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Removal of Lead Ions from Water Using Pellet Generated from *Bacillus subtilis* Isolated from Gold Mining Site in Niger State

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Abstract
This work concentrated on the isolation of lead tolerant strains of bacteria, identification of the isolated strain with the highest lead tolerance capacity using microgen identification kit. Also, the efficacy of the generated pellet (dead cell) in the bioremoval of lead from aqueous solutions was determined. A total of nine bacteria were isolated from soil collected from gold mining site in Kontagora metropolis, Niger State. Of the nine isolates, only *Bacillus subtilis* (KO1) possess high tolerance capacity for high levels of lead ions. The pellet generated from the *Bacillus subtilis* (KO1) strain was then used to adsorb lead ions from synthetic ion solutions. The isolate's removal efficiency was enhanced by optimizing several physical conditions (pH, temperature, initial lead concentration and contact time). The best optimized adsorption removal efficiency (>90%) was found at pH 3, temperature 40°C with 100 mg/L of initial concentration of lead after 3 hours of treatment. The use of the pellet generated from eco-friendly *Bacillus subtilis* (KO1) has great potential and additional benefits in terms of lead removal.
Keywords: *Bacillus subtilis*; lead; adsorption; pellet; optimization

INTRODUCTION

The accumulation of heavy metals in the environment has become a serious threat to our ecosystem and thus, necessitating immediate action. This has a deteriorating effect on the quality of water sources, the atmosphere, and agricultural crops and thus, has become a great risk to public health (Dong *et al.*, 2011; Acosta *et al.*, 2011). Heavy metal pollution is a direct consequence of both natural and man-made sources (Ako *et al.*, 2014; Lima *et al.*, 2016). Several studies have shown that man-made sources, particularly metal ores mining and smelting operations, are responsible for the majority of heavy metal pollutions in the natural environment (Facchinelli *et al.*, 2001; Ako *et al.*, 2014; Lima *et al.*, 2016). Due to mining activities in various countries, the upsurge in heavy metals contamination of water and lands has become a significant global challenge (Ehsan *et al.*, 2013; Li *et al.*, 2014; Paul, 2017). Furthermore, large amounts of untreated soil resulting from mining and smelting processes end up creating another massive issue that can pose a risk to the natural environment (Klein *et al.*, 2014).

One of several heavy metals being discharged into the natural environment is lead (Ehsan *et al.*, 2013; Paul, 2017). Lead is famous for its numerous industrial uses, and it can be found in our natural environment at the conclusion of many industrial operations. Only a trace concentration of lead is found naturally in the environment (Shivani *et al.*, 2020). However, the threshold has risen solely through pollution caused by anthropogenic sources (Naja and Volesky, 2009; Jaishankar *et al.*, 2014; Shivani *et al.*, 2020). Lead is most frequently ingested by children and adults through lead-based paints and work environment respectively. Ingestion of lead through external sources causes excess adverse effects in the human body, including kidney and brain damage, blood pressure elevation, infertility in men, intellectual disabilities and psychosocial distraction in children (Tong *et al.*, 2000; Morais *et al.*, 2012; Rodriguez-Tirado *et al.*, 2012; Sardar *et al.*, 2013).

Membrane technologies, ion exchange, electrochemical methods, filtration, precipitation and chemical redox reactions are all common techniques for the removal of heavy metals, but they are costly and ineffective, particularly, when the metal amount in solution is low (within 1–100 mg/L) (Witek-Krowiak, 2013; Shivani *et al.*, 2020). As a result, developing eco-friendly separation methods that are effective, economically cheaper, and selective for the elimination of lead ions present in the environment is highly imperative and necessary.

In a metal-polluted environment, microorganisms (bacteria, fungi and algae) show tolerance to the toxic effects of existing heavy metals on their own and are effective in heavy metal sequestration from the polluted environment (Wong *et al.*, 1993; Giller *et al.*, 1998). The separation of toxic heavy metals from contaminated commercial and domestic wastewater has already begun on an industrial scale with the use of specific microorganisms (Igiri *et al.*, 2018). At high levels of potentially toxic metals, these microorganisms are known to adapt or mutate in order to survive (Mishra *et al.*, 2017).Microorganisms that are exposed to heavy metal stress on a regular basis have developed mechanisms to adapt to the metal contaminants. Transport across the cell membrane, precipitation, entrapment in extracellular capsules, adsorption to the cell walls, redox reactions and complexation are some of the biological responses microorganisms have to these molecules (Mishra *et al.*, 2017; Rehan and Alsohim, 2019). The availability of multiple anionic structures, like chitin and glucan provide microorganisms' cell surfaces a negative charge, allowing them to bind metal cations (Maghsoodi *et al.*, 2007).

