



Abilities of Three Microorganisms for Cleaning Cadmium Contaminated Soils

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The abilities of three indigenous bacteria for bioremediation of cadmium contaminated soils collected from Agbabu Farm Settlement close to mining sites in Ondo state, Nigeria was studied to provide helpful information for soils remediation and soils health management in this sub-region for Millennium Development Goals accomplishment. *Bacillus subtilis*, *Proteus mirabilis*, and *Escherichia coli* isolated from the soils were inoculated into different soil samples conditioned with optimized factors determined from the first phase experiments. The conditioned samples were experimented for residual cadmium concentration with time in days using Atomic Absorption Spectrophotometer. The soil cadmium attenuation from the initial concentration of 70.21 mg/kg to below the maximum allowable of 3 mg/kg was hard for the organisms. *Bacillus subtilis* performed correction at time 35 days with an efficiency of 96.10 % and residual concentration of 2.74 mg/kg. *Proteus mirabilis* and *Escherichia coli* with respective, high efficiencies of 85.05% and 79.35% failed. The removal rate capacities were -0.131d^{-1} for *B. subtilis*; -0.111d^{-1} for *P. mirabilis*; -0.105d^{-1} for *E. coli*. Four kinetic models fitted described the experimental data well. The models assessment revealed the removals to be transport controlled as diffusion process was the rate-controlling step.

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INTRODUCTION

Heavy metals are capable of causing damage to vital organs and systems in living organisms. Access of these metals to arable soil should be prevented because they are not breakable to non-toxic form, they have negative impact on ecology and human health, and are difficult to eliminate from soils [1–4].

Cadmium (Cd) is a heavy metal with adverse effects of renal dysfunction, decreased hemoglobin levels, disturbed liver function, lung emphysema, short breath, and increased excretion of calcium [5].

Researchers work had till date combed for remediation alternatives that proffer green technology solution to the problem of soil pollution with heavy metals.

Physical and chemical soil remediation methods have been proven to have great disadvantages. They give birth to strange toxic mid-way and end products of greater toxicity; less eco-friendly; and are very expensive-unsuitable for effective treatment at low metals concentrations [3, 6]. Bioremediation clears the disadvantages of physical-chemical methods of remediating metals contaminated soils. Bioremediation applicability requires the adjustment of

environmental parameters to stimulate high microbial activities to allow metals removal proceed rapidly [7, 8]. Factors like pH, temperature and biomass loading have been discovered to influence bioremediation [9–11].

This work is a laboratory study of the performances of indigenous *Bacillus subtilis* (*B. subtilis*), *Proteus mirabilis* (*P. mirabilis*), and *Escherichia coli* (*E. coli*) for bioremediation of cadmium contaminated soils collected from Agbabu Farm Settlement close to mining sites in Ondo state, Nigeria. Agbabu settlers are predominantly farmers of food crops and vegetables for both home consumption and market purposes outside Ondo state. This work also incorporated a detailed investigation of the optimum factors required for these organisms to bring cadmium ion to below the maximum allowable concentration (3 mg/kg) set for agricultural soils by regulatory bodies reported by Chiroma et al. [12].

It looked at the rate controlling steps of the metal removal by fitting the laboratory data with four kinetics models. The work furnished information on the comparative remediation capabilities of these indigenous organisms and help in addressing remediation problem of Cd contaminated arable soils in the sub-region in pursuit of the Millennium Development Goals.

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MATERIALS AND METHODS

Preparation of nutrients

Solutions of triple sugar iron agar, Simon Citrate agar, peptone water powder and MacConkey agar were prepared according to manufacturers' instruction and the methods are available in literature [13]. The amount of 65, 24, 15, and 52 grams of defined media powders were respectively weighed into 1 liter of distilled water each. The respective mixtures were left untouched for 10 min and then swirled to allow for proper mixing before subjected to an autoclave at temperature of 121°C and 1.5 psi pressure for 15 minutes and then cooled to 45°C.

Identification of organisms

The microbial isolation and identification was conducted in the Delta State University microbiology laboratory in Abraka, Nigeria on cadmium contaminated soil samples collected from agro soils at Agbabu Farm Settlement close to mining sites in Ondo state.

