CHEMICAL ENGINEERING TRANSACTIONS
VOL. 96, 2022
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.cetjournal.it
Guest Editors: David Bogle, Flavio Manenti, Piero Salatino

ISBN 978-88-95608-95-2; **ISSN** 2283-9216
DOI: 10.3303/CET2296063

Biosorption of Aqueous Pb(II) by a Metabolically Inactive Industrial *Klebsiella pneumoniae* Strain

Patrick Y. Kpai[a], Evans M. N. Chirwa[b], Hendrik G. Brink[c,*]

Department of Chemical Engineering, Faculty of Engineering, Built Environment and Information Technology, University of Pretoria, Pretoria, South Africa.
deon.brink@up.ac.za

A microbial consortium which was obtained from lead contaminated soil at a battery recycling plant in South Africa was previously proved to be effective at removing approximately 50% of Pb(II) from solution within the first 3 h at 80 and 500 ppm Pb(II). *Klebsiella pneumoniae* was determined to be the dominant species responsible for Pb(II) bioprecipitation which was the main lead removal mechanism. In the current study, the purified *K. pneumoniae* was metabolically deactivated by drying and the resulting biomass tested for Pb(II) adsorption properties. Results demonstrated that the metabolically inactive *K. pneumoniae* biomass removed approximately 39% of a 100 ppm Pb(II) solution in 3h. pH measurements and FTIR spectroscopy indicated cation exchange of H^+-ions for Pb(II) as well as adsorption interactions with functional groups (hydroxyl compound and amide) as being responsible for this removal. These results confirmed biosorption being responsible for the initial phase of Pb(II) removal which acts as a vehicle for concentrating Pb(II) on the surface of the bacteria before bioprecipitation takes place. These results provide further insights into the Pb(II) removal mechanisms involved in the Pb(II) bioremediation processes required for eventual scaling of these processes.

1. Introduction

Rapid industrialization and unplanned urbanization have introduced heavy metals into the environment through improper dumping of industrial wastes directly on land and into water bodies (Dixit et al., 2015). Consequently, environmental contamination by heavy metals has emerged as a major concern (Hashem et al., 2017) and is associated with environmental pollution and bio-toxicity issues attributed to their ability to inhibit biodegradation activities (Masindi and Muedi, 2018).

Heavy metal contamination, especially lead, has been a constant common problem worldwide (Tong et al., 2000). Chronic exposure to heavy metals such as lead poses a major threat to soil, water, and food safety because of their inherent toxicity to living organisms, especially humans (Fewtrell et al., 2004).

The permissible limit of lead in drinking water is 5 µg/L (Waihung et al., 1999). The presence of lead in drinking water above the permissible limit causes diseases such as anaemia, encephalopathy, hepatitis, and nephrotic syndrome (Panchanadikar and Das, 1994).

Due to the increasing concern about the public health and environmental problems caused by lead contamination, developing highly efficient and stable treatment methods is necessary (Jeong et al., 2019). Conventional techniques such as membrane filtration, adsorption, chemical precipitation, ion exchange and electrodialysis are employed in addressing lead pollution from waste streams by converting Pb(II) ions to a less harmful state but requires supplementary treatment in the recovery of Pb (0) (Van Veenhuyzen et al., 2021a). Most of these treatment techniques are advantageous due to high selectivity but are too costly in the treatment of waste streams with low Pb(II) concentrations (Fu and Wang, 2011). The traditional approaches for the removal of Pb(II) include reduction, extraction, ion exchange, precipitation, and membrane filtration which suffer the problems of low efficiency and high operating costs (He et al., 2019). Adsorption has become one of the alternative treatments due to its low-cost, high performance and wide pH range, in recent years, the search for low-cost adsorbents that have metal-binding capacities has intensified (Leung et al., 2000). The adsorbents may be of mineral, organic or biological origin, zeolites, industrial by-products, agricultural wastes, biomass, and polymeric materials (Kurniawan et al., 2005).

