REMOVAL OF LEAD BY *BACILLUS SUBTILIS AND STAPHYLOCOCCUS AUREUS* ISOLATED FROM E-WASTE DUMPSITE

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Abstract
Soil samples obtained from e-waste dumpsite in Kansuwan Gwari, Minna, Nigeria were used for the isolation of microorganisms used in lead (Pb) biosorption studies. The soil sample was cultured for the test organisms (Bacillus subtilis and Staphylococcus aureus). Box Behnken design was used to optimize the removal of lead by the test organisms. The factors used in the Box Behnken design included: temperature, pH and lead concentration. For Bacillus subtilis, the analysis of variance for the response(lead removal) gave a probability value of 0.651 for the regression, which showed the model was not quite fit for the design since the p-value(0.651) was greater than 0.05. For Staphylococcus aureus, the results of the analysis of variance for the response indicated that the probability value of 0.983 for the regression showed that the model was not a good fit for the design since the p-value (0.983) was equally greater than 0.05. More models could be applied to various fields in microbiology as it reduces the time for lengthy experiments and tends to narrow the experiment to how effectively the results can be achieved. This study can be applied to the bigger picture of the effective pH, temperature and other parameters for the clean-up of pollution in the environment.

Keywords: Biosorption, Lead, Temperature, E-waste, Isolation, Organisms

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1. INTRODUCTION

Electronic waste may be described as discarded computer, office electronics equipment, entertainment device electronics, mobile phones, TV sets and refrigerators. E-waste includes a wide range of products, almost any household or business item with circuitry or electrical components with power or battery supply (Yuan*et al.*,2007). Some components used in electronics are toxic to human,for example lead is commonly used in the electrical and electronics industry in solder, lead-acid batteries, electronic components, cable sheathing, glass of CRTs. Short-term exposure to high levels of lead can cause vomiting, diarrhea, convulsions, coma or even death. Other symptoms are appetite loss, abdominal pain, constipation, fatigue, sleeplessness, irritability and headache. Continued excessive exposure, as in an industrial setting, can affect the kidneys. It is particularly dangerous for young children because it can damage nervous connections and cause blood and brain disorders(Singh and Chopra, 2014).).

Although ion exchange resins and activated carbons have long been recognized as effective commercial adsorbents for treating industrial wastewaters containing adsorptive pollutants, their high cost and low efficiency have limited their commercial use in actual industrial scenarios (Vijayaraghavan and Yun, 2008). Although the ability of microorganisms to take up metals from aqueous solution was investigated as early as 18thand 19th centuries (Modak and Natarajan, 1995), it is only during the last 3 decades that living or non-living microorganisms have been used as adsorbents for the removal and recovery of materials from aqueous solutions.

Biosorption, which is based on bioremediation that is defined as a natural process whereby various microorganisms are capable of removing organic and inorganic contaminants in a given environment, and this allows mainly the reuse of the contaminated area and the recovery of the ecological balance (Deuren*et* *al.*, 1997).Biosorption of heavy metals by microbial cells has been recognized as a potential alternative to existing technologies for recovery of heavy metals from industrial waste streams. Many aquatic microorganisms such as bacteria, yeast and algae, can take up dissolved metals from their surroundings onto their bodies and can be used for removing heavy metal ions successfully (Aksu*et al.*,1992). The major advantages of biosorption over conventional treatment methods include low cost, high efficiency, minimization of chemical biological sludge, re-generation of biosorbent and possibility of metal recovery (Michalak*et al.*,2013; Thiyarakajan*et al*., 2015).

Box and Behnken proposed three level designs for fitting response surfaces. These designs are formed by combining 2k factorials with incomplete block designs. According to the principle of Box-Behnken design, concentration of lead, pH and temperature were identified to have strong effects on the response in preliminary one-factor-at-a-time experiments, were taken as the variables tested in a 15-run experiment to determine their optimum levels. The three factors chosen for this study were designated as A, B and C and prescribed into three levels, coded +1, 0,−1 for high, intermediate and low value successively. The aim of the study was to use bacteria isolated from an e-waste dumpsite for the biosorption of lead.

2. MATERIALS AND METHODS

Heavy metal used in the study

Lead nitrate was obtained from Panlac Scientific Equipment Store Minna, Niger State, Nigeria. The lead nitrate was of commercial grade, it had the following specifications: Formula: $Pb(NO_3)_2$, Molar mass: 331.2g/mol, Density: 4.53g/cm^3(20^{0}C) and Melting point: 270^{0}C.

