*UJMR, Volume 5 Number 1, June, 2020, pp 43 - 48* *ISSN: 2616 – 0668*
https://doi.org/10.47430/ujmr.2051.007

**Received:** 12th Nov, 2019 **Accepted:** 18th Dec, 2019

# Optimization of Culture Condition for Biosorption of Lead using *Pseudomonas aeruginosa* isolated from Gold Mining Site of Anka, Zamfara State

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***Abstract***
*Pseudomonas aeruginosa* was used as a biosorbent for biosorption of lead from heavy metal contaminated gold mining soil of Anka, in Zamfara State. The effects on the degree of biosorption by *Pseudomonas aeruginosa* were studied: these include Contact time, pH, Temperature, Biomass load and Agitation speed. The effects of contact time were studied at 24, 48, and 72h. Results show highest uptake (98.3%) of lead at 48h and the least (97.8%) at 24h. Effect of pH studied at pH 3, 4, 5, 6, and 7, with pH 6 recording the highest lead removal of 99.0% while pH 3 recorded the least percentage biosorption. The effect of Temperature was studied at 25, 35, 45, 55, and 65$^0$C, the highest percentage biosorption (99.3%) recorded at 55$^0$C and the lowest 98.1%) was at both 35 and 45$^0$C. Effect of biomass load was studied using different volumes (0.5, 1.0, 1.5, 2.0, and 2.5ml) of the innoculum: generally the values obtained did not show variation with change in biomass load. Effect of agitation speed was studied at 2000, 2500, and 3000rpm; the highest lead removal was at 2000 rpm. Biosorption of heavy metals is an excellent technology and represents a potentially cost-effective way for heavy metal decontamination from the environment.
**Key words**: Biosorption, Lead, Heavy metals, Soil, Innoculum.

## INTRODUCTION

Soil being a universal sink holds the largest portion of environmental contaminants, contamination occurs from several routes including gold mining, electroplating, accidental discharge and by-products or residues from industries etc. In particular wastes from metallurgy and mining industries contain various heavy metal ions. These waste are discarded which result in detrimental soil contamination. The accumulation of heavy metals in soils can cause reduction in soil fertility through their adverse impact on heterogeneous microbial communities inhabiting the soils. Heavy metals are also known to cause undesirable effect on both plants and animals via food chains (Dutton and Fisher 2011; Takahash *et al*., 2012). Most heavy metals are hazardous to humans and they include lead (Pb), mercury (Hg), cadmium (Cd), arsenic (As), copper (Cu), zinc (Zn), and chromium (Cr).

Such metals are found naturally in soil in trace amounts and when concentrated in particular area, they present a danger. Arsenic and cadmium, for instance can cause cancer, mercury can cause mutations and genetic damage while lead, copper and mercury can cause brain and bone damage and are accumulated in the kidneys, neurological tissues and the liver (Tchounou, 2003). Lead is one of the most precious heavy metal used widely, in many industries in the production of electronics crystal glasses and batteries. Lead consumption has been increasing at a high rate globally due to the increase demand of cars and power assisted machines. Recently a relationship between high blood levels and lead pollution in gold mining areas has been proclaimed as a serious problem.

Some reports have suggested that many children living in the areas near mining industries developed symptoms of lead poisoning. Therefore the remediation of soil contaminated with lead is of paramount importance.

The increasing concern about the contamination of soil by heavy metals has stimulated a large number of researches to find possible ways to remove these toxic substances from the environment. To overcome the heavy metal pollution researchers are often interested in the most optimum and cost effective method to remove heavy metals from environment. Some of the methods employed, or studied, include precipitation, filtration, coagulation, ion-exchange, magnetic fields, fluidized bed reactor, ion flotation, reverse osmosis and adsorption. However, there are some disadvantages with these approaches.

Incomplete metal removal, high reagent and energy requirement, and generation of toxic sludge or other wastes that require careful disposal. Hence the need for imperative for a cost effective treatment method that is capable of removing heavy metals from the environment, cannot be overemphasized; (Ahalya, *et al.*, 2003), (Das, *et al.*, 2008). One of the methods that is receiving attention is biosorption. Various biomaterials like bacteria (Samarth, *et al.*, 2012), and Fungi (Pavani, 2013), *et cetera.*, have been investigated in prior research.

The purpose of this current research is to assess the potential of *Pseudomonas aeruginosa* in bioremediation of lead contaminated soil of Anka in Zamfara State.

