

# Microbial Pb(II) precipitation: the role of biosorption as a Pb(II) removal mechanism

Brandon van Veenhuizen<sup>a</sup>, Evans M.N. Chirwa<sup>a</sup>, Hendrik G. Brink<sup>a,\*</sup>

<sup>a</sup>Department of Chemical Engineering, Faculty of Engineering, Built Environment and Information Technology, University of Pretoria, Pretoria, South Africa  
[deon.brink@up.ac.za](mailto:deon.brink@up.ac.za)

The role of a metabolically independent lead removal mechanism in an industrially obtained lead-precipitating consortium was investigated. Cultures were prepared under anaerobic conditions for 24 hours in batch reactors starting with 20 g L<sup>-1</sup> tryptone, 10 g L<sup>-1</sup> yeast extract, 1.0 g L<sup>-1</sup> NaCl and 0.43 g L<sup>-1</sup> NaNO<sub>3</sub>. Bacteria were suspended in 50 mM of sodium azide (NaN<sub>3</sub>) solution for 3 h to successfully inhibit the microbial respiratory chain, thereby preventing bacteria growth and activity. Fourier-transform infrared spectroscopy (FTIR) was used to inspect whether NaN<sub>3</sub> deformed the structure of bacteria cell walls and changed material characteristics.

Reactors containing 100 mL of 80 mg L<sup>-1</sup> Pb(II) and 1.0 g/L NaCl were spiked with 1 mL of NaN<sub>3</sub>-sterilized bacteria culture and sampled over a 3 h period. Bulk Pb(II) concentration and metabolic activity were measured. Results showed that NaN<sub>3</sub> was an effective means to cease metabolic activity of the consortium without altering the surface properties. Pb(II) is still removed from solution (61.7 %) by dead bacteria after NaN<sub>3</sub> sterilization, indicating that the initial removal of Pb(II) from solution by the lead-precipitating consortium is an abiotic process. FTIR analysis revealed functional groups such as carboxyl, amine, and phosphate playing a role in Pb(II) biosorption.

Conclusions drawn from this study allow for future experimentation, modelling, and optimization of the biosorption mechanism in an industrially obtained lead-precipitating consortium.

## 1. Introduction

Modern industrial activities, such as mining and battery manufacturing, continue to introduce lead pollutants into the environment. Lead is highly toxic and has been found to accumulate through different trophic levels of ecosystems (Naik et al., 2013). It serves no biological purpose, but rather harms organisms directly by damaging cell structure or indirectly by impairing enzymes and substituting cationic nutrients.

Most conventional approaches to addressing lead pollution involve immobilizing Pb(II) ions from waste water streams or converting it to a less harmful state, but require additional processing to confront the recovery of Pb(0). These include adsorption, membrane filtration, electrodialysis, ion exchange, chemical precipitation, and electrochemical treatment. Most of these techniques are favourable due to high selectivity, but are burdened with high operating costs and prove uneconomical for treating waste water with low concentrations of Pb(II) (Fu and Wang, 2011). Bioremediation, on the other hand, has proven to be an attractive alternative treatment method due to low operating cost and high remediation efficiency (Kang et al., 2015).

A microbial consortium sourced from lead contaminated soil at a battery recycling plant in South Africa has been shown to remove 90 % of Pb(II) from an 80 mg L<sup>-1</sup> solution over a period of 7 days (Brink et al., 2017). It was shown that ionic lead in solution was precipitated out as PbS and elemental Pb by the microbes.

Various Pb-resistance strategies are present in micro-organisms, including intracellular bioaccumulation, extracellular sequestration, surface biosorption, bioprecipitation, cell morphology alteration and metal-ligand complex formation (Naik et al., 2013). SEM analyses on the battery recycling plant consortium indicated that surface Pb(II) bioprecipitation was the dominant lead removal mechanism (Hörstmann et al., 2020).

