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Microbial lead(II) Precipitation: The Influence of Aqueous Zn(II) and Cu(II)

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The current study focused on the impact of heavy metals Zn(II) and Cu(II), regularly found in conjunction with lead in industrially polluted areas, on the Pb(II) bioprecipitation capabilities and metabolic activity of an industrially isolated microbial consortium. The experiments were performed with Pb(II) concentrations of 80 ppm and varying concentrations of Zn(II) (40 ppm and 80 ppm) and Cu(II) (40 ppm, 100 ppm, and 150 ppm). Different conditions were tested namely; Pb(II)&Zn(II), Pb(II)&Cu(II), Zn(II) only, and Cu(II) only. The experiments were run for a period of 7 days, where the residual aqueous Pb(II), Zn(II), and Cu(II) measured the degree of removal of each metal. The growth activities for each combination of metals were quantified using CFU plate count analysis by plating the final sample of the 40 ppm Zn(II) and Cu(II) runs (including and excluding Pb(II)) on agar plates spiked with a constant 80 ppm Pb(II). The results were compared to previous experiments conducted with samples containing only 80ppm Pb(II).
Pb(II) concentrations decreased by 69% and 25% in the presence of 40 ppm and 80 ppm Zn(II), respectively. A grey precipitate was only observed in the presence of 40 ppm Zn, with no precipitate observed with 80 ppm Zn(II). Additionally, a limited attenuation in the Zn(II) concentrations of 12 % and 7% were measured for the 40 ppm Zn(II)&Pb(II) and 80 ppm Zn(II)&Pb(II) runs respectively. The results suggest different removal mechanisms present in the 40 ppm and 80 ppm Zn(II) runs, with a precipitation mechanism at 40 ppm Zn and a biosorption mechanism at 80 ppm of Zn(II).
Pb(II) concentrations decreased by 0%, 32%, and 26% for the 40 ppm, 100 ppm, and 150 ppm Cu(II)&Pb(II) runs respectively. The corresponding Cu(II) concentrations decreased by 50%, 63%, and 71% respectively, indicating a competitive removal mechanism with no observed production of coloured precipitate, such as biosorption. The Cu(II) only runs exhibited removal percentages of 64%, 63%, and 53% for the 40 ppm, 100 ppm, and 150 ppm runs. During growth activity analysis it was observed that the samples containing Pb(II)&Zn(II) and Pb(II)&Cu(II) showed significantly less growth than that of the Pb(II) only plates previously tested at $5.47\pm0.83 \times 10^8$ CFU/mL, compared to $1.131\pm0.065 \times 10^7$ CFU/mL and $5.98\pm1.86 \times 10^6$ CFU/mL respectively. It can be concluded that the bioprecipitation mechanism of Pb(II) as previously observed are severely inhibited by elevated concentrations of Zn(II) and Cu(II), resulting in an adsorption mechanism dominating. Additionally, it was found that Pb(II) promotes metabolic activity while Zn(II) and Cu(II) inhibits metabolic activity. This is possibly as a result of inhibition of the Pb(II) precipitation mechanism. These results indicate that Zn(II) and Cu(II) ions need to be removed prior to bioprecipitation and recovery of Pb(II) using the specific industrial consortium.

1. Introduction

Lead is a well-documented environmental pollutant. It is an extremely toxic heavy metal found rarely in pure form in nature (Rosenberg, Silverman & Strain, 1979). Pb is considered one of the most nefarious human neurotoxins; small children are the most vulnerable to Pb exposure with the effects of Pb poisoning evident even at relatively low concentrations (Mathee et al., 2013). The amount of global lead reserves decreases daily, and it is estimated that 17 years' supply of global reserves remains (Statista, 2017). Major application of lead include rechargeable batteries, antiknock agents in gasoline, soldering wire, wire insulation, and for radiation shields. It is also used for ammunition and pigments (Zhang, Wilson, Hou & Meng, 2015).

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Lead is commonly found in ores in conjunction with zinc, copper, and silver (Snodgrass, 1986). Lead, zinc, and copper are thus commonly found in wastewater streams together and bioremediation of this water will entail an interaction of these metals. Bioremediation is considered a cost-effective alternative to existing remediation technologies for the removal and/or recovery of toxic metals from wastewaters before releasing the recovered water into natural water bodies (Mtimunye & Chirwa, 2014).