Paper Received: 4 January 2022; Revised: 12 July 2022; Accepted: 28 August 2022
Please cite this article as: Kpai P.Y., Chirwa E.M.N., Brink H., 2022, Biosorption of Aqueous Pb(II) by a Metabolically Inactive Industrial Klebsiella Pneumoniae Strain, Chemical Engineering Transactions, 96, 373-378 DOI:10.3303/CET2296063

The most recent estimate of current lead ore reserves (88 Mt) means that raw lead could potentially be depleted by 2035 (Statista, 2019). Lead recovery is of ultimate importance as it does not only lead to an environmentally friendly cycle, but also results in the diminution of the adverse effects of lead mining.
A microbial consortium which was obtained from lead contaminated soil at a battery recycling plant in South Africa has been demonstrated to remove 90 % of Pb(II) from an 80 mg/L solution over a period of 7 days (Brink et al., 2017). The consortium was effective at precipitating Pb(II) from solution and was shown to remove approximately 50 % of Pb(II) at conditions of 80 and 500 ppm within the first 3 hours (Hörstmann et al., 2020). Study shows that the ionic lead in solution was precipitated out as PbS and elemental Pb by the microbes (Van Veenhuyzen et al., 2021a). Further study on the battery recycling plant consortium shows that *K. pneumoniae* was the dominant species responsible for Pb(II) bio precipitation which was the main lead removal mechanism (Hörstmann et al., 2020). Non-living bacteria removed 61.7 ± 4.86 % of Pb(II) in 3 hours, and FTIR spectroscopy supported the chemisorption of lead onto functional groups as being responsible for this removal confirming biosorption being responsible for the initial phase of Pb(II) removal which acts as a vehicle for concentrating Pb(II) on the surface of the bacteria before bioprecipitation takes place (Van Veenhuyzen et al., 2021a).
The main purpose of this study is to investigate the bioremediation removal effectiveness of *K. pneumoniae*, an isolated microbial strain of the consortium to determine its contribution to Pb(II) removal. This method of bio-removal could serve as the first step towards the design of a continuous reactor for large-scale implementation in various industries as a simple cost-effective method to remediate and regenerate Pb-containing effluents.

2. Materials and methods

2.1 Material preparation

K. pneumoniae was prepared from a battery recycling plant consortium frozen at -60 °C. The 100 mL pure culture was prepared under aerobic conditions in a batch reactor starting with 20 g/L tryptone, 10 g/L yeast extract and 1 ml of 100 g/L NaCl (Hörstmann et al., 2020). The culture was left to grow in a shaker-incubator for 24 h, 35 °C and 120 rpm. The pure culture was then centrifuged at 9000 rpm for 10 minutes at 4 °C, rinsing with ultrapure water, and centrifuging again before being oven dried at 74 °C for 24 h to successfully inhibit microbial respiratory chain and ensuring Pb(II) removal through biosorption alone.

2.2 Optical density measurement

A spectrophotometer was used in reading the degree of light dispersed by the pure culture. For optical density reading (OD_{600}), the sample was diluted 4 times before measurement was made at 600 nm.

2.3 Metabolic activity measurement

Metabolic activity was measured with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) which is a yellow dye reduced to formazan crystals by the dehydrogenase system of viable gram-negative bacterial cells (Van Veenhuyzen et al., 2021a).
For metabolic activity readings, 0.5 mL filtered (0.45 µm) of the sample was added to 0.2 mL MTT and 1.3 mL sterilized ultrapure water. Also, 0.5 mL unfiltered sample was added to 0.2 mL MTT and 1.3 mL sterilized ultrapure water. Dimethyl sulfoxide was added to the solution after an hour incubation to dissolve the formazan crystals (Van Veenhuyzen et al., 2021a).
A spectrophotometer was used in measuring light absorbed at 550 nm between the unfiltered and filtered samples to infer metabolic activity differences (Peens, 2018).