This study serves to investigate whether biosorption is responsible for an initial phase of Pb(II) removal that acts as a vehicle for concentrating Pb(II) on the surface of the bacteria before bioprecipitation takes place.

The biosorption of heavy metals with bacteria has been widely studied. Several species of bacteria have demonstrated significant Pb(II) biosorption, including *Bacillus firmus* (Salehizadeh and Shojaosadati, 2003), *Micrococcus luteus* (Puyen et al., 2012), *Pseudomonas putida* (Uslu and Tanyol, 2006), *Pseudomonas aeruginosa* (Chang et al., 1997), and *Streptomyces rimosus* (Selatnia et al., 2004). The chemical composition of bacteria surfaces for both Gram-positive and Gram-negative bacteria are rich in negatively charged functional groups that result in an overall negative surface charge and facilitate the attraction of positively charged metal cations like ionic lead (Vijayaraghavan and Yun, 2008). These functional groups also allow for chemisorption to take place, where hydrogen ions are exchanged for Pb(II) ions (Lu et al., 2012). Chemisorption not only prevents lead from entering cells, but allows for the concentration of lead to be used as a terminal electron acceptor in bioprecipitation (Haas et al., 2001).

## 2. Materials and methods

### 2.1 Material preparation

Cultures were prepared in sterile batch reactors from 0.2 mL of battery recycling plant consortium frozen at - 60 °C. The 100 mL growth suspension contained 20 g L<sup>-1</sup> tryptone, 10 g L<sup>-1</sup> yeast extract, and 1.0 g L<sup>-1</sup> NaCl (Hörstmann et al., 2020). 41 mg L<sup>-1</sup> NaNO<sub>3</sub> was added to ensure nitrates were present equivalent to the concentration provided by 80 mg L<sup>-1</sup> Pb(NO<sub>3</sub>)<sub>2</sub>. Batch reactors were purged with nitrogen for 3 min to ensure anaerobic conditions (Peens et al., 2018) and left to grow in a shaker-incubator for 24 h, 35 °C and 120 rpm. To successfully inhibit the microbial respiratory chain and ensure Pb(II) removal through biosorption alone, the culture was exposed to 50 mM of NaN<sub>3</sub> (Cabrol et al., 2017) for 3 h after the 24 h growth period.

### 2.2 Metabolic activity measurement

Metabolic activity was measured with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). MTT is a yellow dye which is reduced to formazan crystals by the dehydrogenase system of viable gram-negative bacterial cells. MTT solution was prepared using 5 g L<sup>-1</sup> MTT in ultrapure water. For metabolic activity readings, filtered (0.45 µm) and unfiltered samples were diluted 4 times and mixed with MTT to form a 10% MTT solution. The solution was incubated for an hour, after which formazan crystals were dissolved by dimethyl sulfoxide. Light absorption measurements at 550 nm were performed to infer metabolic activity differences between filtered and unfiltered samples (Peens, 2018).

### 2.3 FTIR analysis

Fourier transform infrared (FTIR) spectra of the consortium were measured after three consecutive processes. The first measurement was recorded after a 24 h growth period. The second measurement was recorded following 3 h sterilization of the consortium with 50 mM NaN<sub>3</sub>. Finally, the third measurement was taken after exposure of the sterilized consortium to 200 mg L<sup>-1</sup> Pb(NO<sub>3</sub>)<sub>2</sub>. Spectra were recorded on a Perkin Elmer Spectrum 2000GX FTIR spectrometer using an attenuated total reflection (ATR) attachment. All FTIR spectra were recorded at a resolution of 2 cm<sup>-1</sup> for 30 scans from 4000 to 550 cm<sup>-1</sup> and represent the average of 30 scans.

### 2.4 Lead removal experiments

Sterilized reactors containing 100 mL of ultrapure water with 1.0 g L<sup>-1</sup> NaCl were prepared. The reactors were spiked with NaN<sub>3</sub>-sterilized bacteria and various concentrations of Pb(II).