Previous studies have been published on the bioprecipitation of lead(II) especially under anaerobic conditions which were highly effective with the use of a rich growth medium LB (Luria Bertani) broth (Brink & Mahlangu, 2018). Previous work also demonstrated the potential of an industrially obtained consortium to precipitate Pb(II) ions from solution under the same conditions as the previously mentioned study, for concentrations of up to 1000 ppm Pb(II) (Peens, Wu & Brink, 2018). Previously a 90% – 95% removal of 80 ppm Pb(II) was observed in 7 days under these conditions with a final metabolic activity of $5.47 \pm 0.83 \times 10^8$ $CFU.mL^{-1}$.Currently there are no research available on the effects of other heavy metals, specifically zinc and copper on the bioprecipitation capabilities by this microbial consortium of lead(II).

The experiments were conducted using the previously isolate consortium (Peens, Wu & Brink, 2018) under anaerobic conditions in 100 mL batch reactors using commercial LB (Luria Bertani) broth. Different conditions were tested: Pb(II)&Zn(II), Pb(II)&Cu(II), Zn(II) only, and Cu(II) only. The experiments were run for a period of 7 days and the residual aqueous Pb(II), Zn(II) and Cu(II) concentrations were measured as the degree of removal of each metal. Atomic absorption spectroscopy was used to determine the remaining individual metal concentrations in solution at various time intervals. The removal capabilities were compared to results previously gathered on samples containing only 80 ppm lead (II) by a masters student from this research team (Peens, 2019). The growth activity for each of the combinations of metals was quantified using CFU plate count analysis by plating the samples taken on the last day of the 40 ppm Zn(II) and 40 ppm Cu(II) runs, in combination with and without Pb(II). All the runs were plated on agar plates spiked with a constant 80 ppm Pb(II). The CFU results found were finally compared to a previous experiment conducted in the same way as the current, but only using samples containing 80 ppm Pb(II).

2. Materials and methods

2.1 Materials

Each of the batch reactors was set up with the use of 100 mL serum bottles. A rich growth media, standard Miller Luria Bertani Broth (LB broth sourced from Sigma Aldrich, St Louis, MO) was used, with a final concentration of 25 mg/L. The lead stock solution was prepared using $Pb(NO_3)_2$, the copper with $Cu(NO_3)_2 \cdot 3H_2O$, and the zinc with $Zn(NO_3)_2 \cdot 6H_2O$.

2.2 Microbial culture preparation

A lead resistant consortium was obtained from a borehole at an automotive-battery recycling plant in Gauteng, South Africa. An inoculum was prepared by adding 1 g of Pb contaminated soil to a mixture of LB (Luria Bertani) broth and 80 ppm Pb(II) in a 100 mL serum bottle, which was then incubated for 24 hours at 32 °C under anaerobic conditions at 120 rpm. Glycerol was added to a final ratio of 20% v/v and cryogenically stored at −77 °C (Peens, Wu & Brink, 2018).

A preculture was prepared from the abovementioned 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(II) in a 100 mL serum bottle. The serum bottle was purged with nitrogen gas for 3 minutes and sealed to ensure anaerobic conditions, then incubated at 30°C and 120 rpm for three days before inoculation of the experiments took place.

2.3 Experimental

Control experiments were conducted during all the experimental runs to confirm that any precipitation and/or decrease in metal concentration was the result of biotic activity. Each of the batch reactors was set up with the use of 100 mL serum bottles. A rich growth medium, LB broth was used, with a final concentration of 25 mg/L. The various metal stock solutions and LB broth (already in serum bottles) were prepared and then autoclaved separately, after which they were left for cooling. When cooled (to about 25°C), the various metal stock solutions were added to the growth media under sterile conditions. The serum bottles were then inoculated with a loop from the prepared preculture, temporarily sealed with parafilm, purged with N_2 gas for 3 minutes, sealed with a rubber stopper and lastly clamped with a metal cap to ensure anaerobic conditions. The batch reactors were placed in a shaker at 120 rpm and 30°C for a period of 7 days.

2.4 Sampling

Samples were taken periodically from time zero under sterile conditions, taking place every day at approximately the same time for 7 days. The sealed serum bottles were shaken well before sampling. A hypodermic needle and sterile syringe were then used to pierce the rubber stopper. Two 2 mL samples were extracted from each reactor, on each time interval and stored in sterile cryogenic vials. One of the 2 mL samples, which will be used for metal content analysis, were centrifuged for 10 minutes at 9 000 rpm and 20°C. The supernatant was decanted from the solid precipitate (pellet) and stored in another cryogenic vail. The other sample was to be used for CFU plate count analysis. The vails with the samples (2 mL sample, ± 2 mL supernatant and a pellet) was stored at 5°C to await analysis.