## MATERIALS AND METHOD

**Collection of Sample:** Soil sample was collected in a clean polyethylene bags from the surface of the gold mining area of Anka, in Zamfara State Nigeria.

**Isolation of Organism**: The collected soil sample obtained was used to prepare a soil slurry by adding 1gram of the soil to 9ml of sterile distilled water and was mixed for 15minute, 0.1ml of the serially diluted sample were cultured on nutrient agar by method of spread plate and incubated at 37◦C for to 24h. The colony was sub-cultured to obtain a pure culture and was stored in an agar slant for further use.

**Preparation of metal solution**

Stock solutions of lead nitrate, $(Pb(NO_3)_2$ was prepared by dissolving 0.1g of the heavy metal salts in 1000 ml of distilled water in a conical flasks. The flask was shaken vigorously for fifteen minutes and then allowed to stand for one day in order to obtain a whole dissolution of the salts. The initial lead concentration was determined using the AAS (Atomic Absorption Spectrophotometer).The pH of the solution was adjusted to pH 7 using sodium hydroxide (NaOH) and hydrochloric acid (HCl). The solution was stored in refrigerator for further use.

**Experimental Design**

The soil sample was dried at $100^0$C for 24h and autoclaved for 30 minutes at $124^0$ C to kill the indigenous microorganism. The experiment was carried out in 250 ml flasks to which 50 g of the soil sample was weighed in three different conical flask containing 100 milliliter of sterile nutrient broth, contaminated with heavy metal (Pb). Sample was inoculated with 15 ml biomass of *Pseudomonas aeruginosa* from exponential phase. The metal pH solution was adjusted to the pH value of 7 before the addition of the biomass to the solution. This was done by adding appropriate amount of NaOH and HCl respectively, and the pH reading was taken using pH meter. The conical flasks were incubated at a temperature of $37^O$C. Fifteen (15 ml) of the samples were withdrawn from each conical flask at specific intervals of 1, 7, 14, and 21, days of inoculation, Each experiment was carried out twice and the mean values were recorded , centrifugation was done at 4000rpm for 5 minutes. After centrifuging, the supernatant was digested in correspondence to their varying concentration using nitric acid of 4ml for the metal solution. The concentration of metal was determined by AAS. The biosorption percentage was determined by Beer Lambert's law. The remaining concentrations of metals in the supernatant were analysed by Flame Atomic Absorbance Spectrophotometer AA 6300ASC (Abioye, 2015).

$$\text{Percentage biosorption (\%)} = \frac{\text{Initial metal concentration} - \text{Final metal concentration}}{\text{Final metal concentration}} \times 100$$

**Optimization of Culture Condition**

One variable-at-a-time optimization of biosorption conditions was carried out in accordance with Abioye (2015).

**Effect of contact time**

The effect of contact time was determined at different time intervals. Two milliliter of freshly prepared inoculums (*Pseudomonas aeruginosa*) was dispersed in 50 ml nutrient broth prepared in three different conical flasks, each of the solution containing 5 mg/l of lead, concentration in 250ml conical flask. The pH was adjusted to 7.5. Cultures were incubated at $37^0$ C for 24, 48 and 72h, Flasks were allowed to attain equilibrium and centrifuged at 4000 rpm for 5 minutes, and the supernatant were analysed for the residual metal content using Flame Atomic Absorbance Spectrophotometer AA6300ASC the mean value was recorded and the mean value was recorded (Abioye, 2015).

**Effect of pH**

To study the effect of pH on the growth of the bacterial isolate, the metal sorption was monitored at pH range 3, 4, 5, 6, and 7. Then 0.1M of NaOH or 0.5M of Hcl was used as pH regulators. Two milliliter of freshly prepared inoculums (*Pseudomonas aeruginosa*) was dispersed in 50 ml each of nutrient broth prepared in three different conical flasks containing 5mg/l of lead concentration in 250ml conical flask. All flasks were maintained at different pH values for 48h in an incubator at temperature of $37^0$C.

Fifteen (15ml) of the solution were withdrawn and centrifuged at 4000rpm for 5 minutes, the supernatant were analysed for the residual concentrations of the metal ions using the Flame Atomic Absorbance Spectrophotometer AA6300ASC and the mean value was recorded (Abioye, 2015).