2.4 FTIR analysis

Fourier-transform infrared (FTIR) spectra of the pure culture were measured after four successive processes. The first measurement was taken after 24 h growth period of the bacteria. The second measurement was taken after 24 h oven drying of the bacteria at 74 °C. The third measurement was taken after the bacteria was exposed to 100 ppm of $Pb(NO_3)_2$. The last measurement was taken 14 h after the addition of 100 ppm $Pb(NO_3)_2$ to the pure culture. An attenuated total reflection (ATR) attachment was used in recording spectra on a Perkin Elmer Spectrum 100 FTIR spectrometer. All FTIR spectra were recorded on a wavelength from 4000 to 900 cm^{-1} and represent an average of 30 scans.

2.5 Lead removal experiments

Sterilized reactors containing 100 mL of ultrapure water, 1 ml of 1.711 M $NaNO_3$ salt substitute with oven dried-sterilized bacteria, and 100 ppm of Pb(II) was prepared. This will serve as the concentration for the basis of comparison with other microbial strains of the consortium. The reactor was triplicated to ensure repeatability and the Pb(II) removal over a 14 h period was investigated.

Reactors were sampled at various time intervals and filtered (0.45 µm) and initial and final pH readings of the samples were measured. The Pb(II) concentration in samples was measured using an atomic absorption spectroscopy (Perkin Elmer AAnalyst 400, Waltham, Massachusetts).
The dry mass of bacteria per mL of pure culture was determined by centrifuging the pure culture at 9000 rpm for 10 min at 4 °C, rinsing with distilled water, and centrifuging again (Van Veenhuyzen et al., 2021a) before being oven dried at 74 °C for 24 h.

3. Results and discussion

3.1 FTIR analysis

FTIR analysis for functional groups revealed the presence of two functional groups in the samples. The wavelength and functional group obtained from the spectra are presented in Table 1.

Table 1: FTIR spectra of K. pneumoniae

Wavelength (cm^{-1})	Functional group	Reference
3298	Hydroxyl compound	Deepashree et al., 2013
1640	Amide	Y. Liu et al., 2016

The presence of hydroxyl compound was revealed due to the occurrence of the broad peak found at 3298 cm^{-1} which was caused by O-H stretching (Deepashree et al., 2013). This finding aligns with literature which shows that hydroxyl functional groups are mainly responsible for the adsorption of Pb(II) (Xiaoping & Xiaoning, 2013). The band occurring at 1640 cm^{-1} was attributed to the occurrence of -C=O in amide I (Y. Liu et al., 2016).
No difference was observed in any of the four spectra which shows that the surface characteristics of the bacteria remain unchanged and oven drying the bacteria at 74 °C for 24 h did not rupture the cell wall of the bacteria.