Bacillus subtilis is an aerobic bacterium that has a broad variety of industrial uses and is capable of producing enzymes such as proteases and amylase (Da Silva *et al.*, 2014). Because of its simple genetic nature, it was used often times as a prototype agent in laboratory experiments (Harwood, 1992; Roy *et al.*, 2012; Da Silva *et al.*, 2014). Despite the fact that there are some studies in the literature on the adsorption of heavy metals, along with lead, by various strains of *B. subtilis*, no information on the use of generated pellets of indigenous strains of *B. subtilis* from Nigeria has been reported for lead removal. Hence, in this study, the adsorption ability of pellet of *B. subtilis* isolated from gold mining site to adsorb lead ions that appear most frequently in polluted sites and water was investigated. Furthermore, physical conditions influencing adsorption such as pH, temperature, initial heavy metal concentration and contact time were also investigated.

MATERIALS AND METHODS

Collection of soil samples

Ten soil samples were collected from Kontagora gold mining site, Niger state. At every 10 m interval, one soil sample of 10 g each was collected from the top layer (5–10 cm) using a soil auger. Thereafter, all collected samples were thoroughly mixed in a bowl to create a composite sample (Stefani, *et al.*, 2015). The composite sample of about 100g was placed in a polythene bag and transported immediately to the environmental laboratory of Department of Microbiology, Ahmadu Bello University, Zaria for microbiological analysis.

Isolation of lead tolerant bacteria by enrichment

The method described by Jiang *et al.* (2017) was used with some modifications to isolate lead tolerant bacteria using enrichment technique. Ten grams of the composite sample was added into 100 mL of sterilized distilled water and 2 mL of the solution was inoculated in 20 mL Luria Bertani (LB) (Peptone 10g/L, yeast extract 5g/L, NaCl 10g/L) medium supplemented with 100 mg/L concentration of lead as $[Pb(NO_3)_2]$.The culture was incubated on a rotatory shaker at 28ºC with 160 rpm for 4 hours. After incubation, 0.1 mL of the culture was then plated on LB agar plates supplemented with 100 mg/L concentration of lead as $[Pb(NO_3)_2]$ using spread plate method and incubated at 28ºC for 24 to 48 h. A single strain capable of growing at this condition was selected for further experiments. The isolate was kept in LB agar slants at 4ºC.

Determination of lead tolerance of the isolated population

On LB solid medium, the metal tolerance range for growth was determined. Lead ion (Pb^{2+}) tolerance was tested using final metal concentrations of 100, 150, 200 250 and 300 mg/L. Pure cultures were spread onto LB enriched with each metal concentrations to determine growth, then incubated at 25ºC for 72 h and observed daily. Growth on the plate was then assessed (Jaafar *et al.*, 2016).

Biochemical Characterisation of the Isolate with the best lead tolerance capacity

Gram staining and spore staining procedures were used to determine the morphological properties. Motility, catalase, oxidase, Methyl red-Voges Proskaeur, hydrogen sulphide test, and starch hydrolysis tests were performed (Bergey, 2004; Willey *et al.*, 2008).

Microgen Bacillus-ID for Confirmation of the Bacillus isolate

The Microgen Bacillus-ID identification was used according to the manufacturers' instructions. The substrates included in the test panel were chosen based on a computer-based analysis of all available substrates for the identification of organisms. Each isolates was identified by recording the results of a colour change after 48 h incubation at 30°C and adding appropriate reagents (Indole, Nitrate and VP tests) after 48 hours. The Microgen Identification System Software (MID-60) was then used to analyze the results.

Generation of Bacterial pellet

The method described by Peterson *et al*. (2012) was used to generate bacterial pellet. A single colony from overnight LB plate was inoculated into 100 mL of LB medium and cultured for 24 h at 28°C on a rotatory shaker at 150 rpm. Bacterial pellet was harvested by centrifugation at 9000 rpm for 10min. The supernatant was discarded, and the cells were re-suspended in sterilized phosphate buffered saline (NaCl 8g/L, KCl 0.2g/L, Na_2HPO_4 1.44g/L and KH_2PO_4 0.245 g/L) for washing before being centrifuged twice. The pellet was heat killed for 24 hrsin hot air oven at 60°C and was used for the adsorption experiments.