A 0.1 ml from 10⁻³ of serial diluted sample was introduced into MacConkey agar in sterile Petri-dishes by the pure plate technique [14, 15]. The plates were incubated at temperature of 37°C for 24 hours [13]. The grown colonies were sub cultured and characterized in accordance to the method discussed in literature [13–16].

Screening of factors for bioremediation studies

Vital factors have been discovered to have significant influence on bioremediation process and adsorption rate [9, 10]. The immense scientific significance of these factors at their optimal levels requires that they be carefully studied, screened and selected for a particular bioremediation study.

This study was carried out by adopting the batch remediation methods in triplicate [17, 18]. Distinct values of factors (1, 2, 3, 4, 5, 6, 7, 8, 9, and 10) of pH; (10, 20, 30, 40, 50, and 60°C) of temperature; (2, 4, 6, 8, 10, and 12 ml) of nutrient; (1, 2, 3, 4, 5, and 6g) of organisms' weights; and [0, 1, 2, 3, 4 and 5 per week (pw)] of stirring frequency were respectively set to condition 4g of soil samples each in 102 50 ml capacity beakers. Thirty-four samples for each organism (that is 10 for pH, 6 for temperature, 6 for dosage of nutrient, 6 for organisms' weight, and 6 for stirring frequency). The organisms were allowed to interact in the soil for 14 days, and the soil samples were evaluated for residual Cd ion [9, 18] using Atomic Absorption Spectrophotometer (GBC SensAA, Model no. A6358) after centrifuging.

Cadmium ion removal determination

Soil samples of 4g each measured into 50 ml capacity beakers each were inoculated with each organism to make up 63 inoculated soil samples for the complete experiment in triplicate [17, 18]. The experimental soil samples were conditioned with the values selected for the optimum factors as pH of 8 for *B. subtilis*; and 6, 5 for *P. mirabilis* and *E. coli*, respectively; temperature of 30°C for the organisms; nutrient of 8 ml for the organisms; optimum organism's weight of 4g for *B. subtilis*, 3g for *P. mirabilis*, and 4g for *E. coli*; and stirring frequency of 5pw for the organisms.

The residual ionic concentration of cadmium in the soil samples were evaluated on the 5th, 10th, 15st, 20th, 25th, 30th and 35th days using AAS after centrifuging.

The ionic concentration (mg/kg) removed with time, efficiency of organisms' performance (%), and ionic concentration (mg/kg) removed at equilibrium were determined from Equations (1) to (3) [19, 20].

$$q_t = \frac{(C_o - C_t)}{m} \cdot V \quad (1)$$

$$\varepsilon = \frac{(C_o - C_f)}{C_o} \cdot 100 \quad (2)$$

$$q_e = \frac{(C_o - C_e)}{m} \cdot V \quad (3)$$

C_o, C_e, C_t, C_f, q_e, q_t, V and m are the initial metal ionic concentration (mg/kg); residual metal ionic concentration (mg/kg) at equilibrium, residual ionic concentration (mg/kg) with time final ionic concentration (mg/kg), ionic removal (mg/kg) at equilibrium, ionic removal (mg/kg) with time, volume (m³) of soil and mass (g) of organisms.

A 2-ways analysis of variance at (P < 0.05) was conducted with Microsoft Excel 2016 on the removal data to investigate significant difference in the removal performances between the organisms; and to investigate removal differences with experimental times. The rates of removal capacities of Cd by the respective organisms were studied with pseudo-first order kinetic model.

Rate-limiting step determination

This aspect of the work focused on determination of controlling processes of the metal removal. These were studied from the rate controlling steps (chemisorption or diffusion) of the removal established with removal kinetics employed to study batch experimental data of Cd removal. Four mathematical kinetics models: pseudo first and second order kinetic models, the simple elovich model, and the intraparticle diffusion models presented by Equations (4) to (7) [21–23] were linearized and fitted with the batch laboratory experimental data.

$$\frac{dq_t}{dt} = k(q_e - q_t) \quad (4)$$

$$\frac{dq_t}{dt} = k_1(q_e - q_t)^2 \quad (5)$$

$$\frac{dq_t}{dt} = \alpha \exp(-\beta q_t) \quad (6)$$

$$q_t = K_2 t^{\frac{1}{2}} + X \quad (7)$$

The parameters k₁ and k₂ are rate constants of pseudo second order and intra-particle diffusion models expressed in (kg.mg⁻¹.d⁻¹) and (mg/kg.d^{1/2}) respectively; α and β are the initial removal rate in mg.kg⁻¹ and desorption rate constant in mg.kg⁻¹.d⁻¹, respectively.