2.5 Analysis

Two sets of analysis were conducted on the samples gathered throughout the experiment. The residual aqueous Pb(II), Zn(II), and Cu(II) measured the degree of removal of each metal, and the growth activities for each combination of metals were quantified using CFU plate count analysis by plating the final sample of the 40 ppm Zn(II) and Cu(II) runs that include and exclude Pb(II), on agar plates spiked with 80 ppm Pb(II).
Photographs were taken throughout the experiments of the batch reactors to compare changes in appearance. Growth kinetics were quantified with the use of CFU (colony forming unit) counts. The spread plate method was used to plate the final sample (day 7) of the 40 ppm Zn(II) and Cu(II) runs, which included and excluding Pb(II), on agar plates spiked with 80 ppm Pb(II). The results were compared to a previous experiment which was conducted in the same way, but with samples containing only 80 ppm Pb and taken on day 7. The CFU plates were plated on LB Agar which consists of 25 g/L LB broth and 15 g/L Agar. The plates were grown in airtight jars under anaerobic conditions with the use of anaerobic indicators (Oxoid, Thermo Scientific, Basingstoke, Hampshire) and AneroGenTM sachets (Oxoid, Thermo Scientific, Basingstoke, Hampshire). The plates were incubated at 35°C for 24 hours. The colonies were counted with the aid of image analysis software, ImageJ (Grishagin, 2015).
The residual aqueous Pb(II), Zn(II), and Cu(II) were measured using an atomic absorption spectrometer (Perkin Elmer AAnalyst 400, Waltham, Massachusetts), with a Pb, Cu and Zn Lumina hollow cathode lamp. The supernatants collected throughout the experiment was used. These results were compared to a previous study done by a masters student using samples that contained only 80 ppm Pb(II) (Peens, 2019).

3. Results and discussion

The control experiments did not exhibit any visual changes and therefore indicated that any changes that occurred were of biotic origin. The visual changes of the inoculated batch reactors are presented in Figure 1 below. The first image was taken on day zero and the second on day 7. The initial photo was taken of the first run only, as all the initial reactors presented the same. The formation of a dark grey precipitate was observed in the reactors containing a mixture of Pb(II)&Zn(II) at concentrations of 80 ppm & 40 ppm respectively, compared to no precipitation observed with Pb(II)&Zn(II) concentrations of 80 ppm & 80 ppm (Figure 2). Figure 2 compares the reactor containing 80 ppm Pb(II) & 40 ppm Zn(II) (left) to the reactor containing 80 ppm Pb(II) & 80 ppm Zn(II) (right). In figure 1, a white precipitate was observed in the reactors containing only 40 ppm Zn(II). The Pb(II)&Cu(II) reactors at Cu(II) concentrations of 80 ppm and 40 ppm respectively visually changed into a brown-red colour, forming a precipitate that was mostly white with a slight trace of brown residue. The same was observed in reactors containing Pb(II)&Cu(II), at Cu(II) concentrations of both 100 ppm and 150 ppm respectively. A similar observation was made for the reactors containing only Cu(II) at 40 ppm, 100 ppm, and 150 ppm. Colony forming unit count analysis was done on the set of lower concentrations which consisted of initial Pb(II) concentrations of 80 ppm, Zn(II) concentrations of 40 ppm and Cu(II) concentrations of 40 ppm. The analysis was done on the samples taken on day 7. The results are presented in Figure 3 above. The CFU's counted for the samples containing the mixture of Pb(II)&Zn(II) produced $1.13\pm0.065 \times 10^7$ CFU/mL, Zn(II) on its own produced $5.78\pm0.82 \times 10^6$ CFU/mL, Pb(II)&Cu(II) produced $5.98\pm1.86 \times 10^6$ CFU/mL and Cu(II) on its own produced a CFU reading of $4.61\pm3.80 \times 10^6$ CFU/mL. From a previous study done on samples containing only 80 ppm Pb(II) and taken on the seventh day of analysis, a CFU count of $5.47\pm0.83 \times 10^8$ CFU/mL was observed. These results were compared in Figure 3 with the results gathered in the current investigation. It is clearly observed that the growth was considerably higher on the plates that contained samples of only 80 ppm Pb(II) compared to the plates containing any of the other metals.