The removal of Pb(II) over a 3 h period was investigated by spiking triplicate reactors containing 80 mg L<sup>-1</sup> Pb(II) with 1.0 mL of bacteria. Reactors were sampled at various time intervals and filtered (0.45 µm).

The effect of initial Pb(II) concentration and bacteria concentration on Pb(II) removal was determined by setting up reactors with 1.0 g L<sup>-1</sup> NaCl, 50 – 400 mg L<sup>-1</sup> Pb(II), and 1.2 – 3.4 mL of bacteria culture. The dry mass of bacteria per mL of culture was determined by centrifuging the culture at 9000 rpm for 10 minutes at 4 °C, rinsing with distilled water, and centrifuging again before being oven dried at 50 °C for 24 h.

The Pb(II) concentration in samples was determined by atomic absorption spectroscopy (Perkin Elmer Analyst 400, Waltham, Massachusetts).

### 3. Results

#### 3.1 FTIR analysis

The presence of functional groups was confirmed by FTIR analysis. In Figure 1, the broad peak found around  $3298\text{ cm}^{-1}$  was attributed to O-H stretching (Francioso et al., 2010), whereas the band at  $1640\text{ cm}^{-1}$  was assigned to the presence of  $\text{-C=O}$  in amide I (Y. Liu et al., 2016). The addition of  $\text{NaN}_3$  appears to do little to affect the surface properties of the consortium. This is seen in Figure 1a) and Figure 1b). A difference between the two spectra is observed at  $2040\text{ cm}^{-1}$  in Figure 1b), with this band signaling the presence of  $\text{N}\equiv\text{N}$  from  $\text{NaN}_3$  (Tao et al., 2011). The addition of  $\text{Pb(II)}$  results in a significant disruption in transmittance between  $1550$  and  $900\text{ cm}^{-1}$ , as observed in Figure 1c). The appearance of new peaks could be indicative of the cell wall rupturing, resulting in the exposure of various functional groups from the inside the cell. This consortium has been recorded growing in  $\text{Pb(II)}$  concentrations around  $1000\text{ mg L}^{-1}$  (Peens et al., 2018), making such destructive effects of  $200\text{ mg L}^{-1}$   $\text{Pb(II)}$  on cell walls unlikely.

Alternatively, the appearance and strengthening of weak bands could show chemisorption of  $\text{Pb(II)}$  to particular surface functional groups. Bands are described as appearing if they were not discernable in the spectra of  $\text{NaN}_3$ -sterilized cultures but became apparent in Figure 1c) **Figure 3**.

The appearance of a band at  $1537\text{ cm}^{-1}$  may be indicative of interference or deformation of N-H bending in amide II, and is suggestive of  $\text{Pb(II)}$  binding (Masoumi et al., 2016). The strengthening of the band at  $1451\text{ cm}^{-1}$  and subsequent shift to  $1454\text{ cm}^{-1}$  could signal  $\text{Pb(II)}$  interactions with  $\text{CH}_3$  and  $\text{CH}_2$  groups of lipids and proteins (Z. Liu et al., 2016). The strengthening and shift of the band at  $1406\text{ cm}^{-1}$  to  $1393\text{ cm}^{-1}$  is likely a result of  $\text{COO}^-$  groups (Dittrich and Sibling, 2005) binding with  $\text{Pb(II)}$ . Additionally, the strengthening and shift of the  $1240\text{ cm}^{-1}$  band to  $1235\text{ cm}^{-1}$  could arise from lead binding with  $\text{P=O}$  functional groups of phosphorylated proteins or polyphosphate storage products (Dittrich and Sibling, 2005). The appearance of a band at  $1030\text{ cm}^{-1}$  may be attributed to a variety of mixed modes of carbohydrates (Z. Liu et al., 2016) or carboxyl groups (Lu et al., 2012). Finally, the appearance of a band at  $956\text{ cm}^{-1}$  is likely due to  $\text{Pb(II)}$  interfering with  $\text{C=C}$  bending in alkenes.