The four models' R² were assessed to ascertain the best fit indicating the rate-limiting step, and the controlling process.

RESULTS AND DISCUSSION

Organisms identification

Microbiological analysis conducted on sample for isolating and identifying the organisms yielded the selected organisms from the biochemical scrutiny of microbial characters among the colonies of 3.6 x 10³ cfu/ml that developed after incubation. The organisms were identified with gram staining

and the respective biochemical indications of catalase, oxidase, indole, citrate, glucose, sucrose, lactose, and motility as *E. coli* (negative, positive, negative, negative, negative, positive, negative and negative); *B. subtilis* (positive, positive, negative, negative, positive, positive, negative and positive); *P. mirabilis* (positive, negative, negative, negative, positive, positive, positive and positive). The summary of microbial identification is shown in Table 1.

Optimum factors

Vital factors have been discovered to have significant influence on bioremediation process and adsorption kinetic rate [9, 10]. The immense scientific significance of these factors at their optimal levels requires that they be carefully studied, screened and selected for a particular bioremediation study - as these factors at their optimum values enhance the effective removal of metals by microorganisms [17]. This has necessitated that these factors were studied with an aim to select their optimum values necessary for this bioremediation study.

The impacts of 2, 4, 6, 8, 10 and 12 ml of nutrient; 1, 2, 3, 4, 5 and 6g organisms; 10, 20, 30, 40, 50 and 60°C temperature; 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 of pH; and 0, 1, 2, 3, 4, 5 and 6 per week (pw) of stirring frequency on the metals removal by the organisms were studied for 14 days to obtain optimum values through screening.

A criterion of least residual concentration was engaged in identifying these optimum factors. The respective optimum values of nutrient's volumes, organisms' weights, temperature, pH and stirring frequency were recognized and selected at the respective least residual concentrations.

Optimum pH

pH impacts on the connectivity of the negative charges on cells responsible for bioremediation; cell wall chemistry; and the hydrolysis and physiochemistry of metals [11, 24]; thus, the pH becomes the most critical parameter for metals bioremediation [11]. Figure 1 shows the impact of pH values of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 on the removal of cadmium by the organisms at 14 days.

The residual concentrations were found to be 14.19 mg/kg for removal by *B. subtilis*; 18.67 mg/kg for removal by *P. mirabilis*; and 23.41 mg/kg for removal by *E. coli*. These concentrations were the least residual for the respective organism and they formed the criteria for identifying the optimum pH values. Relative to these residual concentration values, the optimum pH values were found to be 8 for removal by *B. subtilis*; 6 and 5 for respective removal by *P. mirabilis* and *E. coli* at the respective efficiencies of 79.79%, 74.41% and 62.81%.

For the action of *B. subtilis*, Figure 1 shows a decline in Cd residual concentration curve with increase in pH from 1 to 8 before a rise in the curve. Residual concentration curve for

the action of *P. mirabilis* declined with increases in pH from 1 to 6 before a rise. In the action of *E. coli*, the residual concentration curve declined with increase in pH from 1 to 5 before it began to rise. From these curves, it is obvious that the most favorable pH values were 8, 6 and 5 for the actions of *B. subtilis*, *P. mirabilis* and *E. coli*, respectively. These values are the optimum pH values for the respective organisms.

The change of pH to the respective optimum values (8, 6 and 5) may have caused a rise in the negative charge in walls of the organisms' cells to promote attraction that is electrochemical in nature and consequent removal of Cd [25]. However, low pH was observed to prevent metal removal because of the neutralization of most of the organism's cell wall groups that contain nitrogen [26] and the high ionic concentration of hydrogen contests at the removal sites with cations [27]. Reduction in organisms' performances was noticed after the optimum pH values. This reduction grew worse after pH of 8. This might be as a result of insoluble oxides formation and the development of insoluble hydroxides and carbonates which decreased the available Cd ion [28].