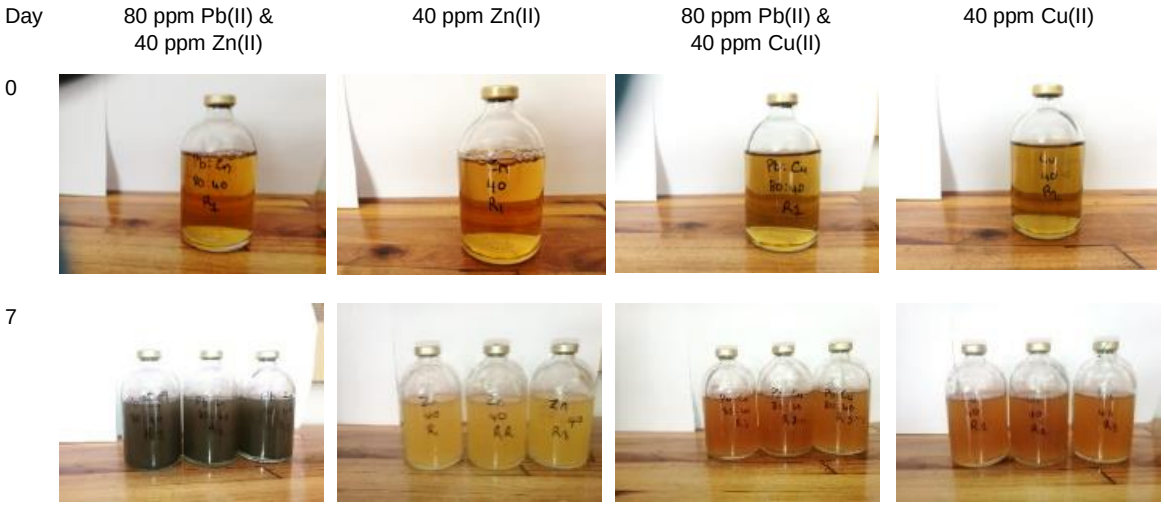

Figure 1: Visual monitoring of the second experiments, photos taken on T_0 and on T_{Final}.

Figure 2: 80 ppm Pb(II) & 40 ppm Zn(II) on the left versus 80 ppm Pb(II) & 80 ppm Zn(II) on the right.

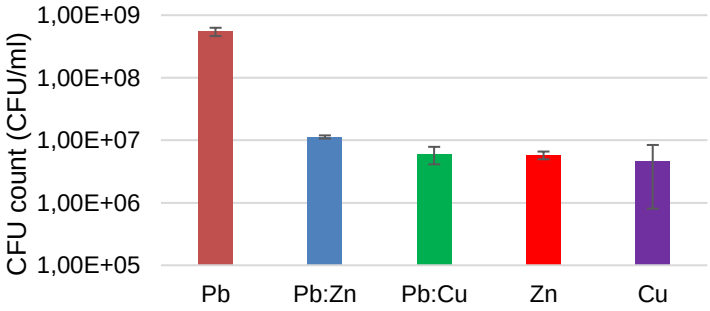

Figure 3: CFU count results taken from day 7 (final sample).

These findings can be supported by a basic double sided T-test that was conducted on the CFU data gathered in Table 1. The probability of the CFU count being comparably the same for the reactors containing Pb(II)&Cu(II) to reactors containing only Zn(II) was 0.894, which is considered a high probability and is corroborated by the findings observed in Figure 3. A similar trend was observed for samples containing only Cu(II) and samples containing only Zn(II), having a probability of 0.6920 of being the same. The probability of samples containing only Pb(II) being comparably the same as any of the samples containing other metals was 0.001, which leads to conclude that this occurrence is highly unlikely. It can be concluded that the CFU readings for samples containing only Pb(II) were considerably higher than the rest of the various readings found in conjunction with other metals.

Table 1: T-test results conducted on the CFU data gathered.

