Journal of Geochemical Exploration 182 (2017) 275–278

Contents lists available at ScienceDirect

Journal of Geochemical Exploration

journal homepage: www.elsevier.com/locate/gexplo

Sustainable remediation of heavy metal polluted soil: A biotechnical interaction with selected bacteria species

Emenike C.U. [a,b,*], Agamuthu P. [b], Fauziah SH. [b]

[a] Institute of Research Management & Monitoring, University of Malaya, 50603 Kuala Lumpur, Malaysia
[b] Institute of Biological Sciences, University of Malaya, 50603 Kuala Lumpur, Malaysia

ARTICLE INFO

Article history:
Received 8 September 2016
Accepted 3 October 2016
Available online 4 October 2016

Keywords:
Bacteria species
Bioaugmentation
Bioremediation
Heavy metals
Landfill leachate
Pollution soil

ABSTRACT

When the inevitable nature of waste generation is considered detrimental to the environment, it becomes imperative to develop waste management options that do not only take care of disposal, but will ensure sustainability and environmental safety. Due to the persistent nature of heavy metals in landfill leachate contaminated soil, resident microbes need bioengineering with the aim of evaluating a biotechnical approach suitable for the bioremoval and/or immobilization of heavy metals in contaminated soil. Utilized bacterial strains optimized the reduction of extractable Al (72%), Cu (88%), Cd (41%), Mn (65%) and Pb (71%) ions from leachate-contaminated soil.

1. Introduction

Waste generation is a common phenomenon associated with human and capital development, industrialization and socio-economic dynamism. When the inevitable nature of waste generation is considered detrimental to the environment, it becomes imperative to develop waste management options that do not only take care of disposal, but will ensure sustainability and environmental safety. In as much as some waste disposal/management options exist, especially incineration and composting, yet the use of landfills remain the most widely adopted option. In fact, in some parts of Asia, especially Malaysia, more than three hundred and six landfills are available as against the very few incinerating plants around, which in most cases fail to perform optimally. Landfills are known to accommodate almost every material in the solid waste stream especially among the developing nations (Agamuthu and Tanaka, 2014), and the municipal solid waste (MSW) is the most significantly disposed waste to landfills.

However, one of the major issues associated with MSW landfilling is the generation of leachate. The presence of this liquid substance, leachate, is often a subject of concern to both landfill managers and the environmental protectionists due to the impact of leachate on the environment, especially, on ground water, surface water and soil (when not properly handled). Leachate composition can vary across landfills regardless of the status/condition of operation, yet its characterization commonly shows the presence of inorganic macro-compounds, dissolved organic matter, high biochemical oxygen demand (BOD) and chemical oxygen demand (COD) loads (Ludwig et al., 2003; Emenike et al., 2012). Furthermore, heavy metals detected show higher concentrations in discharged leachate (Fauziah et al., 2013) and leachate contaminated soils.

Therefore, when the negative impact of heavy metal is reviewed in terms of adverse effect on human physiology and biological systems (Kobya et al., 2005), it becomes necessary to identify option(s) for its removal and/or immobilization within leachate contaminated soil. In most developing nations, where the landfill types are either mere dumpsites or non-sanitary landfills, leachate percolation of soil profile is inevitable and prevalent due to the vertical and lateral migratory nature of leachate (Jaffar et al., 2009). Most heavy metals have high affinity for other elements like sulphur, thereby disrupting enzyme functions of living cells via formation of bond, or even the use of ions to bind cell membranes that initiate interference within the cell transport processes (Manahan, 2004).

Due to the foregoing, the use of a biotechnical and environmentally safe approach is necessary for the remediation of heavy metal contaminated soil, especially in pollution induced by leachate, because it is a heterogeneous liquid. There is no doubt that the adoption of biotechnical approaches such as bioremediation is most welcome due to its sustainability potential, yet many biological techniques are not only relatively new but are inherently difficult to standardize most times due to the involvement of living organisms, especially microbes. Microbes relatively survive in landfill environment and such may suggest that favourable condition for metabolism exists. However, it is still necessary to optimize the impact of bacterial species on the bioremediation

* Corresponding author.
E-mail addresses: emenike@um.edu.my, emenikecu@gmail.com (C.U. Emenike).

http://dx.doi.org/10.1016/j.gexplo.2016.10.002
0375-6742/

Table 1
Isolated microbes and the distribution in the microcosms.