Effects of optimization conditions on adsorption capacity

The effects of physical conditions such as pH, temperature, initial lead concentration and contact time on adsorption capacity of pellet of *B. subtilis* (KO1) were evaluated. The effect of pH was conducted at pH 2, 3, 4, 5, 6 and 7, adjusted with dilute NaOH and HNO_3 (Alladin, Shanghai, China) and the samples were incubated for 6 h at 30°C in a 100 mg/L solution of lead using 0.2g of bacterial pellet. The effect of temperature was carried out at 20, 25, 30, 35 and 40°C with incubating at the optimized pH for 6 h in a 100 mg/L solution of lead using 0.2g of bacterial pellet. Similarly, the effects of initial concentration of lead (100, 150, 200, 250, 300 and 350 mg/L) and contact time (1, 2,3, 4, 5 and 6 h) were carried out at the optimized pH and temperature. All experiments were repeated thrice.

Lead Adsorption assay

To achieve a final bacterial concentration of 1 g/L, 0.2g of bacterial pellets was suspended in deionized water containing 100mg/L solution of lead as lead nitrate (Alladin, Shanghai, China). The adsorption experiment was carried out in Erhlenmeyer flasks of 250 mL. The mixture was stirred at 150 rpm at 28°C and 10 mL of sample was collected at intervals (1, 2,3, 4, 5 and 6 h), centrifuged at 3000 rpm for 10 min and the supernatant was decanted. The supernatant was then used to measure the remaining concentration of metals by the flame atomic absorbance spectrophotometer. Metal ions removal efficiency Q (%) was calculated using equation (1) (Dai *et al*., 2019).

$$Q = \frac{(Co - Ce)}{Co} \times 100 \quad (1)$$

Co and Ce are the initial and final lead ion concentrations (mg/L).

RESULTS

After 48 hours of incubation, exuberant growth on Lead supplemented 100 mg/L LB agar medium revealed that the collected sample contains lead tolerant bacteria. Nine bacterial isolates were isolated from Kontagora gold mining site, and were selected for further study. The majority (88%) of the nine isolates was sensitive to high concentrations of lead, only one isolate was found to grow richly at higher concentration (250 mg/L) of lead (Table 1).

The cultural, morphological, biochemical characteristics of the highest lead tolerant isolate (KO1) were used to identify the organism, as well as Microgen identification. In comparison with standard description of Bergey's Manual of determinative bacteriology 9th edition, the isolate was characterized to belong to *Bacillus subtilis* (Bergey, 2004; Willey *et al*., 2008).

Figure 1 depicts the effect of pH on the percentage adsorption capacity of pellet generated from *B. subtilis* (KO1). The adsorption increased from 70% at pH 2 to 90% at pH 4, and then decreased significantly as the pH was raised. The experiment result for the effect of temperature is shown in Figure 2. At 40°C, the maximum adsorption was observed to be around 93%. The percentage of sorption increased as the temperature rose. Figure 3 shows the effect of initial lead concentration on the adsorption capacity of pellet generated from *B. subtilis* (KO1). At the initial lead concentration of 100 mg/L, the maximum adsorption was observed to be around 92%. This finding indicates that the sorption percentage decreased as the initial lead concentration increased. Adsorption experiments conducted for various contact times at optimized pH, temperature, and initial lead concentration as shown in Figure 4 illustrates the percent adsorption as a function of time. The pellet's adsorption of lead increased over time. At 3 h, the maximum adsorption of 94% was achieved.

Table 1. Metal tolerance of the isolated strains (most tolerant strain in italics)

Isolates	Lead tolerance (mg/L)				
	100	150	200	250	300
KO1	+++	+++	+++	+++	-
KO2	+++	+++	-	-	-
KO3	+++	+++	++	-	-
KO4	+++	++	-	-	-
KO5	+++	-	-	-	-
KO6	+++	++	-	-	-
KO7	+++	++	-	-	-
KO8	+++	-	-	-	-
KO9	+++	+	-	-	-

Key:- means no growth, +: poor growth, ++: good growth and +++: rich growth.
KO means strains from Kontogora and numbers 1-9 represent the isolate number.