	Pb	Pb&Zn	Pb&Cu	Zn	Cu
Pb	1.000	0.001	0.001	0.001	0.001
Pb&Zn	0.001	1.000	0.019	0.002	0.070
Pb&Cu	0.001	0.019	1.000	0.894	0.670
Zn	0.001	0.002	0.894	1.000	0.692
Cu	0.001	0.070	0.670	0.692	1.000

The percentage removal on day 7 for the various metals in their various mixtures are presented in Figure 4. The change in concertation over time of each metal are individually presented in Figures 5 to 7 below. The highest Pb(II) removal was observed in reactors containing only Pb(II) at 86.2% after 7 days (Peens, 2018). A decrease in the Pb(II) concentration was observed with 69% lead removal in the presence of 40 ppm Zn(II), as compared to 25% with concentrations of 80 ppm Zn(II) (Figure 4 a). This coincides with the grey precipitate

that was only observed in the presence of 40 ppm Zn(II), and not with 80 ppm Zn(II). Figure 4 b presents the amount of Zn(II) removed, with limited removal of 12 % and 7% measured for reactors containing 80 ppm Pb(II) & 40 ppm Zn(II) and 80 ppm Pb(II) & 80 ppm Zn(II) runs respectively. A minimal amount of Zinc(II) removal was observed with 19% and 2% in the presence of only 80 ppm and 40 ppm Zinc(II) respectively.
The Pb(II) concentrations decreased with 32%, and 26% in the presence of 100 ppm Cu(II), and 150 ppm Cu(II) runs respectively (Figure 4 a). No Pb(II) was removed in the presence of 40 ppm Cu(II).
The Cu(II) concentrations decreased with 71%,61% and 52% in runs containing Pb(II) & 40 ppm Cu(II), Pb(II) & 100 ppm Cu(II) and Pb(II) & 150 ppm Cu(II) respectively (Figure 4 c). In the reactors containing only Cu(II), it was observed that 53%, 63% and 64% for the 100 ppm, 150 ppm, and 40 ppm Cu(II) was removed respectively.

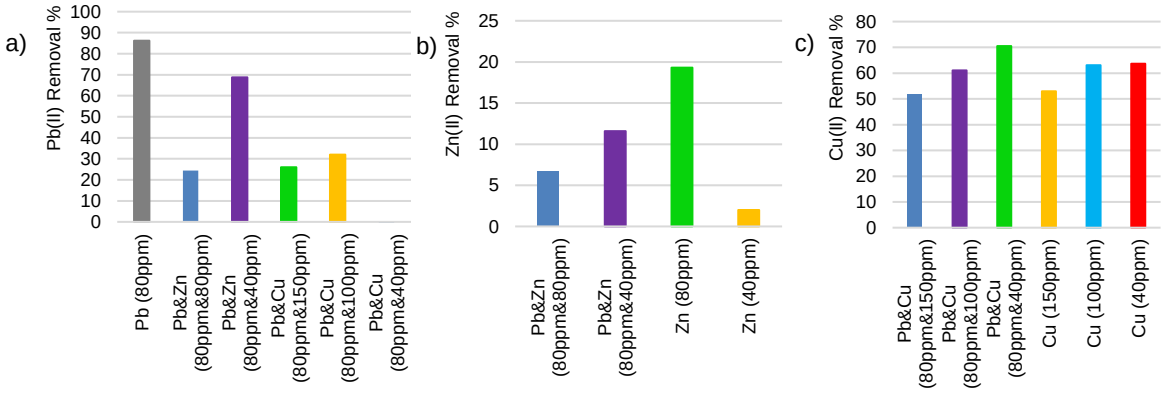

Figure 4: Percentage removal for various metals in various mixtures a) Pb(II) removed, b) Zn(II) removed, c) Cu(II) removed.

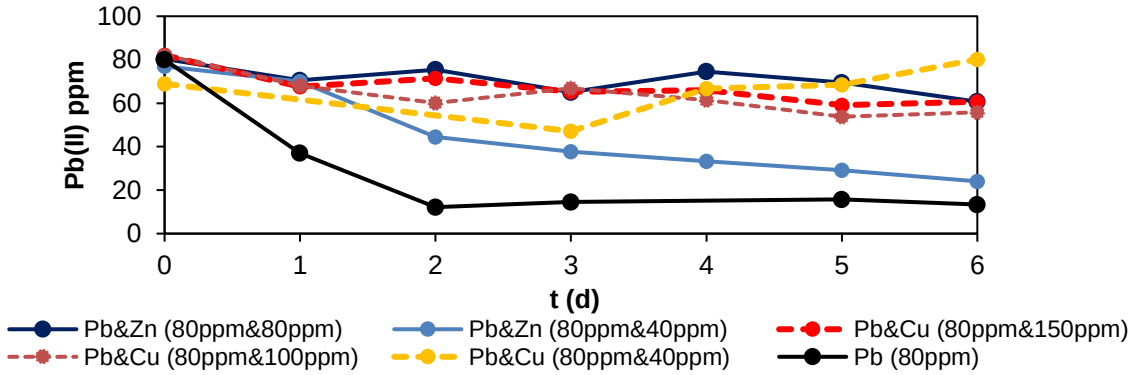

Figure 5: Change in Pb(II) concentrations with time in the presence of Zn(II) or Cu(II) at various combinations of concentrations.