**Effect of Temperature**

The effect of temperatures was studied at temperature of 25, 35, 45, 55 and 65°C, for 48h. pH of 6.0 was used to monitor the temperature effect on biosorption. One milliliter of freshly prepared inoculums (*Pseudomonas aeruginosa*) was dispersed in 50 milliliter nutrient broth, prepared in conical flasks, each of the solution containing 5mg/l of lead. The samples were incubated for 48h. Fifteen milliliters of each sample was then collected and centrifuged at 4000 rpm for five minutes and the supernatant analysed for residual metal concentration using AAS.

**Effect of biomass Load**

Different biomass load of 0.5, 1.0, 1.5, 2.0, and 2.5ml of innoculum (*Pseudomonas aeruginosa)* were dispersed in 50ml nutrient broth containing 5mg/l metal (pb) concentration in 250ml conical flask. The pH of the solutions was adjusted to 6.0. Flasks were incubated for 48h at temperature of 55ºC. Fifteen (15ml) of the solution were withdrawn and centrifuged at 4000rpm for 5mins. The supernatant were analysed and residual metal concentration were determined by AAS (Abioye 2015).

**Effect of Agitation rate**

The effect of agitation speed was determined at varying agitation rate of 2000 rpm, 2500 rpm, and 3000 rpm for 48h at pH 6.0. One milliliter of the innoculums was dispersed in 50 ml nutrient broth prepared in three different conical flasks, each containing 5mg/l of lead, Thereafter, 15 ml of the solutions were centrifuged at 4000 rpm for 5 minutes and supernatants analysed for residual metal concentration using AAS (Abioye, 2015).

## RESULT AND DISCUSSION

Bioremediation of heavy metal contaminated soil through biosorption ability of *Pseudomonas aeruginosa* was studied under different culture conditions. The effect of contact time shows a slight variation in percentage removal of lead with 97.6% at the 24$^{th}$ hour, and thereafter increased to 98.3% at the 48$^{th}$ hour, and this was the maximum removal obtained as shown in Figure 1. Generally, uptake of metal increased with increase in contact time and then ceased due to the saturation of the binding sites on the cell surface. This finding is inconsistent with the report of Shazia and Sumera (2015) who stated that high removal of lead by *Aspergillus flavus* occurred at the initial time and declined with increase in time, and the report of the report of Ogunsile and Babrinde (2013) who recorded initial uptake at the first few minutes, and this remained constant with increase in time.

pH is a vital factor to be considered in a biosorption study, This was studied at pH 3, 4, 5, 6 and 7 and the highest lead removal was recorded at pH 6 with 99.0% and the least was recorded at pH 3 (Figure 2). This finding mimics that of Shridhar *et al*. (2017) who reported maximum uptake of lead at pH5.

Temperature effect was studied at temperature ranges of 25- 65$^0$C the highest lead removal (99.3%)was recorded at 55$^0$C and the least removal (98.1%)was at both 35 and 45$^0$C (Figure 3).This findings disagree with the report of AL- Homaidan *et al.*, 2016, where the highest lead removal was at 26$^0$C.

Effect of biomass load was studied using 0.5, 1.0, 2.0, and 2.5ml, of innoculum;- Results obtained did not show much variation in percentage removal with change in the biomass load;- The same value of 99.8% was recorded for 0.5, 1.5, and 2.5ml while a small difference was recorded for 2.0 ml with 97.8% removal figure 4.This may be due to overlapping of adsorption site as a result of overcrowding of biomass. Findings by Kabir, *et al.*, (2018), went contrary to this when they reported that reduction efficiency increase with increase in biomass concentration. This is expected due to complex interaction between the analyte and the binding sites. Further increase had very little or no effect on the process when the binding sites were already saturated. Similar observation was made by Olukanni *et al.*, (2014), but contradicts with Wahab *et al.*, (2017), who reported that decreased biosorption capacity at increased biosorbent dose due to the interference on the binding sites. The effect of agitation speed was also optimized at 3000, 2500 and 2000rpm and the highest percentage removal (99.9%) recorded was at 2000rpm and the least biosorption was at 2500rpm (figure 5).AL-Ashesh and Duvnjak, (1995), and Purainik and Pakniker, (1999) have reported that the rate of metal uptake is influenced by agitation rate or shaking.Wahab *et al.*, (2017) reported low biosorption at a higher speed.