Optimum temperature

Temperature is the major influence of bioremediation of heavy metals [10]. It changes affect the factors which are vital to heavy metals bioremediation [29]; and the magnitude of the heat provided for the process is a significant criterion for the efficient removal of metals from a medium [10].

Impacts of various degrees of temperature shown in Figure 2 revealed an optimum temperature of 30°C for the maximum removal of Cd ion by the three mentioned organisms.

The decreasing order in which the studied temperature values affected the performances of the organisms is 30, 40, 50, 60, 20 and 10°C. The respective residual concentration for removals at the optimum temperature where 11.79 mg/kg for *B. subtilis*; 16.17 mg/kg for *P. mirabilis*; and 21.41 mg/kg for *E. coli* at the respective removal efficiency of 83.21%, 76.97% and 69.51%. The residual concentrations were the least residual for the organisms' activities, and they formed the criteria for identifying the optimum temperature values.

It is obvious that temperature variation had notable influences on the performances of the organisms. The residual concentration curve in Figure 2 declined as temperature grew from 10°C to 30°C before it began to rise. This shows that 30°C is the temperature that stimulated the greatest performances of the organisms; and that temperature was selected as the optimum temperature of the study. The maximum performances of organisms at 30°C might be due to cell metabolism that are likely affected adversely by low temperature [28].

TABLE 1. Summary of biochemical indicators

Microorganism	Catalase	Oxidase	Indole	Citrate	Glucose	Sucrose	Lactose	Motility
<i>E. coli</i>	-	+	-	-	-	+	-	-
<i>B. subtilis</i>	+	+	-	-	+	+	-	+
<i>P. mirabilis</i>	+	-	-	-	+	+	+	+

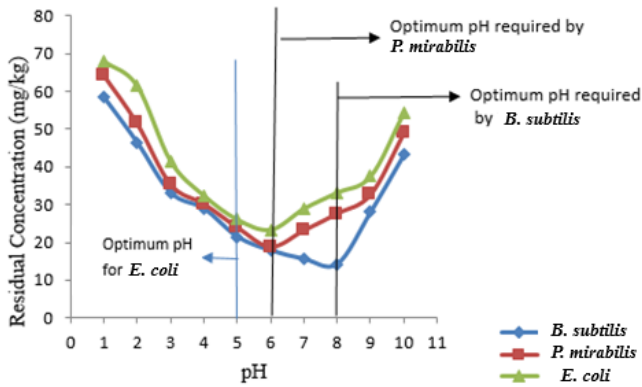


Figure 1. Impact of pH on cadmium removal

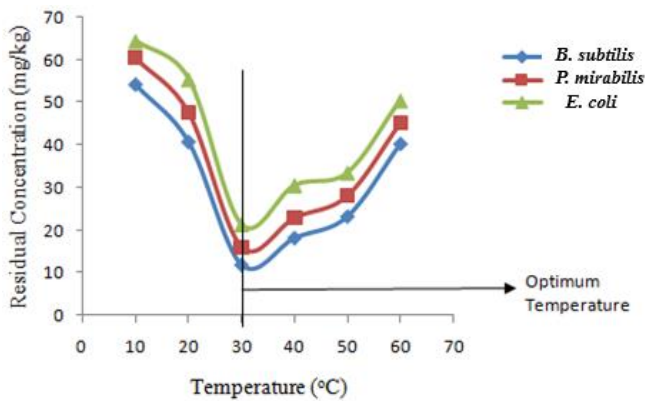


Figure 2. Impact of temperature on cadmium removal

Optimum volumes of nutrient

Effects of 2, 4, 6, 8, 10 and 12 ml of nutrient on Cd removal by *B. subtilis*, *E. coli* and *P. mirabilis* were carefully studied for 14 days. The decreasing impact order of the applied nutrient volumes are 8, 6, 10, 12, 4 and 2 ml for the action of *B. subtilis*; 8, 10, 6, 12, 4 and 2 ml for the removal by *P. mirabilis*; and 8, 10, 6, 12, 4 and 2 ml for the action of *E. coli*. These orders of nutrient impact showed an optimum nutrient volume of 8 ml for the performances of the organisms.