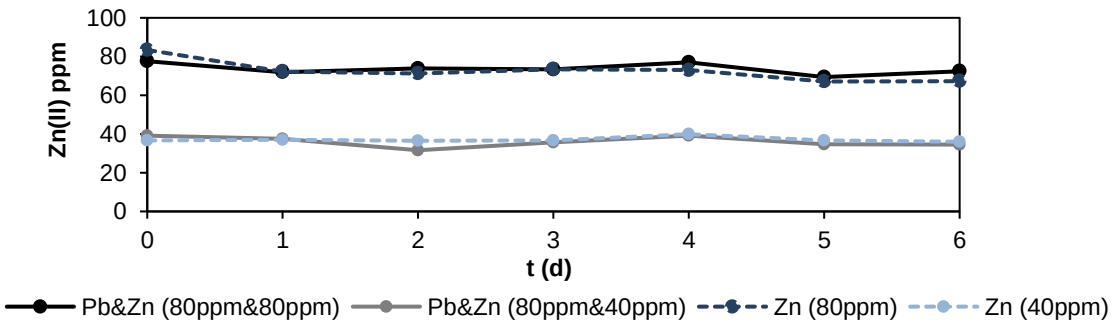

Figure 6: Change in Zn(II) concentrations with time in the presence of Pb(II) or on its own in various combinations of concentrations.

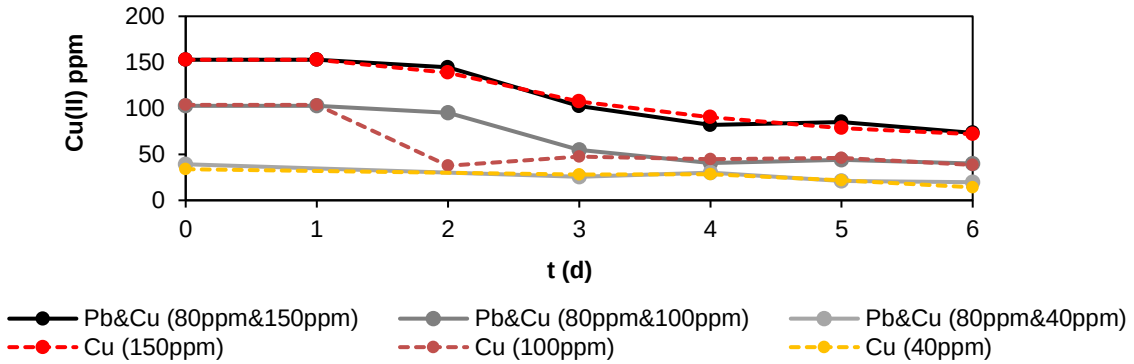

Figure 7: Change in Cu(II) concentrations with time in the presence of Pb(II) or on its own in various combinations of concentrations.

4. Conclusions

Growth was most inhibited in the reactors containing only Cu(II), although it was observed that the consortium had the capability to remove a considerable amount of Cu(II) in the presence of 80 ppm Pb(II) at 71% at a Cu(II) concentration of 40 ppm. The Pb(II) concentrations did not decrease as much as Cu(II), indicating a _competitive removal mechanism such as biosorption. No significant amount of coloured precipitate was observed and biosorption onto biomass can thus be explanatory. The highest amount of growth was observed in the reactors that contained only Pb(II), followed by Pb(II)&Zn(II) at lower Zn(II) concentrations (40 ppm). This agreed with the higher amount of lead removal (68%) and the formation of a grey precipitate. No precipitate was formed in the reactors containing elevated amounts of Zn(II)&Pb(II), but the removal of lead was measured (25%). The results suggest different removal mechanisms present in the reactors containing 40 ppm and 80 ppm Zn(II), with a precipitation mechanism at 40 ppm Zn(II) and a biosorption mechanism at 80 ppm Zn(II). It was found that Pb(II) promotes metabolic activity while Zn(II) and Cu(II) inhibits metabolic activity. This is most probably due to inhibition of a lead precipitating mechanism. The results indicate that Zn(II) and Cu(II) ions need to be removed prior to bioprecipitation and recovery of Pb(II) using another specific industrial consortium.

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