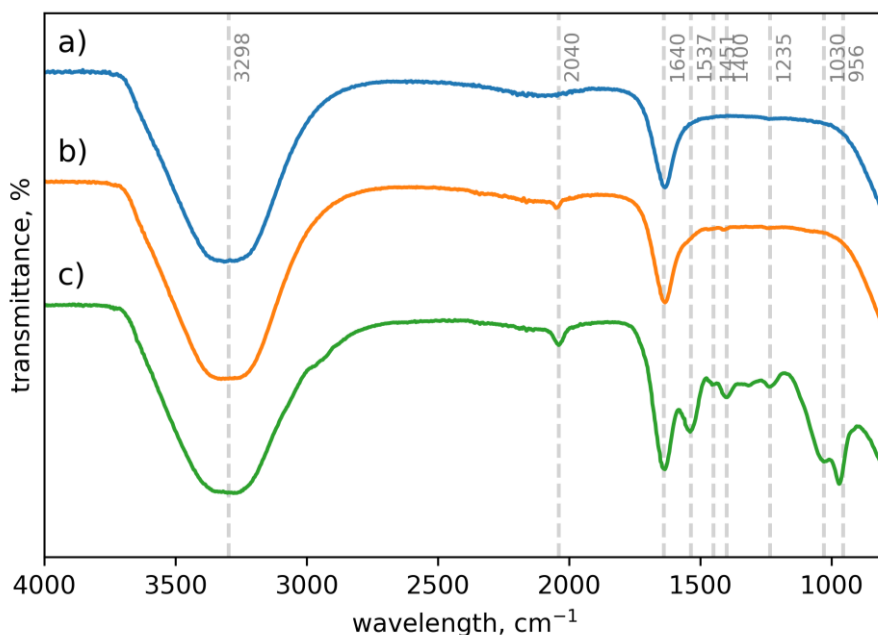


Figure 1: FTIR spectra of bacteria consortium a) after 24 h growth period, b) after 3 h exposure to  $\text{NaN}_3$ , and c) after exposure to  $\text{NaN}_3$  and  $\text{Pb(NO}_3)_2$ .

These findings are consistent with literature reports of functional groups involved in the chemisorption of heavy metals to biomass, including carboxyl groups (Fomina and Gadd, 2014), amine (Fein et al., 1997), phosphate (Ngwenya et al., 2003), and carbonyl (Mathew and Krishnamurthy, 2018).

### 3.2 Lead removal experiments

Following 3 h,  $61.7 \pm 4.86$  % of Pb(II) was removed from solution by  $\text{NaN}_3$ -sterilized bacteria (Figure 2). In addition, no metabolic activity was detected in any of the reactors using MTT. This indicates that a passive mechanism was involved in lead removal. The concentrations used were also found to be within the solubility range of  $\text{Pb}(\text{N}_3)_2$  (Lieber et al., 1966), confirming that lead removal was caused by the presence of the battery recycling plant consortium alone. No black/grey precipitate was discernable following a 24 h period, signaling a lack of PbS or Pb(0) formation.

As shown in Figure 2: *Percent lead remaining in solution as a function of time after the addition of the  $\text{NaN}_3$ -sterilized consortium.*

Table 1: Comparison of lead removal by various bacteria.