Isolated bacteria	Treatment A (TA)	Treatment B (TB) (Control experiments)
Bacillus sp.	*Bacillus sp.*	NU
Psuedomonas sp.	NU	NU
Stenotrophomonas sp.	NU	NU
Flavimonas sp.	NU	NU
Lysinibacillus sp.	*Lysinibacillus sp.*	NU
Acinetobacter sp.	NU	NU
Brevundimonas sp.	NU	NU
Microbacterium sp.	NU	NU
Rhodococcus sp.	*Rhodococcus sp.*	NU

"NU" means not used (meaning that the isolated bacteria species was not part of a particular treatment)

of soil contaminated with metals. Sometimes, metals distinctively perturb soil microbial biomass and activity and even reduce the composition and diversity of the microbial community of soil (Xu et al., 2015). Hence, this study evaluated the potentials of landfill resident microbes towards the bioremoval and/or immobilization of heavy metals in contaminated soil.

2. Materials & methods

2.1. Soil samples collection and characterization

The experiment undertaken at a laboratory scale involved the use of two soil sources; leachate soaked soil from landfill (3°13.78′N, 101°39.72″E) and non-contaminated garden soil (3°7′724.15′N, 101°39′16.79″E). While the first soil source was required for microbial isolations, the later was utilized for the setup of the bioremediation microcosm. Soil samples collected were in accordance to 2004 ASTM E-1197 standard guidelines for conducting terrestrial soil-core microcosm test (Sprocati et al., 2011). Samples were adequately replicated to accommodate variability and ensure homogeneity.

2.2. Microbial isolation

In order to identify the possibility of microbial survival in leachate-contaminated environment, microbial isolation was carried out prior to the study presented here. This is because the presence of microbes in the landfill environment may imply the persistent nature of the microbes, hence the potential involvement in some biological processes taking place within the contaminated soil. Hence, 1 g of soil was previously mixed with 0.9% NaCl and the suspension vortexed for 2 h at 150 rpm using Lab-line 3521 orbit shaker. Serial dilutions were plated (Kauppi et al., 2011) on nutrient agar (NA) and subsequently incubated for 48 h at 33 °C. Single colonies were grown separately on freshly prepared NA to obtain discrete pure cultures that were eventually identified using Biolog GEN III MicroPlate protocol (Bochner, 1989a; Bochner, 1989b).

Table 2
Initial and residual mean concentrations of heavy metals from the bioremediation of leachate contaminated soil.

Heavy metals	Initial concentrations (mg/kg)	Mean residual concentrations (mg/kg) and level of reduction (%)			
		Treatment A		Treatment B	
Al	51,200	14,143	72%	20,967	59%
Cd	1.70	1.00	41%	1.00	41%
Cu	24.10	3.00	88%	11.00	54%
Mn	129	45.00	65%	98.00	24%
Pb	206.8	60	71%	121	41%

n = 3

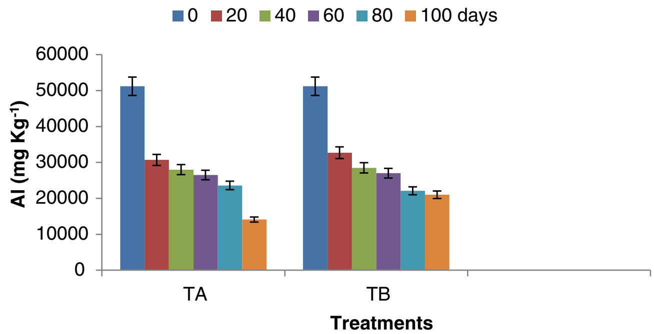

Fig. 1. Reduction of Al across experimental duration (100 days).