Collection of soil samples

The 100g of soil sample was collected from electronic waste dumpsites in Kasuwan Gwari located at Minna, Niger State, Nigeria The study site contained discarded electronics of various kinds including refrigerators, discarded DVD players, air conditioners, radios, dismantled televisions. The study area was a beehive of activities; burning activities to retrieve copper wires could be observed on the ground of the study site. The soil was collected into a glass jar using a sterile spatula from three different dumpsite. The soil samples were transported to the Microbiology laboratory of the Federal University of Technology, Minna where the samples were analysed microbiologically.

Isolation and identification of bacteria from soil sample

The soil sample was serially diluted using sterile distilled water up to 10^{-5} dilution factor. The standard pour plate method as described by Fawole and Oso (2007) was used for the isolation of bacteria. Each colony that appeared on the plate was sub-cultured repeatedly on nutrient agar to get a pure culture and was later stored in agar slant containing nutrient agar. The organisms were characterized morphologically and on the basis of biochemical reactions. They were transferred weekly to new medium in order to keep metabolic activities for further characterization and identification (Nazir, 2007).

Metal solution preparation

Stock solution (500ml) was prepared by dissolving 0.79g of lead nitrate [$Pb(NO_3)_2$] in deionised water, shaking it for 15minutes and then allowed to stand for 24hours to obtain complete dissolution. Solution was adjusted to desired pH values (6 and 7) with 0.1M of NaOH and HCl. The initial lead concentration was measured at the beginning of all experiments using Atomic Adsorption Spectrophotometer (AAS WIN 500, UK). AAS is used for quantifying the concentration of chemical elements through their absorption of optical radiation.

Bacteria isolates suspension preparation for biosorption studies

Freshly grown single colonies of the isolates were picked up with an inoculation loop, stirred into 10ml nutrient broth in a test tube incubated at 25^{o}C for 60minutes and maintained as suspension stock for biosorption experiments.

Lead biosorption studies

Aliquots of 1ml suspension of the bacterial were inoculated in 50ml nutrient broth containing different concentrations of lead (0.5, 1.0, 1.5 and 2.0 ppm) (Homaidan *et al*., 2014). Before adding the isolates, the pH of the metal solution was adjusted to the required value with 0.1M NaOH and HCl as the case may be. The conical flasks were incubated at 37^0C in an incubator with a constant shaking. Each flask were taken out every week (i.e. after 7, 14, 21 and 28 days) and were centrifuged at 4000 rpm for 25 minutes. After centrifugation, the supernatant was digested with HNO_3 and metal concentration was determined by Atomic Adsorption Spectrophotometer (AAS).

Effect of incubation temperature on biosorption rate: Nutrient broth medium (50ml) containing 0.5ppm of lead with 1ml aliquot of *B. subtilis* and *S. aureus* bacteria suspension (24 hours old) in conical flasks were incubated at different temperatures of 25 and 37^oC(Homaidan *et al*.,2014) with constant shaking respectively. All experiments were conducted in triplicates. The lead concentration in the digested supernatant was measured using AAS.

Effect of pH on biosorption Rate: Nutrient broth medium (50ml) containing varying amounts of
lead (0.5, 1.0, 1.5 and 2.0ppm) and pH 6 and 7, was inoculated with 1ml 24 hours aliquot of *B. subtilis* and *S. aureus* bacteria suspension (Homaidan *et al.*, 2014). The experimental set up was incubated at 37^oC and heavy metal concentration in the digested supernatant was measured as previously described.

Effect of heavy metal concentration biosorption rate: *B. subtilis* and *S. aureus* (bacteria isolates) were inoculated into nutrient broth medium (50ml) containing different concentrations (ppm) of lead (0.5, 1.0, 1.5 and 2.0) respectively. The pH of the medium was adjusted to 7 and was incubated at 37^oC. The heavy metal concentration in the digested supernatant was analyzed using Atomic Absorption Spectrophotometer (AAS).Percentage of biosorption was calculated by using the Beer Lambert's law in the concentration range studied.

% biosorption = [(Initial metal concentration – final metal concentration)/ Initial metal sorption] X 100

Statistical analysis

The analysis of variance (ANOVA) was used to check the adequacy of the developed models at 95% confidence level (Altin*et al*., 2007; Pawade*et al*., 2008). The criteria followed in this technique is that if the calculated value of the F-ratio of the regression model is more than the standard value specified (F-Table) for 95% confidence level, and then the model is considered adequate within the confidence limit (Liao and Shiue, 1996).