Bacteria	Mass (mg)	Original Pb(II) concentration (mg L <sup>-1</sup> )	Time (h)	Percent removal	Source
<i>Acinetobacter junii</i> L. 606 Pb1	2000	100	6	74	Kushwaha et al (2017)
<i>Bacillus sp.</i> (ATS-1)	-	100	0.25	74	Tunali et al. (2006)
Battery recycling plant consortium	-	80	3	50	Hörstmann et al. (2020)
Battery recycling plant consortium	47.5	150	3	63	This study
<i>Rhodococcus sp.</i> HX-2	750	200	0.5	47	Hu et al., (2020)
<i>Streptomyces rimosus</i>	3000	500	3	20	(Selatnia et al., 2004)

, an increase in bacteria mass dosing was found to produce a clear increase in Pb(II) removal. This is presumably due to an increase in available sorption sites. An increase in initial Pb(II) concentration from 0 mg L<sup>-1</sup> to 100 mg L<sup>-1</sup> shows greater Pb(II) removal, most likely due to a stronger concentration gradient. A similar effect was seen with Pb(II) adsorption onto carbon nanofibers (Ahmed et al., 2010). Above 150 mg L<sup>-1</sup> a decrease in adsorption is observed with increases in initial Pb(II) concentration, indicating the limitation of adsorption sites being reached at these concentrations. As seen in Table 1, lead removal by the battery recycling plant consortium requires significantly less biomass when compared to several previous studies.

These results suggest that biosorption is responsible for the initial, rapid removal of Pb(II) from solution. Since both biosorption and precipitation of Pb(II) occur on the surface, it is likely that the biosorption of lead ions onto bacteria cell walls is a necessary precursor to the precipitation of Pb(II) to PbS or Pb(0). More intensive biosorption investigations should be done to enable a detailed biosorption model that can be incorporated into models of microbial Pb(II) precipitation.

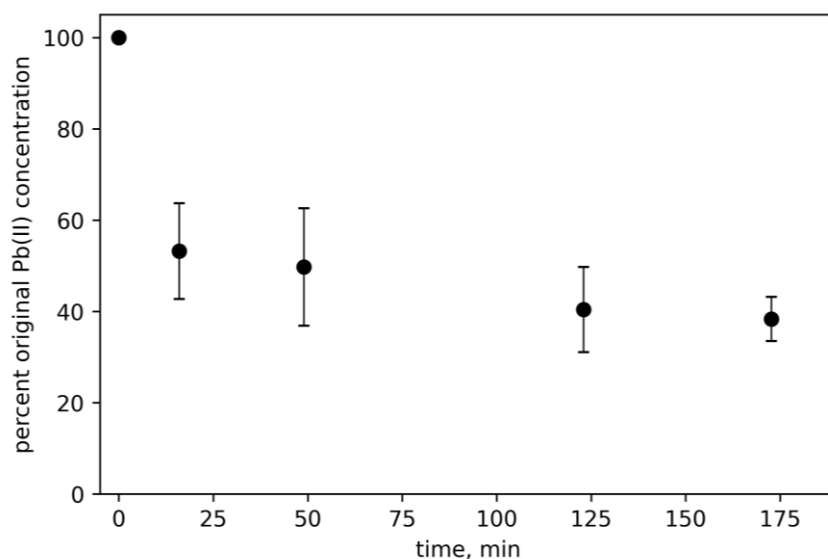


Figure 2: Percent lead remaining in solution as a function of time after the addition of the  $\text{NaN}_3$ -sterilized consortium.

Table 1: Comparison of lead removal by various bacteria.

Bacteria	Mass (mg)	Original Pb(II) concentration (mg L <sup>-1</sup> )	Time (h)	Percent removal	Source
<i>Acinetobacter junii</i> L. 606 Pb1		100	6	74	Kushwaha et al (2017)
<i>Bacillus sp.</i> (ATS-1)	2000	100	0.25	74	Tunali et al. (2006)
Battery recycling plant consortium	-	80	3	50	Hörstmann et al. (2020)
Battery recycling plant consortium	47.5	150	3	63	This study
<i>Rhodococcus sp.</i> HX-2	750	200	0.5	47	Hu et al., (2020)
<i>Streptomyces rimosus</i>	3000	500	3	20	(Selatnia et al., 2004)