The residual concentrations at this optimum nutrient value were 15.79 mg/kg, 20.67 mg/kg and 25.01 mg/kg for removals by *B. subtilis*, *P. mirabilis* and *E. coli*, respectively; and the efficiencies of removals were 74.35%, 79.05% and 67.05% for *P. mirabilis*, *B. subtilis* and *E. coli*, respectively. These residual concentrations were the least residual for each organism. They were relevant for identifying the optimum nutrient dosages.

The relevance of nutrients supply is to stimulate the indigenous organisms towards enhanced performances. The 8 ml optimum nutrient is the nutrient dosage at which the organisms influenced the most on the process of bio-stimulation of the organisms. The idea of bio-stimulation is the addition of nutrient to promote organism’s growth and replication for increased bioremediation rate [30]. Bio-removal of metal was reported possible under anaerobic conditions by the addition of adequate carbon and energy sources [31].

Optimum weights of organisms

The collective weight of organisms is related to the population of the organisms brought in contact with contaminated medium. The activities and effectiveness of organisms used for bioremediation of metals in soils is significantly affected by the population of the organisms involved. Therefore, it is of immense significance to engage an optimum population in a bioremediation study.

The study of the influences of 1, 2, 3, 4, 5 and 6 g of the respective organism on the metal removal showed the respective minimum residual concentration of Cd to occur at 4g of *B. subtilis*; 3g of *P. mirabilis*; 4g of *E. coli*. These values in grams were the optimum weights of the organisms needed to remediate the contaminated soil.

The influences of the tested weights of the organisms were in the decreasing order of 4, 5, 3, 6, 2 and 1g for removal by *B. subtilis*; 3, 5, 4, 6, 2 and 1g for removal by *P. mirabilis*; and 4, 5, 3, 6, 2 and 1g for removal by *E. coli*. The respective residual concentrations of Cd at the optimum weights where 14.71 mg/kg for *B. subtilis*; 18.01 mg/kg for *P. mirabilis*; and 23.11 mg/kg for *E. coli*. These concentrations were the lowest residual per organism and they were pointers to identifying the optimum weights of the organisms.

In general, bio-removal rate of microorganisms that are alive should depend strongly on the cells population [32]. However, there is a need for optimum population to strike a balance between the supplied optimum nutrient dosage and the cells population - and the cells pollution vary with the cells collective weight. In this sense, the optimum weight of organisms reflects the optimum population engaged in the bioremediation for optimum results. The respective optimum weights of 4g, 3g, and 4g for *B. subtilis*, *P. mirabilis* and *E. coli* would satisfy the required harmony with nutrient dosage, pH, temperature and stirring frequency for optimum remediation performances of the organisms.

Optimum stirring frequency

Figure 3 shows the influence of stirring frequency on the removal of cadmium by the organisms. An optimal value of 5pw at 120 revolutions per minute (rpm) for *P. mirabilis*; and 5pw at 150 (rpm) for *B. subtilis* and *E. coli* were selected from the screened values of 0, 1, 2, 3, 4 and 5 (pw). The minimum residual concentration observed at the 5 (pw) were 14.19 mg/kg for *B. subtilis*; 19.69 mg/kg for *P. mirabilis*; and

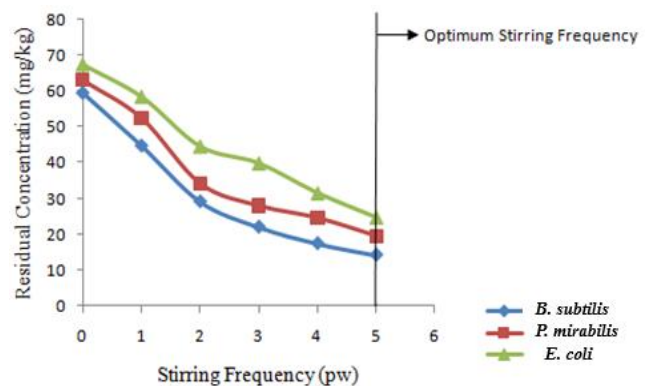


Figure 3. Impact of stirring frequency on cadmium removal

24.71 mg/kg for *E. coli*. These were instrumental for identifying the optimum frequencies. Stirring of the soil samples was important to promote oxygen diffusivity necessary for aerobic activities of the microorganisms.