3. RESULTS AND DISCUSSION

Biosorption of heavy metals by Isolates

The bacterial isolates were *B. subtilis* and *S. aureus*. *B. subtilis* was Gram positive rod, capable of utilizing citrate, starch and producing oxidase. *S. aureus* was a Gram positive coccus, capable of utilizing citrate and producing indole and coagulase. Both bacteria were catalase positive.The effect of lead concentration on biosorption of lead by *B. subtilis* and *S. aureus is* shown in Table 2. After 7 days of incubation, the highest lead uptake by *B. subtilis* was 81.8% at 0.5 ppm while the least was 68.7% at 2 ppm . There was a gradual decrease in the uptake of lead by *B. subtilis* as the days progress, with highest lead uptake at day 28 (68.4%) at 0.5 ppm lead concentration and the least was 64.5 % at 1.5 ppm. The effects of concentration on lead biosorption by *S. aureus* is shown in Table 2. At each day of incubation (7, 14, 21, 28) and varying lead concentration (0.5, 1, 2.5 and 2 ppm), *B. subtilis* appeared to have a higher rate of lead uptake compared to *S. aureus*. The highest value recorded for *S. aureus* was 53.4 % of 0.5 ppm lead concentration after 7 days while the lowest value was 28.3% of 2 ppm lead concentration at day 28.

Box Behnken design

All results of this research work were tested with probability value of 5% (0.05). The probability value shows the level at which the hypothesis tested was either accepted or rejected. A model was constructed for each of the organism considered in this research work, which include; *B. subtilis* and *S. aureus***.** Minitab 17 version was used for analysing the data. The equation used for the coded value -1, 0 and 1 was,

$$Y = \beta_0 + \beta_1 A_1 + \beta_2 B_2 + \beta_3 A_3 + \beta_{12} A_1 B_2 + \beta_{13} A_1 C_3 + \beta_{23} B_2 C_3 + \beta_{11} A_1^2 + \beta_{22} B_2^2 + \beta_{33} C_3^2$$

(Dong *et al.*, 2008).

Where,**A=** pH value, **B=**temperature, **C=**lead concentration, A, B and C are the factors considered.

This is further illustrated in Table 1.

Table 1. Three factor Box Behnken design used in this study

	Experimental runs			Hypothetical values		
S/N	A (pH)	B(Temp)	C (Lead conc)	Observed	Predicted	Diff/Error
1	-1	1	0	74.20	72.55	1.65
2	-1	1	0	70.20	66.77	3.43
3	-1	1	0	67.17	65.40	1.77
4	-1	1	0	66.06	66.77	-0.71
5	1	0	-1	66.13	67.57	-1.44
6	1	0	-1	64.17	62.72	1.45
7	1	0	-1	62.76	62.96	-0.2
8	1	0	-1	60.80	62.77	-1.97
9	0	-1	1	64.06	66.77	-2.71
10	0	-1	1	62.33	62.12	0.21
11	0	-1	1	61.10	64.51	-3.41
12	0	-1	1	59.50	56.08	3.42
13	0	0	0	60.73	58.76	1.97
14	0	0	0	51.20	52.96	-1.76
15	0	0	0	57.63	59.27	-1.64

Table 2: Effect of lead concentration on biosorption by *Bacillus subtilis* and *Staphylococcus aureus*

	Concentration of lead (ppm)			
Time (Days)	0.5	1.0	1.5	2.0
B. subtilis				
7	81.8± 1.5	80.7±1.9	74.6±2.4	68.7±3.1
14	80.8±0.86	76.6±3.6	72.5±2.2	68.4±6.3
21	78.6±5.6	72.1 ±5.0	69.7±4.0	60.9±6.9
28	68.4± 12.5	67.4±6.9	64.5±6.6	65.4±7.7
S. aureus				
7	53.4±2.4	51.3±1.3	45.3±2.5	39.3±1.4
14	51.2±3.2	48.9±1.6	40.1±1.2	31.6±4.5
21	49.8±2.3	44.2±4.6	31.2±5.0	29.4 ±6.2
28	45.5±1.2	41.3±2.0	30.2±1.4	28.3±4.2