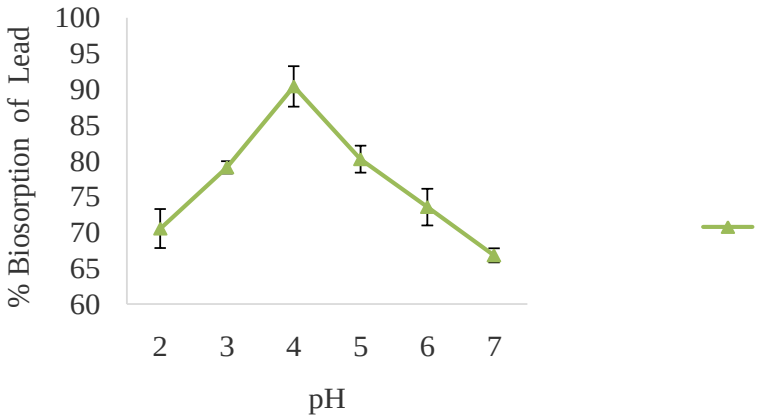

Fig 1: Percentage adsorption of lead ions at different pH

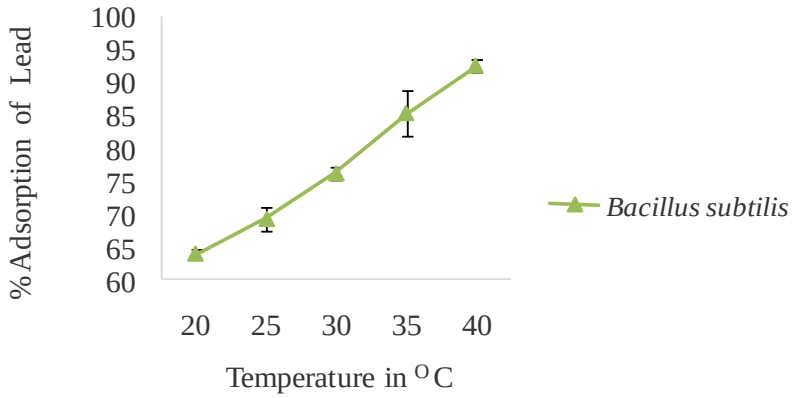

Fig 2: Percentage adsorption of lead ions at different temperature.

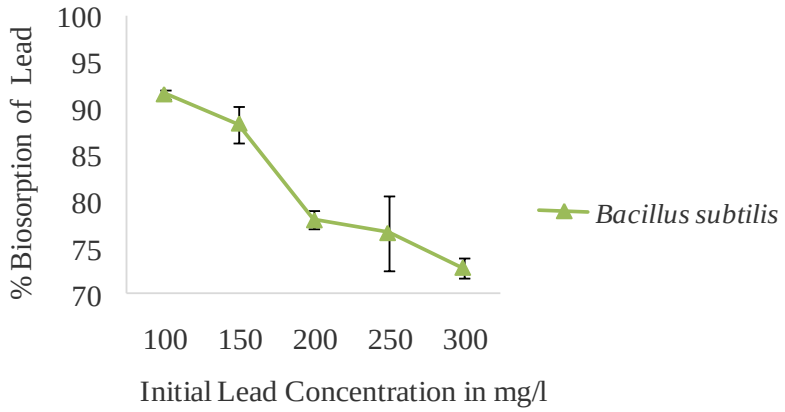

Fig 3: Percentage adsorption of lead ions at different initial lead concentrations.

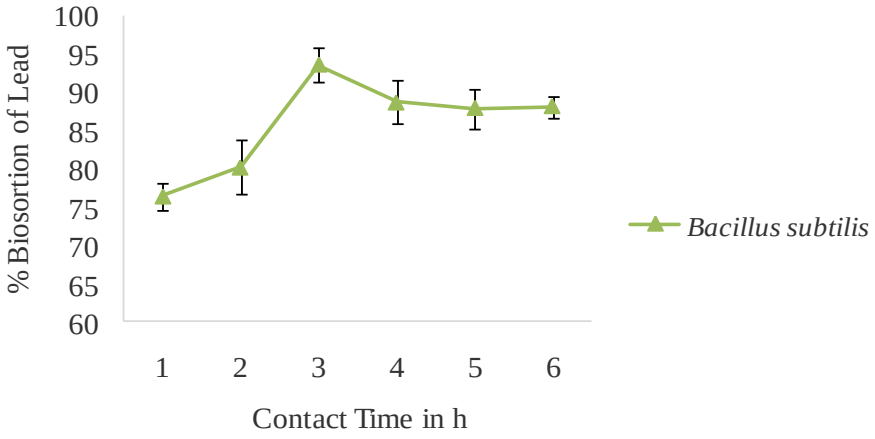

Fig 4: Percentage adsorption of lead ions at different contact time.