2.3. Microbial formulation and bioaugmentation set-up

Bioaugmentation was the preferred method of bioremediation adopted in this study considering that sometimes microbes require some manipulation in order to optimally metabolize in presence of pollutants. The formula used in the bioaugmentation experiment contained three strains of bacteria isolated from the leachate soaked soil (contaminated landfill site). Each strain was re-grown as a pure culture and discrete suspensions at the same physiological phase (1.3 ABS at 600 nm) were then pooled in equal proportions to set-up inoculum for bioaugmentation. Soil microcosms of two treatments (A & B) were prepared by introducing 10% *v*/*w* of leachate concentration into the non-contaminated garden soil. Treatment A was inoculated with the three bacterial strains, whereas treatment B had no microbial addition in order to serve as a control. Portions of soil microcosms were sacrificed every 20 days (until 100 days) for onward metal analysis. Reported duration was to capture the most active period of the microbes (Emenike et al., 2016). Each soil sample taken for analysis was acid-digested (Hseu et al., 2002) using Multiwave 3000 microwave digester, while Optima 530,00 DV was used to obtain the elemental concentrations of Al, Cd, Cu, Mn and Pb according to USEPA 3050 B.

Data obtained were further processed to calculate the percentage of heavy metals removal using;

$$\%\text{of heavy metal removal} = \left(\frac{C_{0(x)} - C_{F(x)}}{C_{0(x)}}\right) \times 100\% \quad (1)$$

where

$C_{0(x)}$ = initial concentration of metal "x" (Al, Cd, Cu, Mn or Pb) in the soil at the start of experiment

$C_{F(x)}$ = final concentration of metal "x" (Al, Cd, Cu, Mn or Pb) in the soil at the end of experiment.

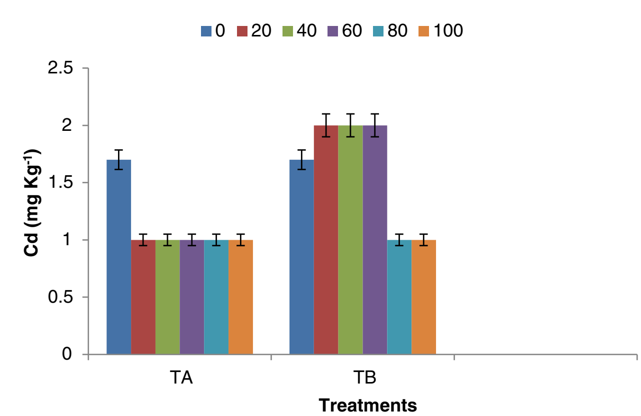

Fig. 2. Reduction of Cd across experimental duration (100 days).

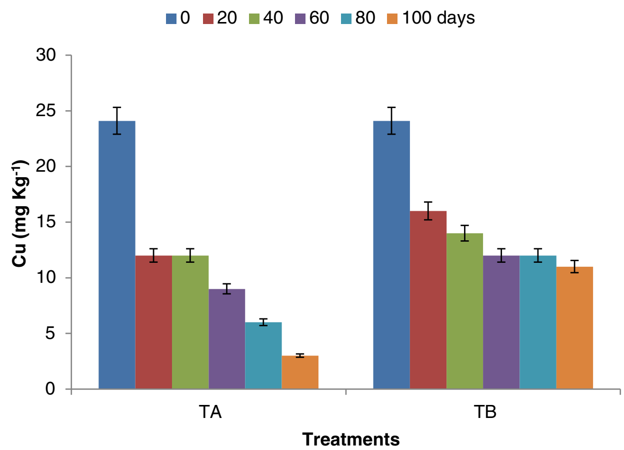

Fig. 3. Reduction of Cu across experimental duration (100 days).

Similarly, first order kinetic model used was to compare the heavy metals removal rate constant;

$$K = -\frac{1}{t}\left(\ln\frac{C}{C_0}\right) \quad (2)$$

where

K = first order rate constant for metal uptake per day

t = time in days

C = concentration of residual metal in the soil (mg/Kg)

C_0 = initial concentration of metal in the soil (mg/Kg).

Following the generation of the heavy metals removal rate constant, the Half-life ($t_{1/2}$) was calculated according to Eq. (3) as shown and the model is based on the assumption that removal rate of heavy metals correlated with heavy metals pool size in soil;

$$\text{Half-life}\ (t_{1/2}) = {}^{\ln(2)}\!\Big/_{K} \quad (3)$$

3. Result and discussion

Result of the microbial isolation from the previous study showed that nine (9) bacterial species were found (Table 1). Therefore, three (3) of these species, namely *Bacillus sp*., *Lysinibacillus* sp. and *Rhodococcus* sp. were selected for this study. These species were gram-positive microbes, and the previous use of all the nine species did not perform optimally in heavy metal remediation (Emenike et al., 2013).