Microorganisms have been used in the removal of heavy metals from the environments. Selvi *et al*. (2012) reported higher reduction of Pb (90%) from tannery effluent using *Pseudomonas* sp. Kumar *et al.* (2010) reported lower reduction of heavy metals using *Pseudomonas* and *Bacillus*. In this report, *B. subtilis* and *S. aureus* demonstrated the ability to remove lead, although, *B. subtilis* had a higher potential for the removal of lead at different concentrations examined (Table 2). Previous report Sujitha and Jayanthi (2014) revealed that immobilized cells of *B. subtilis* showed maximum heavy metal adsorption (74.2 mg/L for Pb^{2+}) among all the bacterial isolates while *S. aureus* showed the least adsorption (47.4 mg/L for Pb^{2+}). Earlier studies carried out by Singh and Chopra (2014), on the removal of Zn^{2+} and Pb^{2+} revealed the potential of *Bacillus* sp. and *B. subtilis* to remove Zn^{2+} and Pb^{2+} of the paper mill effluent up to 73.29% and 85.64% respectively. Although, Singh and Chopra (2014) suggests that the process for the removal of the heavy metals by the organisms were through biosorption. According to Mahmoud *et al*. (2016), the ability of *B. subtilis* and *S. aureus* to remove Pb may be as a result of the presence of some acidic components on their peptidoglycan cell wall such as teichoic acids and carboxylic acids, which act as a binding site on the bacterial biomass surface and interact with the heavy metal ion.

Effects of Temperature on biosorption of lead by *B. subtilis* and *S. aureus*

The incubation temperature has effect on the biosorption of lead by *B. subtilis* and *S. aureus* (Table 3). At 37^{o}C, *B. subtilis* had a higher biosorption capacity when compared to temperature of 25^{o}C on days 7, 14, 21 and 28. Similarly, at each day, *S. aureus* had a higher percentage of lead uptake at 37^{o}C compared to values obtained at 25^{o}C. In this report, the influence of temperature (25°C and 37°C) on the rate of biosorption by the test organisms was examined. The result obtained showed that there was a more efficient lead removal at temperature 37°C compared to 25°C. According to Kamsonlian *et al.* (2011), changes in temperature influence a number of factors essential in biosorption of heavy metals. This is because every organism has an optimum temperature for their growth, activity and internal biochemical reactions (Pclczar *et al*., 2014). This is similar to the report of Singh and Chopra (2014) that emphasized the effect of temperature on the removal of Zn^{2+} and Pb^{2+} by *Bacillus* sp. and *B. subtilis*. They reported an optimum removal of lead at temperature of 35±1.0°C. Mahmoud *et al*. (2016), also asserted that there was a gradual increase in biosorption of Pb^{2+}, Ni^{2+} and Cu^{2+}, by *S. aureus* as the temperature of the reaction gradually increases from 10 to 50^{o}C indicating that the reaction proceeds *via* endothermal reactions.

Effects of pH on percentage biosorption by *B. subtilis* and *S. aureus*

The pH also had an effect on the rate of biosorption by the test organisms. At pH 6, *B. subtilis* and *S. aureus* had a higher values of lead uptake than at pH 7. The exception to this was at 14 days when *S. aureus* had lead uptake value of 43.4% at pH 6 while at pH 7 the lead uptake was 45.4% (Table 4).The response of pH, temperature on biosorption of lead by *Bacillus subtilis* and *Staphylococcus aureus*, shown in Tables 4 and 5 indicate that the linear, quadratic and the interaction among the factors considered are not statistically significant considering the high value of each probability values of the individual factors.(p-value > 0.05). The pH also had an effect on the rate of biosorption by *B. subtilis* and *S. aureus*. The isolates appeared to have a higher Pb uptake at pH 6 compared to pH 7, although there was no significant difference between them. Kumar *et al.* (2010) reported an optimum pH of 6.5 for *Staphylococcus* and pH 7 for *Flavobacterium* for the removal of Pb and Cu respectively from tannery effluent.

Table 3. Effects of temperature (°C) on lead biosorption by *B. subtilis* and *S. aureus*

Days	*B. subtilis*		*S. aureus*	
	25°C	37°C	25°C	37°C
7	68.5± 3.0	80.1±2.4	46.2±3.2	50.4±5.2
14	66.3± 4.3	72.3±1.3	45.2±2.1	50.3±3.1
21	65.3±4.2	70.5±1.2	44.4±0.8	52.3±1.8
28	58.3±1.2	63.2±2.8	39.3±4.0	51.2±2.0

Table 4. Effects of pH on percentage biosorption by *B. subtilis* and *S. aureus*.

