were sampled, the dissolved Pb concentrations were quantified, and the pH conditions were measured using the methods as described previously in Brink et al. (2017b).

3. Results and Discussion

3.1 Glucose/Xylose-supplemented experiments

The results from the glucose and xylose experiments without pH buffering (experiments 1 and 2 in Table 1) are shown in Figures 1 and 2. Evident are significant reductions in aqueous Pb concentrations and decreasing trends in the overall Pb removal with increasing sugar concentration (Figures 1 and 2). No precipitate was observed in any of the sugar-supplemented runs, as compared to the control run without any sugar supplementation. Figure 3 clearly shows that the reactor without added sugar (specifically glucose for Figure 3) exhibited a dark grey precipitate (far left), while the vials containing sugar had significant biological growth, but no observable precipitate formation (four vials to the right in Figure 3). The observed results from Figure 3 correspond well with observations presented previously (Brink et al. 2017b). The xylose experiments had similar observations for the results in the absence and presence of xylose. All the sugar supplemented runs were accompanied by a significant production of gas, which caused the rubber stoppers to pop open leading to premature termination of the experiments.

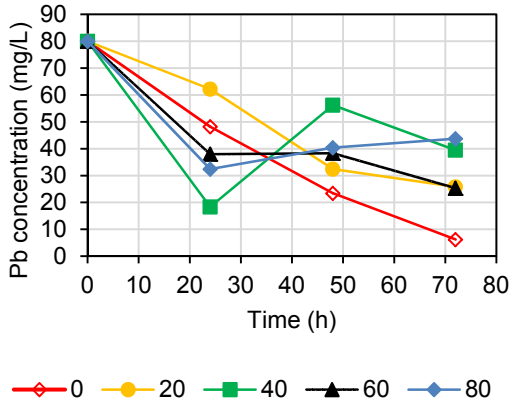

Figure 1: Measured Pb-concentrations vs. time in the glucose-supplemented runs. The legend denotes feed glucose concentrations in g/L

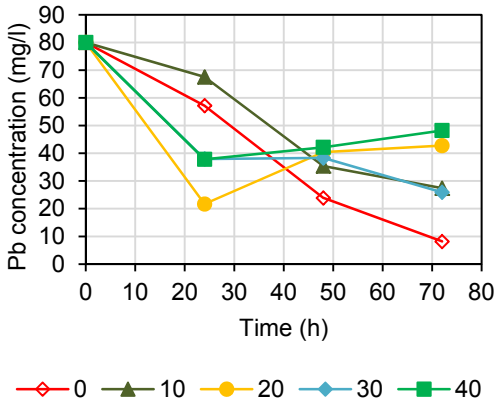

Figure 2: Measured Pb-concentrations vs. time in the xylose-supplemented runs. The legend denotes initial xylose concentrations in g/L

The measured pH values for experiments 1 and 2 are shown in Figure 4 and indicate that the pH values for the sugar-supplemented runs were significantly lower than required for the precipitation of Pb. The values were all below 5 (with the exception of 10 g/L Xylose with a pH of 5.29). The observed and measured results from these experiments indicate the presence of a Pb-resistant population, which has the ability to produce a gas with corresponding reduction in pH. This is likely a result of anaerobic digestion of the sugar substrate, which follows the route: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Batstone et al, 2002). The anaerobic digestion of the sugar substrate would lead to the production of acids (acidogenesis, acetogenisis) and subsequent production of methane (methanogenesis).
In response to the observed pH decreases during experiments 1 and 2, it was decided to buffer the pH of the media using $CaCO_3$. The amount of $CaCO_3$ required to buffer the solutions was calculated as the HCO_3^-

required to neutralise the system should all the sugar be converted to acetic acid (i.e. approximately 1.6 g $CaCO_3$ per g sugar). The aqueous Pb measurements for the glucose- and xylose-supplemented runs with $CaCO_3$ buffers are shown in Figures 6 and 7 respectively. The results indicated that significant reduction in Pb concentrations were observed after only 48 hours of fermentation (75% – 97% removal). No dark precipitate was observed for any of the buffered sugar-supplemented runs (Figure 5), even though the pH was within the acceptable range for elemental Pb precipitation (Figure 4).