The isolated microbes have only little known importance towards enhancing bioremediation of both organic and inorganic compounds, yet their survival within a contaminated soil may be an indication of favourable metabolic impact under the influence of pollutants. Since heavy metals have the ability to inhibit the smooth functioning of a biological system (Kobya et al., 2005) and even disrupt enzyme activities in a living cell (Manahan, 2004), it may imply that the isolated bacterial species possess resistance to metal toxicity. Such microbes might enhance chemical transformations that are required for the bioremoval/immobilization of heavy metals from soil compartments.

Therefore, the leachate-induced soil used for the bioremediation microcosms was characterized prior to start of the experiment. The heavy metals concentrations at the start of the experiment and the residual concentrations are in Table 2. The initial concentrations were above the allowable limits for non-contaminated soil when compared with Malaysian guidelines for contaminated lands (Department of Environment, 2009).

The residual concentrations of the heavy metals in Treatment A (TA) were less than the levels found in Treatment B (TB). It is possible that TB depended on normal soil microflora for the heavy metal-soil interaction. Treatment A showed an enhanced metabolic activity due to the bioaugmentation of the microcosm using inoculums from *Bacillus sp*., *Lysinibacillus sp*. and *Rhodococcus sp*. Statistical significant differences ($p < 0.005$) existed when both treatments were compared in relation to the heavy metal reductions; Pb ($p = 0.017$), Mn ($p = 0.008$), Cu ($p = 0.003$), except for Al ($p = 0.24$) and Cd (not established).

Further analysis on the bioremediation across the experiment's duration (20 days intervals) revealed a reduction trend in both treatments (Figs. 1–5). After plotting the Cu, Mn and Pb reductions in both microcosms, TA demonstrated a sharp slope than TB. This might imply the relative importance of microbes towards the mentioned heavy metals. In addition, the influence of the microbes as introduced into the soil microcosm correlated with the fact that the bioremediation of polluted soils

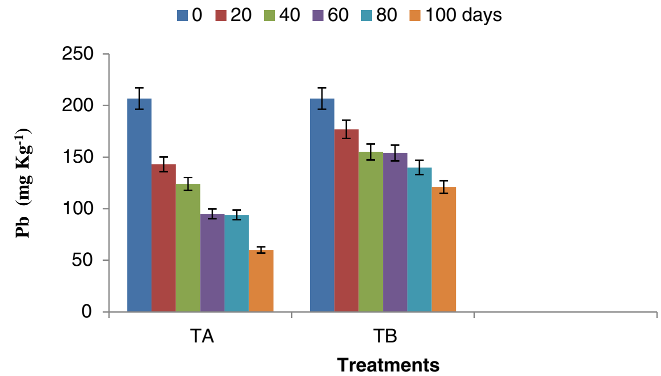

Fig. 5. Reduction of Pb across experimental duration (100 days).

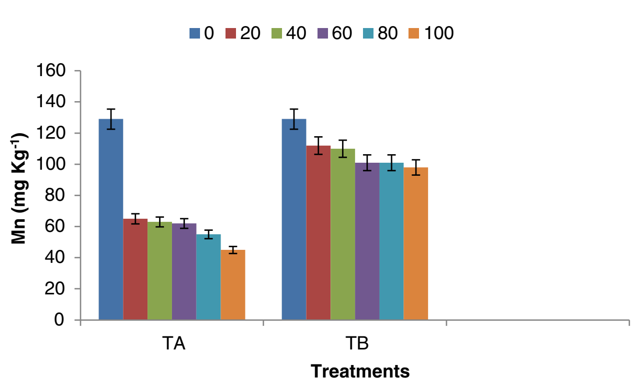

Fig. 4. Reduction of Mn across experimental duration (100 days).