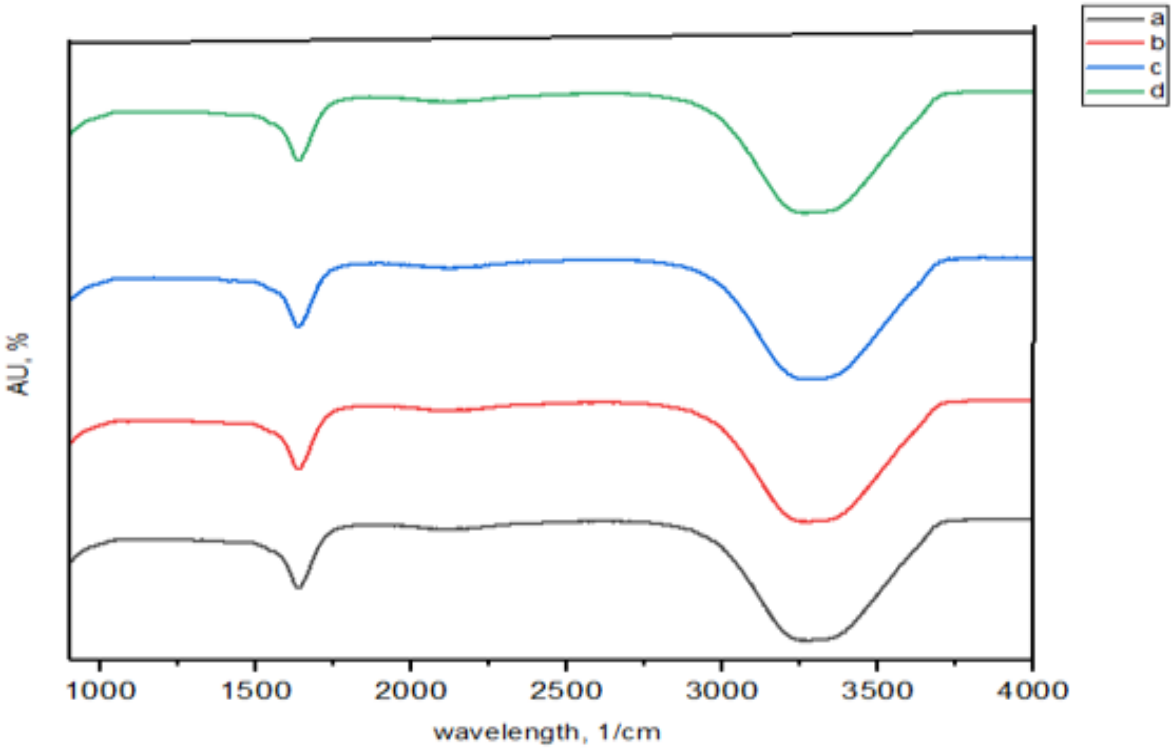

Figure 1: FTIR spectra of K. pneumoniae *a) after a growth period of 24 h, b) after oven drying for 24 h at 74°C, c) after exposure to* $Pb(NO_3)_2$*, and d)14 h after adding* $Pb(NO_3)_2$*.*

3.2 Lead removal experiments

As shown in figure 2, 23.1 mg/g (38.8 %) %) of the Pb(II) was removed by oven dried sterilized bacteria in 3 h. A passive process was responsible for Pb(II) removal from the solution as metabolic activity was not detected using MTT. Black or grey precipitate was not evident in the 14 h period, which indicates that PbS or Pb (0) was not formed. The Initial and final pH values of the reactors were 6.13 and 5.83 respectively. The drop in pH is likely due to the release of protons from the surface of the bacteria because of cation exchange processes in which the H^+-ions are displaced by the Pb(II)-ions on the surface (Van Veenhuyzen et al., 2021b).

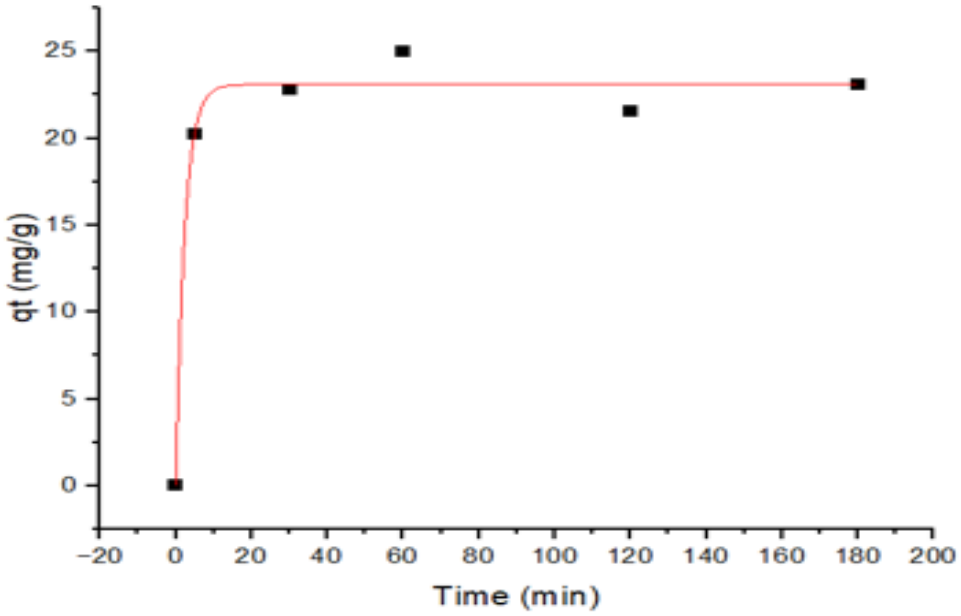

Figure 2: Graph of Pb(II) removal by K. pneumoniae *in mg/g against time in minutes.*