Days	*B. subtilis*		*S. aureus*	
	pH 6	pH 7	pH 6	pH 7
7	80.0±3.2	78.9±8.6	50.1±4.2	44.3±6.9
14	78.3±1.3	76.3±2.1	43.4±7.4	45.4±3.4
21	74.4±3.6	70.2±5.3	42.3±3.3	39.3±6.8
28	69.8±1.1	63.9±4.4	40.4±3.9	36.4±4.4

When analyzed using Response Surface Regression, the coefficient of determination (R^2) for *B. subtilis* was 58.3% (Table 5). It was also observed that the linear, quadratic and the interaction among the factors using Analysis of Variance for response, for *B. subtilis* gave a probability value of 0.651. This showed that the values considered are not significantly different from each other (P>0.05) considering the high probability values of the individual factors. The coefficient of determination (R^2) for *S. aureus* was 25.9% (Table 6). When analysed using Analysis of Variance the probability value of 0.983 was obtained. Thus, the linear, quadratic and the interaction among the factors considered are not significantly different from each other (P>0.05).

In comparison with orthogonal design and variance analysis, Box-Behnken design is one of the designs of the response surface methodology that allows calculations to be made of the response at intermediate levels which were not experimentally studied. A three-level Box-Behnken design was employed in the present study and the optimal conditions were determined through a minimal experiment number compared with other designs. The analysis of response trends using the model is considered to be reasonable. Although many experiments are completed at the canonical analysis (Liu *et al.*, 1999; Kincl *et al.*, 2005) the ridge analysis is very useful to find out the maximum response, which may occur in the experiment when the results of the canonical analysis shows a saddle, stationary point and no unique optimum in the estimated surface (Lin, 1992; Zhuang *et al.*, 2000). Several biosorption studies have been optimized using various models but Box Behnken Design is considered very effective in the study of three factors and three levels (Samuel *et al.*, 2015). From the present experiment, the coefficient of determination (R^2) was 58.3%, which means that the independents variables were able to contribute up to 58.3% for *B. subtilis*. In the other sense, 58.3% of the coefficient of determination (R^2) indicates that the model developed explains 58.3% of the variance in the dependent variable (i.e. response). From analysis of variance (Table 6) for the response, probability value of 0.651 for the regression shows that the model is not a good fit for the design since the p-value is greater than 0.05 (Table 6).

The coefficient of determination (R^2) for *S. aureus* was 25.9%. In other words, 25.9% of the R^2 indicates that the model developed explained 25.9% of the variance in the dependent variable (i.e. response). The analysis of variance of response showed the probability value of 0.983 for the regression. This shows that the model is not a good fit for the design since the p value of 0.983 is greater than 0.05.

Table 5. Response Surface Regression for *B. subtilis*

Term	Coef	StDev	T	P
Constant	86.69	13.956	6.211	0.002
A	-1.41	8.546	-0.164	0.876
B	3.47	8.546	0.406	0.701
C	12.10	8.546	1.416	0.216
A*A	13.07	12.580	1.039	0.346
B*B	-9.77	12.580	-0.776	0.473
C*C	-8.11	12.580	-0.645	0.547
A*B	-2.48	12.086	-0.205	0.846
A*C	-1.35	12.086	-0.112	0.915
B*C	-19.04	12.086	-1.576	0.176
S = 24.17	R-Sq = 58.3%	R-Sq(adj) = 0.0%		

Table 6. Response Surface Regression for *S. aureus*

Term	Coef	StDev	T	P
Constant	58.000	6.134	9.455	0.000
A	0.380	3.757	0.101	0.923
B	-2.778	3.757	-0.739	0.493
C	0.140	3.757	0.037	0.972
A*A	0.884	5.530	0.160	0.879
B*B	-2.086	5.530	-0.377	0.721
C*C	-0.376	5.530	-0.068	0.948
A*B	-4.068	5.313	-0.766	0.478
A*C	-3.257	5.313	-0.613	0.567
B*C	-1.142	5.313	-0.215	0.838
S = 10.63	R-Sq = 25.9%	R-Sq(adj) = 0.0%		

4. CONCLUSIONS

B. subtilis and *S. aureus* were able to uptake lead at varying capacity. *B. subtilis* gave higher rate of biosorption of lead compared to *S. aureus* at various lead concentrations examined and at various incubation days. The temperature and pH had effects on the biosorption by the test organisms.

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