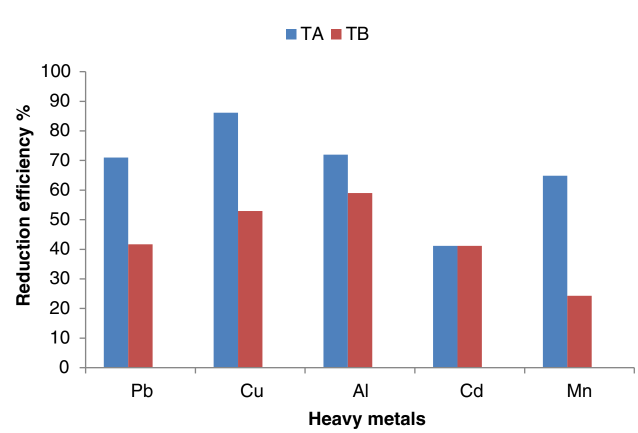

Fig. 6. Percentage of heavy metals reduced during bioremediation.

Table 3
Heavy metals removal rate constants and half-life.

Heavy metals	Treatment A (TA)		Treatment B (TB)	
	Removal rate constant (K) (day^{-1})	Half-life ($t_{1/2}$) (days)	Removal rate constant (K) (day^{-1})	Half-life ($t_{1/2}$) (days)
Al	0.0127	54.59	0.0089	77.88
Cd	0.0053	130.78	0.0053	130.78
Cu	0.0212	32.7	0.0078	88.87
Mn	0.0105	66.01	0.0027	256.72
Pb	0.0124	58.9	0.0053	130.78

using microbes has been widely reported (Emenike et al., 2013; Sprocati et al., 2011).

Similarly, Fig. 6 showed the extents to which both metals were removed from the individual elements. >80% of Cu reduction in the bioaugmented microcosm was evident. In terms of optimal reduction or transformation, the best result was expected in TA due to higher microbial diversity from the inoculums. However, this was not to imply that microbial specificity might be the cause in this experiment.

The mechanism behind the bioremoval in TA might be due to imbued interactions that exist among microbes upon manipulation of diversity and cell concentrations. *Bacillus* sp. previously influenced the removal of Pb (77%) and Cu (8%) from a mine extract (Babu et al., 2013). Similarly, the fact that *Lysinibacillus* sp. is characterized of a hex-histidine tag (His_6-tag) at the C-terminus of its S-layer protein SbpA, may have caused its metal binding property to be more expressed when blended with *Bacillus* sp. and *Rhodococcus sp*.

In addition, the calculated heavy metals removal rate constant (k) and the result of the corresponding half-life ($t_{1/2}$) further buttressed the degree of activities within the experimental set-up (Table 3). It showed that the soil amended with the inoculum (TA) recorded the highest reduction rate of 0.0212 day^{-1} and half-life of approximately 33 days. This might be from the microbes that may discretely have metal removal capacity (Babu et al., 2013). The half-life (time it will take for half of the metal to reduce) is a function of bioremoval rate constant; hence the TA in regards to Cu recorded the least time (approx 33 days). Such development indicates a significant relationship between rate of bioremoval and concentration of heavy metal in the leachate-polluted soil. Hence, the low bioremoval rate and subsequent higher half-life in TB could be from the reduction in the activity of normal soil microbes in the soil at such pollution level (Adesodun and Mbagwu, 2008). The non-amended soil showed some degree of bioremoval which may be due to the presence of normal microflora, but its inability to enhance bioremoval like TA microcosm may be based on the fact that bioremediation is successful in soil remediation only when the pollutant concentration is moderate. Therefore, in higher pollutant situation, bioaugmentation may be required to strengthen microbial activity.

4. Conclusion

Bioremoval and/or immobilization of heavy metals in leachate-polluted soil is possible with use of designate microbes. However, manipulation of bacteria in relation to diversity matching/blending and cell concentration avail more biotechnical mechanism for biotransformation, bioreduction or bioremoval of heavy metals. The study has projected the blending of *Bacillus sp*., *Lysinibacillus* sp. and *Rhodococcus sp*. for the optimal removal of selected metals in leachate-polluted soil of a landfill environment.

Acknowledgement

The authors appreciate the research funds from the Institute of Research Management and Monitoring, University of Malaya (PV067/2012 and RP011A-14SUS), and International Foundation for Science (W/5095-1). Authors declare no conflict of interest.