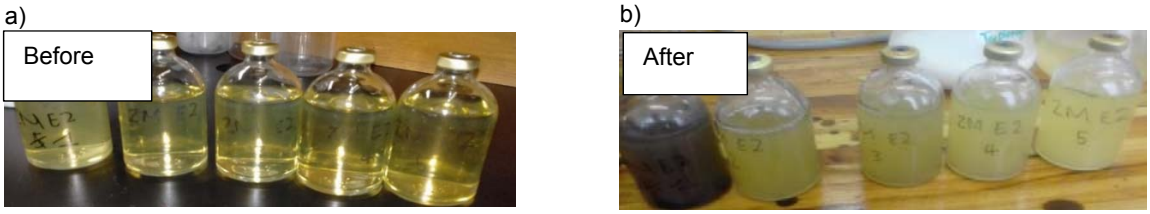

Figure 3: The observed vials in the glucose-supplemented runs: a) before the experiment and b) after 48 hours. The initial glucose concentrations increase from left to right (0 – 80 g/L).

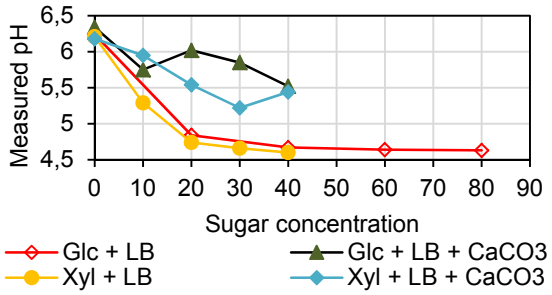

Figure 4: The pH-measurements in the respective glucose (Glc) and xylose (Xyl) supplemented runs vs. initial sugar concentration for the unbuffered (●,◊) and buffered (◆,▲) runs.

Figure 5: The observed experimental vials after 48 hours for the $CaCO_3$ buffered glucose-supplemented runs.

It is known that methanogenesis is inhibited by a low pH (Batstone et al, 2002) which could explain the significant gas build-up, with correspondingly shorter runs, in the pH buffered runs as compared to the unbuffered runs. From the results for both the buffered and unbuffered sugar-supplemented experiments, it appears that the Pb-reducing fraction of the consortium is inhibited to the extent that no observed Pb(II) to elemental Pb reduction was observed. It was verified that the consortia was able to reduce Pb by a control run in each experiment where no sugar was added to the media. These runs exhibited results similar to those observed previously (Brink et al. 2017 a,b), with between 75% removal (48 hours) and 90% removal (72 hours), a pH above 6 (Figure 4), and a dark precipitate (Figure 3).

3.2 LB-broth constituent experiments

The results from the experiments in which the LB-broth constituents were tested (experiments 5–7, 9) are shown in Figure 8. This figure does not include any results from experiment 8 due to the absence of microbial growth in the tryptone-NaCl medium, as shown in Figure 10 d and e. Figure 10 a–c show a representative of the observed results for all the runs that involved yeast extract (experiments 5–7, 9). These figures demonstrate the presence of a dark precipitate forming in the bottom of the vial, while the measured pH values (Figure 9) support the theory that the identity of the precipitate is elemental Pb; all pH values measured were greater than 5.8.

The results for the LB-broth run show a similar trend as that of the control runs (0 g/L sugar) in Figures 1,2,6 and 7; the majority of the Pb-precipitation takes place in the first 48 to 72 hours with a final Pb removal of almost 90% after 144 hours. The runs performed with the different constituents of LB-broth all show a marked drop in Pb present in the aqueous phase.The yeast extract-NaCl run showed fluctuating behavior, removing an amount of Pb after 144 hours (80% removal) comparable to that removed by the commercial LB-broth. The medium in which tryptone was replaced with CSL performed similarly to the yeast extract-NaCl medium; the

overall Pb removal after 144 hours was the same for both media (80% removal). This indicates negligible contribution by the CSL to the overall performance of the medium. The results finally show that the yeast extract-only medium performed markedly worse than the other media (56% removal) and could indicate a requirement for NaCl as supplementary nutrient.