## Conclusions

Sodium azide successfully inhibited metabolic activity of the consortium without damaging the cell walls of bacteria, allowing for Pb(II) removal through abiotic mechanisms to be studied. It was found that  $61.7 \pm 4.86\%$  of Pb(II) is removed in 3 h by non-living bacteria. FTIR spectroscopy supported the chemisorption of lead onto functional groups as being responsible for this removal. The amount of consortium dosed and the initial Pb(II) concentrations used were found to have a non-linear effect on lead removal, with Pb(II) concentration gradient being a limiting factor at concentrations less than 150 mg L<sup>-1</sup> whereas binding sites become the limiting factor above 150 mg L<sup>-1</sup>. These results pave the way for future studies to do more detailed abiotic lead removal experimentation.

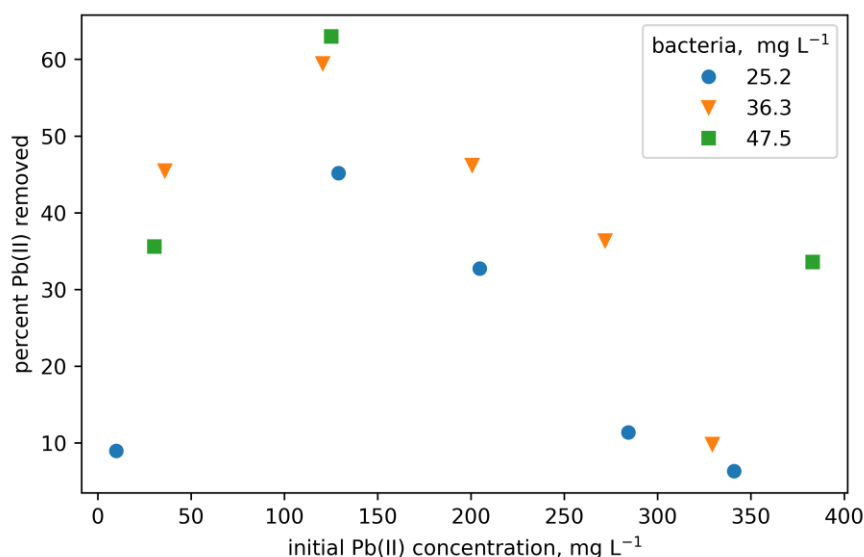


Figure 3: The effects of  $\text{NaN}_3$ -sterilized bacteria concentration and initial Pb(II) concentration on lead removal. The concentration of bacteria is given as a dry mass.

## Acknowledgments

This work is based on the research supported in part by the National Research Foundation of South Africa (Grant Numbers 106938 and 123022).

## References

Ahmed, Y.M., Mamun, A. Al, Muyibi, S.A., Al-Khatib, M.F.R., Jameel, A.T., AlSaadi, M.A., 2010. Effect of