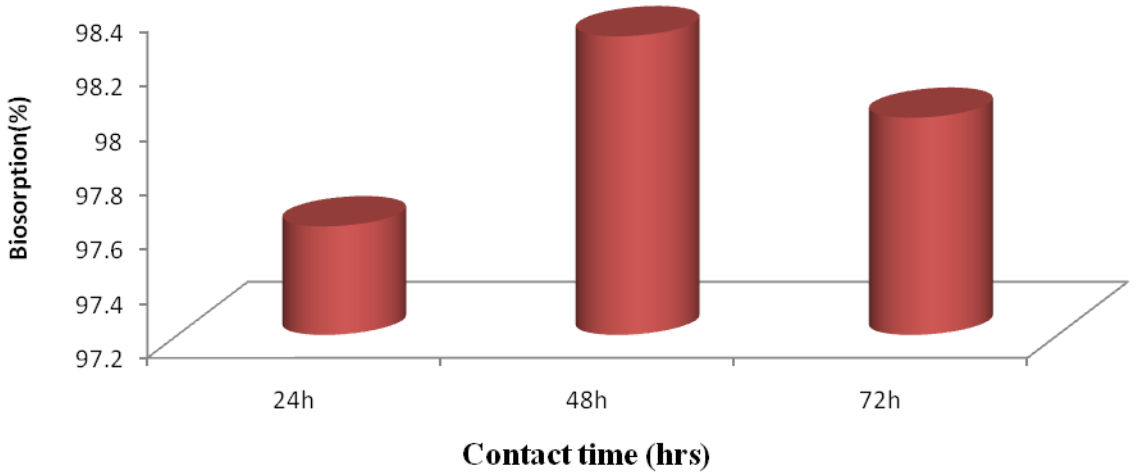


**Figure 1: Effect of Contact time in biosorption of Lead by *Pseudomonas aeruginosa***

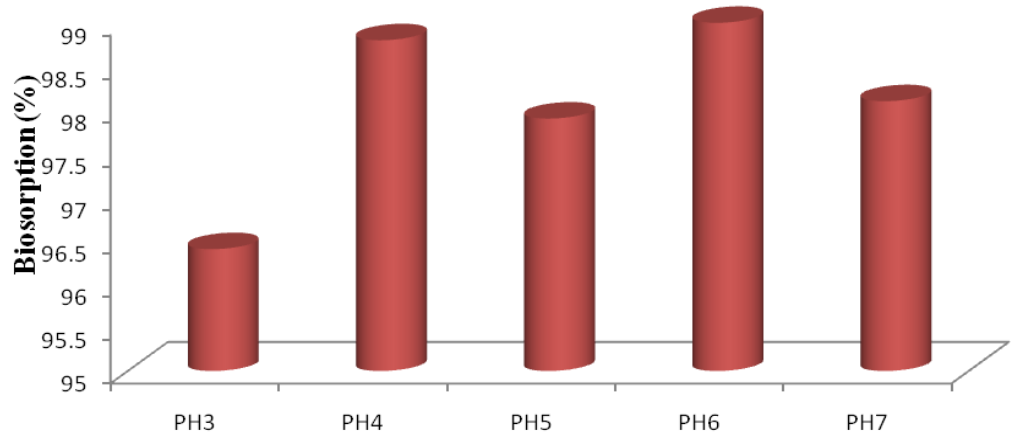


**Figure2: Effect of pH in biosorption of Lead by *Pseudomonas aeruginosa***

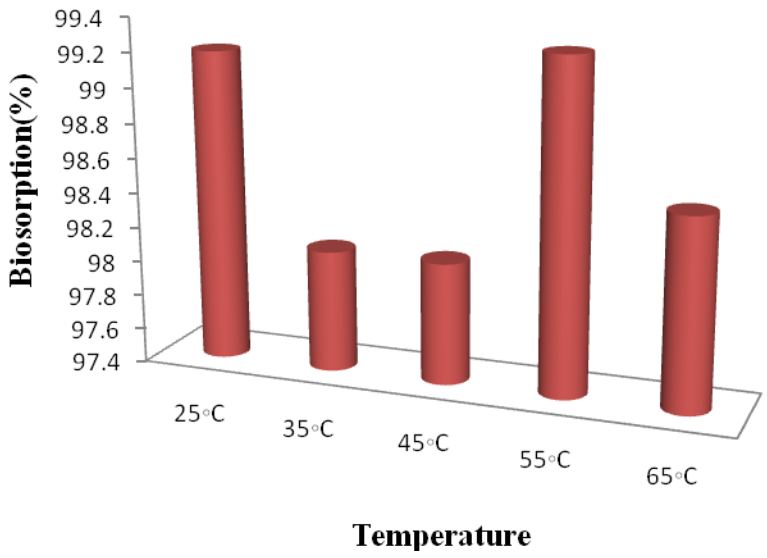

**Figure 2: Effect of Temperature in biosorption of Lead by *Pseudomonas aeruginosa***



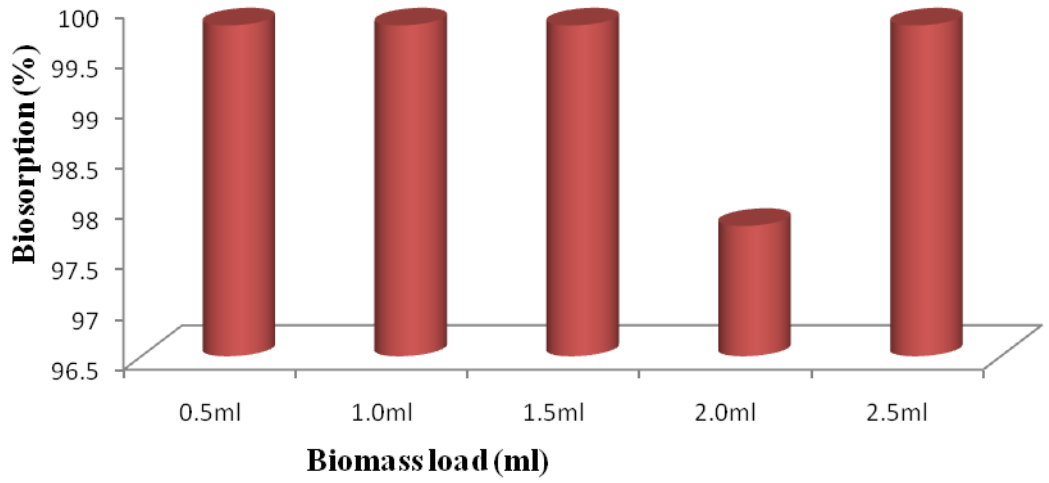


**Figure4: Effect of Biomass load in biosorption of Lead by *Pseudomonas aeruginosa***

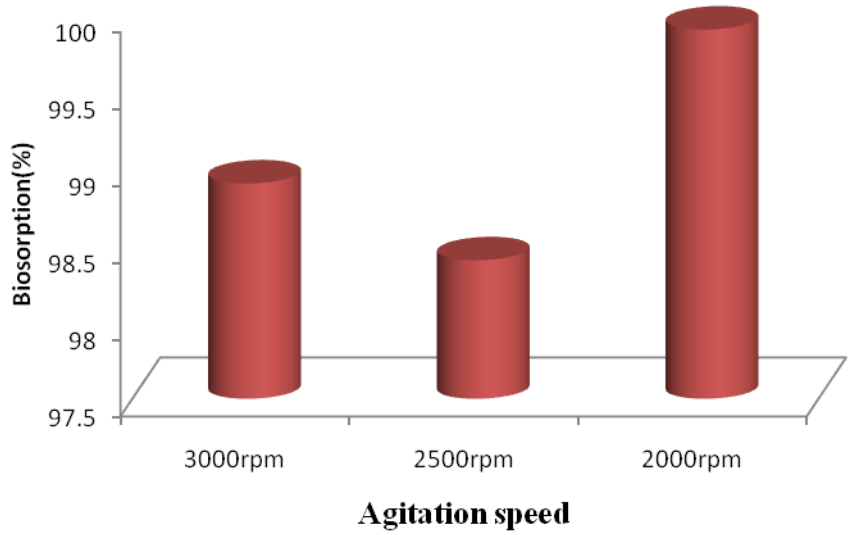


**Figure 5: Effect of Agitation speed in biosorption of Lead by *Pseudomonas aeruginosa***

## CONCLUSION

The efficiency of *Pseudomonas aeruginosa* for bioremediating heavy metal contaminated soil was best at pH 6, temperature 55 °C 2000rpm and 48h. Pseudomonas species used for this study showed excellent ability to accumulate lead on their cell wall and achieved 99.9% removal efficiency under optimized conditions.

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