Table 2 shows a summary of comparable Pb(II) adsorption studies from literature and demonstrates that the current study compares very favourable with studies from literature. This indicates that *K. pneumoniae* has comparable biosorption properties when compared to results from literature. However, comparison of the adsorption capacity of the original consortium studied by Van Veenhuyzen et at (2021a) shows that *K. pneumoniae* had an adsorption capacity an order of magnitude smaller than the overall consortium, indicating that *K. pneumoniae* cannot be considered the dominant biosorbing species in the system.

Table 2: Lead removal assessment by various biomass adsorbents

Biomass properties	Adsorbent Mass (mg)	Original Pb(II) concentration (mg/L)	Time (h)	Removal (%)	Maximum measured adsorption capacity (mg/g)	Reference
Battery recycling plant consortium	47.50	150	3	63	199	Van Veenhuyzen et al., 2021a
Battery recycling plant consortium	-	80	3	50	-	Hörstmann et al., 2020
Rhodococcus sp. HX-2	750	200	0.5	47	88.74	Hu et al., 2020
Streptomyces rimosus	3000	500	3	20	135	Selatnia et al., 2004
Klebsiella pneumoniae	36.2	100	3	38.8	23.1	This study

4. Conclusions

In our research, metabolically inactive *K. pneumoniae* was employed as a biosorbent to eliminate Pb(II) from aqueous solution. It was found that oven drying the bacteria at 74 °C for 24 h inhibited the metabolic activity of the bacteria without rupturing the cell wall. Metabolically inactive *K. pneumoniae* removed 23.1 mg/g (38.8 %) of Pb(II) in 3 h, and FTIR spectroscopy indicated the biosorption of Pb(II) onto functional groups (Hydroxyl compound and Amide) as being responsible for this removal. It was found that, *K. pneumoniae* lowered the pH of the solution by generating molecular hydrogen likely due to the cationic exchange of surface bound protons with Pb(II) ions. Conclusions drawn from this research allow for further study on the comparison of the biosorption effectiveness of the isolated microbial strains to the previously studied consortium to determine the respective contributions of the strains to Pb(II) removal which will be applied in concurrence with a kinetic model to develop a design and implementation strategy for continuous bio-removal and regeneration of Pb(II) from industrial effluents.

Acknowledgments

This work is based on the research supported in part by the National Research Foundation of South Africa (Grant Numbers 120321, 145848, 121891 and 128088)

References

Barakat M. A., 2011, New trends in removing heavy metals from industrial wastewater. Arabian Journal of Chemistry, Volume 4, Issue 4, 361-377.

Brink, H.G., Lategan, M., Naudé, K. and Chirwa, E., 2017, Lead removal using industrially sourced consortia: influence of lead and glucose concentrations.

Deepashree C. L., Komal K. J., Prasad A. G. D., Zarei M., Gopal S., 2013, FTIR Spectroscopic Studies on Cleome Gynandra-Comparative Analysis of Functional Group before and after Extraction. Romanian Journal of Biophysics, 22, 137-143.

Dixit R., Wasiullah M. D., Pandiyan K., Singh U.B., Sahu A., Shukla R., Singh B.P., Rai J.P., Sharma P.K., Lade H., Paul D., 2015, Bioremediation of Heavy Metals from Soil and Aquatic Environment: An Overview of Principles and Criteria of Fundamental Processes Sustain, 2189 – 2212.

Fewtrell L. J., Pruss-Ustun A., Landrigan P., Ayuso-Mateos J. L., 2004, Estimating the Global Burden of Disease of Mild Mental Retardation and Cardiovascular Diseases from Environmental Lead Exposure. Environmental Research, Volume 94, No. 2, 120-133.

Fu F., Wang Q., 2011, Removal of Heavy Metal Ions from Wastewaters. Journal of Environmental Management, Volume 92, 407-418.