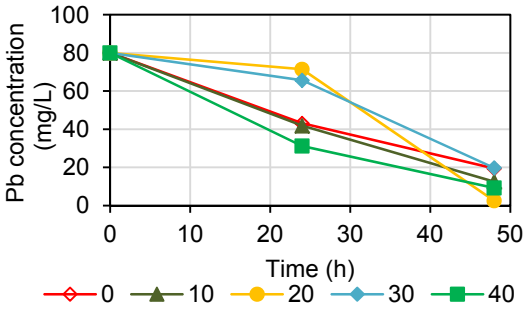

Figure 6: The Pb-concentrations measured in the glucose-supplemented pH-buffered experiments. The legend denotes feed glucose concentrations in g/L

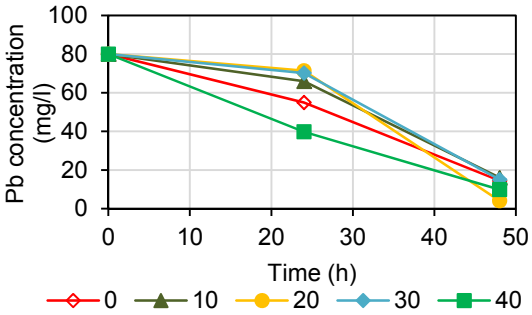

Figure 7: The Pb-concentrations measured in the xylose-supplemented pH-buffered experiments. The legend denotes feed xylose concentrations in g/L

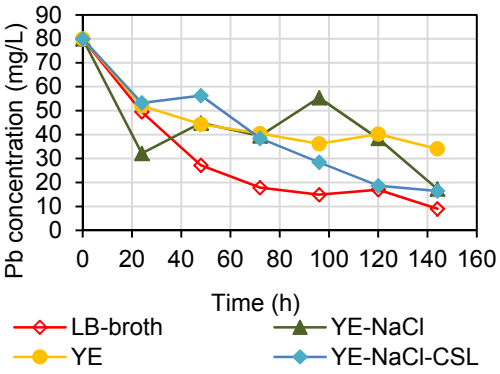

Figure 8: The Pb-concentrations measured in the LB-broth constituent experiments

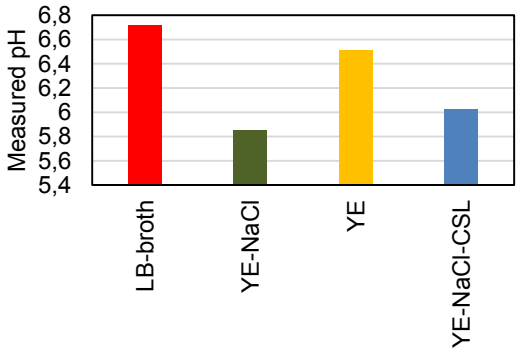

Figure 9:The final pH-values measured in the LB-broth constituent experiments

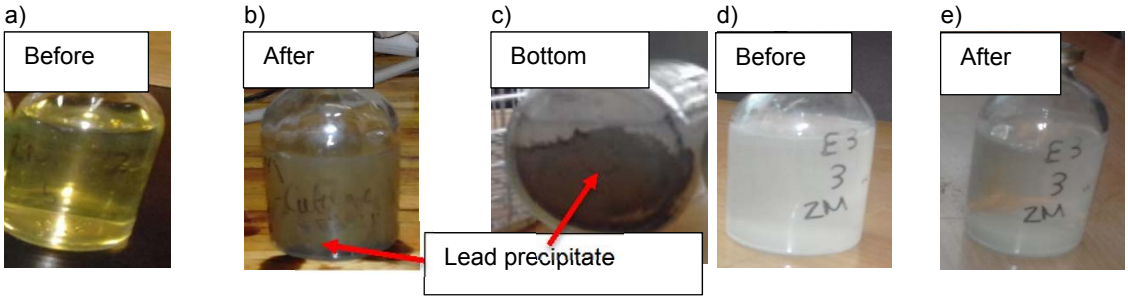

Figure 10: The observed experimental vials in the LB-broth constituent experiments. a) The LB-broth prior to experimentation, b) and c) the LB-broth after 72 hours with dark precipitate indicated. d) and e) The Tryptone-NaCl run before inoculation and after 48 hours indicating no appreciable biological activity.

4. Conclusions

The study showed that any glucose- and xylose-supplementation of the fermentation medium allows the removal of Pb from solution (up to 75–90% in 48–72 hours), while inhibiting the reduction of Pb(II) to elemental Pb. This is possibly due to the promotion of an anaerobic digestion population in the consortium, in preference to the Pb-reducing fraction of the consortium. The mechanism of Pb removal is likely intracellular- or extracellular sequestration. Further, it was found that while LB-broth performed the best (89% Pb removal),

the main constituent in LB-broth required by the Pb-reducing population is the yeast extract, with NaCl as supplementary nutrient (80% removal). Yeast extract alone only removed 56% Pb. It was also shown that clarified CSL used to replace the tryptone in LB-broth does not improve the final removal of Pb when compared to the yeast extract-NaCl medium (80% removal).

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