The order of impacts of the studied stirring frequency is 0, 1, 2, 3, 4 and 5 (pw). This order shows that the higher the stirring frequency, the more the soil was exposed to atmospheric oxygen, and consequently, the greater the remediation results. This is in agreement with the findings in [33, 34] that biosorption is directly related to aeration rate.

Cadmium ion removal

The organisms' removal capabilities for the metal were studied with the optimal factors in a batched approach for 5, 10, 15, 20, 25, 30, 35 days. The maximum allowable concentration of the metal was used as bench-mark to determine the abilities of the organisms to remediate the contaminated soil.

The initial concentration of the metal in the analyzed soil sample is 70.21 mg/kg. At this concentration, the soil was found to be polluted when compared with the maximum allowable concentration value of 3 mg/kg.

Cadmium removal was a big task for the organisms within the experiment time frame of 5 to 35 days. Figure 4 shows the influence of the selected organisms on Cd removal from the soil samples. Along the experiment time, no organism could remove Cd to control level apart from *B. subtilis* at time 35 days with an efficiency of 96.10 % and residual concentration of 2.74 mg/kg. This removal efficiency is greater than 70% efficiency of cadmium removal with *Saccharomyces pombe* [35]. *Proteus mirabilis* and *E. coli* with respective high removal efficiencies of 85.05% and 79.35% failed in removing the metal to control level though their respective minimum residual concentrations were 10.50 mg/kg and 14.42 mg/kg.

These efficiencies of *P. mirabilis* and *E. coli* found greater than 70% efficiency of removal with *Saccharomyces pombe* [35] were not sufficient for Cd removal to the allowable concentration. This could not be tied to the fact that the organisms do not have good expertise for cadmium removal but the initial concentration of 70.21 mg/kg was too high for the organisms to bring to below the maximum allowable of 3 mg/kg with the experimental time.

This is reflective from the great concentration difference ($C_o - C_A$) of 67.21 mg/kg between the initial concentration (C_o) and allowable concentration (C_A); and the rapid drop in concentration with experiment time during the period the organisms were in contact with soils.

Judging from the efficiencies point of view, it is safe to say that the organisms have good expertise for cadmium removal; but the ($C_o - C_A$) value seem to be the great limiting factor which inhibited the *P. mirabilis* and *E. coli* from bringing the concentration to or below 3 mg/kg.

Relationship between the performances of the organisms

A 2-ways analysis of variance employed in analysis at ($P < 0.05$) indicated significant difference in the removal performances between the organisms; and significant differences in performances with times of experiment at less than 0.01 probability level ($P < 0.01$). This implied that one of these three organisms cannot perform like the combination

of either two or the three organisms in agreement with [36]; and showed that shorter intervals between experimental times would yield relevant remediation results.

Difference in 35 days' efficiencies of the organisms are 11.05% between *B. subtilis* and *P. mirabilis*; 5.7% between *P. mirabilis* and *E. coli*; and 16.75% between *B. subtilis* and *E. coli*. The respective mean, maximum and minimum residual concentrations for removal by *B. subtilis* were 24.58 mg/kg, 46.65 mg/kg and 2.74 mg/kg compared with 29.07 mg/kg, 48.46 mg/kg and 10.50 mg/kg for removal by *P. mirabilis*; and 32.19 mg/kg, 51.63 mg/kg and 14.42 mg/kg for the removal by *E. coli*.

Rate of removal

Removal kinetic is significant for determining removal rate for a system [37]. The rate of removal of this metal by the respective organisms was studied with the pseudo-first order kinetic model expressed in Equation (4). The parameter q_e and q_t are the removal capacities at equilibrium and time t in ($\text{mg}\cdot\text{kg}^{-1}$) respectively; while k represents rate constant in [$\text{per day (d}^{-1}\text{)}$].