References

Adesodun, J.K., Mbagwu, J.S.C., 2008. Biodegradation of waste lubricating petroleum oil in a tropical alfisol as mediated by animal droppings. Bioresour. Technol. 99, 5659–5665.
Agamuthu, P., Tanaka, M., 2014. Municipal Solid Waste Management in Asia and the Pacific Islands (Challenges and Strategic Solutions). Springer.
Babu, A.G., Kim, J., Oh, B., 2013. Enhancement of heavy metal phytoremediation by Alnus firma with endophytic Bacillus thuringiensis GDb-1. J. Hazard. Mater. 250–251, 447–483.
Bochner, B.R., 1989a. Sleuthing out bacterial identities. Nature 33, 157–158.
Bochner, B.R., 1989b. "Breathprints" at the microbial level. ASM News 55, 536–539.
Department of Environment (DOE), 2009. Contaminated Land Management and Control Guidelines N0 1: Malaysian Recommended Site Screening Levels for Contaminated Land. Ministry of Natural Resources and Environment, Malaysia (ISBN: 97B-983-3895-19-9).
Emenike, C.U., Agamuthu, P., Fauziah, S.H., 2013. Bioaugmenting leachate polluted soil for optimal removal of heavy metals. Proceedings of the 2013 International Conference on Materials, Architecture and Engineering Technology (ICMAET). Beijing, China (19 – 20th December 2013).
Emenike, C.U., Agamuthu, P., Fauziah, S.H., 2016. Blending Bacillus sp., Lysinibacillus sp. and Rhodococcus sp. for optimal reduction of heavy metals in leachate contaminated soil. Environmental Earth Sciences 75, 26. http://dx.doi.org/10.1007/s12665-015-4805-9.
Emenike, C.U., Fauziah, S.H., Agamuthu, P., 2012. Characterization and toxicological evaluation of leachte from closed sanitary landfill. Waste Manag. Res. 30 (9), 888–897.
Fauziah, S.H., Emenike, C.U., Agamuthu, P., 2013. Leachate risk and identification of accumulated heavy metals in P. sutchi. Waste Manag. Res. 10, 75–80.
Hseu, Z.Y., Chen, Z.S., Tsai, C.C., Tsui, C.C., Cheng, S.F., Liu, C.L., Lin, H.T., 2002. Digestion methods for total heavy metals in sediments and soils. Water Air Soil Pollut. 141, 189–205.
Jaffar, Y.M.A., Lee, Y.H., Salmijah, S., 2009. Toxicity Testing and the effect of landfill leachate in Malaysia on Behaviour of Common Carp (Cyprinus carpio L., 1758; Pisces, Cyprinidae). Am. J. Environ. Sci. 5 (3), 209–217.
Kauppi, S., Sinkkonen, A., Romantschuk, M., 2011. Enhancing bioremediation of diesel-fuel-contaminated soil in a boreal climate: comparison of biostimulation and bioaugmentation. Int. Biodeterior. Biodegrad. 65, 359–368.
Kobya, M., Demirbas, E., Senturk, E., Ince, M., 2005. Adsorption of heavy metal ions from aqueous solutions of activated carbon from apricot stone. Bioresour. Technol. 96, 1518–1521.
Ludwig, C., Hellwed, S., Stucki, S., 2003. Municipal Solid Waste Management. Strategies and Technologies for Sustainable Solutions. Springer-Verlag, Berlin Heidelberg, New York.
Manahan, S.E., 2004. Environmental Chemistry. 8th Ed. CRC Press LLC, Boca Raton.
Sprocati, A.R., Alisi, C., Tasso, F., Marconi, P., Sciullo, A., Pinto, V., Chiavarini, S., Ubaldi, C., Cremisini, C., 2011. Effectiveness of a microbial formula, as a bioaugmentation agent, tailored for bioremediation of diesel oil and heavy metal co-contaminated soil. Process Biochem. 47 (11), 1649–1655.
Xu, C., Peng, C., Sun, L., Zhang, S., Huang, H., Chen, Y., Shi, J., 2015. Distinctive effects of TiO2 and CuO nanoparticles on soil microbes and their community structures in flooded paddy soil. Soil Biol. Biochem. 86, 24–33.