DISCUSSION

Nine strains were isolated using a cultivation-dependent method and lead supplemented media. Heavy metals cause toxicity in microorganisms through a variety of mechanisms. Metal-tolerant bacteria can grow in heavy metal-contaminated environments and they can be isolated and selected for use in adsorption processes (Piotrowska-Seget *et al.*, 2005). The ability of the isolate studied to be tolerant to lead could have resulted from genetic mutation and/or adaptation (Pal *et al.*, 2005; Li *et al.*, 2006; Abou-Shanab *et al.*, 2007). This finding suggests that lead tolerant *Bacillus subtilis* can survive in soils contaminated with high concentrations of lead, indicating a high biosorption potential. This supports previous finding that *Bacillus* sp. was found in greater abundance in soils with the highest levels of heavy metal pollution (Ellis *et al.*, 2003).

Increase in adsorption percentage with increasing pH on lead adsorption could be due to most non-saturated or active sites that are positively charged at low pH which prevents lead ions from binding to these sites (Yousaf *et al.*, 2017). Deprotonation occurs when the pH rises owing to the availability of negatively charged hydroxyl ions and the active sites become accessible for metal ion attachment through the ion exchange mechanism, leading to greater absorption rate. The formation of metal hydroxide and metal-ligand complexes, which considerably decrease the quantity of lead ions adsorbed at high pH, is most likely to be the cause for the lower absorption rate observed in the present study (Yousaf *et al.*, 2017). Different authors have noticed similar trends in pH influence on adsorption (Yousaf *et al.*, 2017; Dai *et al.*, 2019).

The percentage adsorption increased as temperature rose, with the highest percentage adsorption occurring at 40°C, indicating that

the phenomenon was endothermic. This finding backs up studies by Salman *et al*. (2015), Yousaf *et al*. (2017), and Dai *et al*. (2019) on a variety of biosorbents. The increase in adsorption observed as temperature rises could be attributed to an increase in unsaturated sites or an increase in metal ion dispersion onto the biosorbent.

The initial concentration of Pb (II) ions had a significant impact on the biosorption capability of *Bacillus subtilis* KO1 pellets. However, as the initial concentration of Pb (II) ions increased, the percentage biosorption of lead ions decreased. At the lowest concentration of 100 mg/L, the highest percentage adsorption of Pb (II) (92%) was observed. This observation could be due to the fact that there are more active and non-saturated sites available on the biosorbent for lead ions at low concentrations. When the concentration of lead ions was increased, the numbers of active sites in *Bacillus subtilis* KO1 were limited, resulting in a large number of lead ions compared to the number of active sites. As a result, the ratio of unsaturated sites decreased, and more lead ions were unable to combine in the solution, lowering the lead ion adsorption rate (Pal *et al*., 2006). This result is in line with what other researchers have discovered (Pal *et al*., 2006; Dai *et al*., 2019).

For lead biosorption, the sorption percentage increased with increasing time up to 3 h. At 3 h, it had a 94 percent sorption rate and remained almost constant with minor fluctuations. This adsorption trend was in agreement with previous studies on heavy metal biosorption by various biosorbents (Fiol *et al*., 2006; Wang *et al*., 2010; Yousaf *et al*., 2017; Dai *et al*., 2019), but the adsorption equilibrium time differs. The disparities in results could be attributed to the varying quantities of bacteria pellets used, wide variety of bacteria used, the differing experimental designs and many more.

In biosorption experiments, bacterial cell pellets have several advantages over live cells, including the potential to remediate large amounts of water with lower metal levels, less time consumption, accessibility without any significant side effects (Aksu, 2005; Hemambika *et al*., 2011; Shivani *et al*., 2020). Furthermore, the pellets (dead cells) do not require constant nutrient requirements and are not significantly impacted by hazardous wastes. It can be reconstituted and used over and over again. Therefore, the use of the microbial cell pellet in the study provided additional benefits in terms of lead removal from water.

CONCLUSION AND RECOMMENDATIONS

In the present work, the efficacy of pellet generated from *B.subtilis* (KO1) to remove lead ions from aqueous solution was achieved successfully. The optimum conditions for the adsorption of lead ions was found to occur at pH 4, temperature at 40^{o}C, initial lead ion concentration of 100 mg/L and contact time of 3 h. It can be concluded that *B.subtilis* (KO1) can be used as a potential adsorbent material for the removal of lead ions from aqueous solution. Further study is required to investigate the mechanisms of lead ion removal in greater detail, the kinetics modelled as well as the effects of this *B. subtilis* (KO1) against lead ion toxicity *in vivo*.

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