Hashem M.A., Nur-A-Tomal M.S., Mondal N.R., Rahman M.A., 2017, Hair Burning and Liming in Tanneries is a Source of Pollution by Arsenic, Lead, Zinc, Manganese, and Iron Environ. Chem. Lett., 501-506.

He Y., Wu P., Xiao W., Li G., Yi J., He Y., Chen C., Ding P.,Duan Y., 2019, Efficient removal of Pb(II) from aqueous solution by a novel ion imprinted magnetic biosorbent: Adsorption kinetics and mechanisms. PloS one.

Hörstmann C., Brink, H.G. and Chirwa, E.M.N., 2020. Pb(II) Bio-Removal, Viability, and Population Distribution of an Industrial Microbial Consortium: The Effect of Pb(II) and Nutrient Concentrations. Sustainability, p.2511.

Hu X., Cao J., Yang H., Li D., Qiao Y., et al., 2020, Pb^{2+} biosorption from aqueous solutions by live and dead biosorbents of the hydrocarbon-degrading strain Rhodococcus sp. HX-2. PLOS ONE 15(1).

Jeong S.W., Kim H.K., Yang J.E., Choi Y.J., 2019, Removal of Pb(II) by Pellicle-Like Biofilm-Producing Methylobacterium hispanicum EM2 Strain from Aqueous Media.

Kurniawan, T.A., Chan, G.Y., Lo, W.H. and Babel, S., 2006. Comparisons of low-cost adsorbents for treating wastewaters laden with heavy metals. Science of the total environment, 366(2-3), pp.409-426.

Leung, W.C., Wong, M.F., Chua, H., Lo, W., Yu, P.H.F. and Leung, C.K., 2000. Removal and recovery of heavy metals by bacteria isolated from activated sludge treating industrial effluents and municipal wastewater. Water science and technology, 41(12), pp.233-240.

Liu Y., Alessi D.S., Owttrim G.W., Kenney J.P.L., Zhou Q., Lalonde S.V., Konhauser K.O., 2016, Cell Surface Acid-Base Properties of the Cyanobacterium Synechococcus: Influences of Nitrogen Source, Growth Phase and N:P Ratios, Acta 187, 179– 194.

Masindi, V., Muedi, K. L., 2018, 'Environmental Contamination by Heavy Metals', in H. E. M. Saleh, R. F. Aglan (eds.), Heavy Metals, IntechOpen, London. Panchanadikar V.V., Das R.P., 1994, Biosorption Process for Removing Lead (II) ions from Aqueous Effluents Using Pseudomonas sp. International Environmental Studies 46, 243-250.

Peens J., Wu W.Y., Brink H.G., 2018. Chem. Eng. Trans. 64, 439–444.
Selatnia A., Boukazoula A., Kechid N., Bakhti M. Z., Chergui A., Kerchich Y., 2004, Biosorption of lead (II) from aqueous solution by a bacterial dead Streptomyces rimosus biomass. Biochem. Eng. J., 19: 127-135.
Statista., 2019, Lead reserves worldwide by country in million metric tons.
Tong S., von Schirnding Y.E., Prapamontol t., 2000, Environmental Lead Exposure: A Public Health Problem of Global Dimensions. Bulletin of the World Health Organization, Vol. 78, pp. 1068-1077.
Van Veenhuyzen B., Chirwa E.M., Brink H.G., 2021a, Microbial Pb(II) Precipitation: the Role of Biosorption as a Pb(II) Removal Mechanism, Chemical Engineering Transactions, 86, 181-186.
Van Veenhuyzen B., Tichapondwa S., Hörstmann C., Chirwa E., Brink H.G., 2021b, High capacity Pb(II) adsorption characteristics onto raw- and chemically activated waste activated sludge. Journal of Hazardous Materials, 416, 125943.
Waihung L., Hong C., Kim-Hung L., Shu-Ping B.,1999, A comparative investigation on the biosorption of lead by filamentous fungal biomass, Chemosphere Volume 39, 2723-2736.
Xiaoping Y., Xiaoning C., 2013, Adsorption characteristics of Pb(II) on alkali treated tea residue, Water Resources and Industry, Volume 3,1-10.