- Adsorbate Initial Concentration on the Removal of Pb from Aqueous Solutions by Carbon Nanofibers, in: 24th Symposium of Malaysian Chemical Engineers (SOMChE 2010) and 1st International Conference on Process Engineering and Advanced Materials 2010 (ICPEAM2010). Kuala Lumpur, pp. 15–17.
- Brink, H.G., Lategan, M., Naudé, K., Chirwa, E.M.N., 2017. *Int. Conf. Energy, Environ. Clim.*
- Cabrol, L., Quéméneur, M., Misson, B., 2017. *J. Microbiol. Methods* 133, 62–65.
- Chang, J.S., Law, R., Chang, C.C., 1997. *Water Res.* 31, 1651–1658.
- Dittrich, M., Sibling, S., 2005. *J. Colloid Interface Sci.* 286, 487–495.
- Fein, J.B., Daughney, C.J., Yee, N., Davis, T.A., 1997. *Geochim. Cosmochim. Acta* 61, 3319–3328.
- Fomina, M., Gadd, G.M., 2014. *Bioresour. Technol.* 160, 3–14.
- Francioso, O., Rodriguez-Estrada, M.T., Montecchio, D., Salomoni, C., Caputo, A., Palenzona, D., 2010. *J. Hazard. Mater.* 175, 740–746.
- Fu, F., Wang, Q., 2011. *J. Environ. Manage.* 92, 407–418.
- Haas, J.R., Dichristina, T.J., Wade, R., 2001. *Chem. Geol.* 180, 33–54.
- Hörstmann, C., Brink, H.G., Chirwa, E.M.N., 2020. *Sustainability*.
- Hu, X., Cao, J., Yang, H., Li, D., Qiao, Y., Zhao, J., Zhang, Z., Huang, L., 2020. *PLoS One* 15.
- Kang, C.H., Oh, S.J., Shin, Y.J., Han, S.H., Nam, I.H., So, J.S., 2015. *Ecol. Eng.* 74, 402–407.
- Kushwaha, A., Rani, R., Kumar, S., Thomas, T., David, A.A., Ahmed, M., 2017. *Environ. Sci. Pollut. Res.* 24, 10652–10661.
- Lieber, E., Rao, C.N.R., Dingle, H.E., Teetsov, J., 1966. *J. Chem. Eng. Data* 11, 105.
- Liu, Y., Alessi, D.S., Owttrim, G.W., Kenney, J.P.L., Zhou, Q., Lalonde, S. V., Konhauser, K.O., 2016. *Geochim. Cosmochim. Acta* 187, 179–194.
- Liu, Z., Huang, Y., Zhao, G., 2016. *BioResources* 11, 3178–3190.
- Lu, H., Zhang, W., Yang, Y., Huang, X., Wang, S., Qiu, R., 2012. *Water Res.* 46, 854–862.
- Masoumi, F., Khadivinia, E., Alidoust, L., Mansourinejad, Z., Shahryari, S., Safaei, M., Mousavi, A., Salmanian, A.H., Zahiri, H.S., Vali, H., Noghabi, K.A., 2016. *J. Environ. Chem. Eng.* 4, 950–957.
- Mathew, B.B., Krishnamurthy, N.B., 2018. *Groundw. Sustain. Dev.* 7, 313–327.
- Naik, M.M., Khanolkar, D., Dubey, S.K., 2013. *Ecotoxicol. Environ. Saf.* 98, 1–7.
- Ngwenya, B.T., Sutherland, I.W., Kennedy, L., 2003. *Appl. Geochemistry* 18, 527–538.
- Peens, J., 2018. *Pb (II)-Removal From Water Using Microorganisms Naturally Evolved to Tolerate Pb(II)-Toxicity*. University of Pretoria, Pretoria.
- Peens, J., Wu, W.Y., Brink, H.G., 2018. *Chem. Eng. Trans.* 64, 439–444.
- Puyen, Z.M., Villagrasa, E., Maldonado, J., Diestra, E., Esteve, I., Solé, A., 2012. *Bioresour. Technol.* 126, 233–237.
- Salehizadeh, H., Shojaosadati, S.A., 2003. *Water Res.* 37, 4231–4235.
- Selatnia, A., Boukazoula, A., Kechid, N., Bakhti, M.Z., Chergui, A., Kerchich, Y., 2004. *Biochem. Eng. J.* 19, 127–135.
- Tao, P., Li, Y., Rungta, A., Viswanath, A., Gao, J., Benicewicz, B.C., Siegel, R.W., Schadler, L.S., 2011. *J. Mater. Chem.* 21, 18623–18629.
- Tunali, S., Çabuk, A., Akar, T., 2006. *Chem. Eng. J.* 115, 203–211.
- Uslu, G., Tanyol, M., 2006. *J. Hazard. Mater.* 135, 87–93.
- Vijayaraghavan, K., Yun, Y.S., 2008. *Biotechnol. Adv.* 26, 266–291.