A plot of $\ln(q_e - q_t)$ and t in Figure 5 showed that the experimental data fit the model with R^2 values of 0.8031, 0.7626 and 0.7991 for *P. mirabilis*, *E. coli* and *B. subtilis* respectively; and revealed the removal rate capacities of -0.1309d^{-1} for *B. subtilis*; -0.1114d^{-1} for *P. mirabilis*; -0.1054d^{-1} for *E. coli*. This indicated the ion removal rate in the decreasing order of *E. coli*, *P. mirabilis* and then *B. subtilis*.

Rate-limiting step

In biosorption, systems are either reaction controlled or transport controlled; and the study of removal kinetics are significant for this comprehension [9] useful for removal systems design [38].

The controlling processes were studied from the rate-limiting steps assessed from the removal kinetics employed to study experimental data of batch removal. Rate-limiting steps were established from the study of pseudo first and second order kinetic models, the simple elovich model, and the intraparticle diffusion model fitted with the experimental data in Figures 5-8. These Figures reflect the regression equations and the R^2 of the fits. The values of models' parameters k , k_1 , α and k_2 are shown in Table 2.

The performance of pseudo-first order model was studied through the linear fits of $\ln(q_e - q_t)$ and t shown in Figure 5. The order of best fit is *P. mirabilis*, *B. subtilis* and *E. coli* with

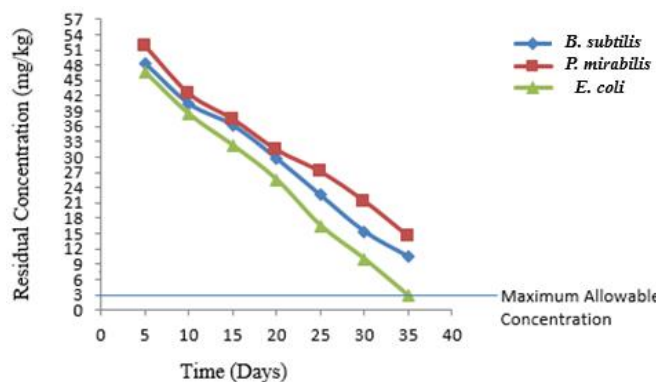


Figure 4. Comparative removal of cadmium

respective R^2 values of 0.8031, 0.7991 and 0.7626; that is, the laboratory results of treatment with *P. mirabilis* followed best the model profile before *B. subtilis*, and *E. coli* came behind. These are signals that chemisorption was present in the processes.

The fits between t/q_t versus t for the pseudo-second order model is shown in Figure 6 in the order of best fit: *E. coli*, *P. mirabilis* and *B. subtilis* deduced from their respective R^2 values of 0.948, 0.9128 and 0.9069. This model described well the removal outputs of the organisms in the stated magnitude order of the R^2 values and informed that chemical reaction was involved the removal processes.

Relationship between q_t versus $\ln(t)$ for elovich model is shown in Figure 7 with R^2 value of 0.9518 for removal with *E. coli*, 0.9245 for *B. subtilis* and 0.9238 for *P. mirabilis*. The experimental results of *E. coli* usage aligned best with the profile of the model, while the results of *P. mirabilis* usage aligned the least with the model profile. The good alignment of all organisms' results with the model profile reflects the

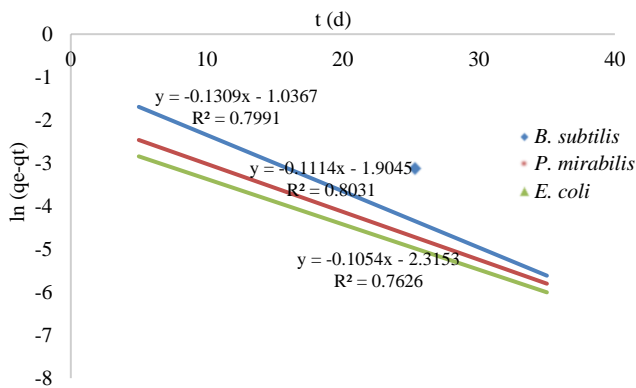


Figure 5. Pseudo-first order plot

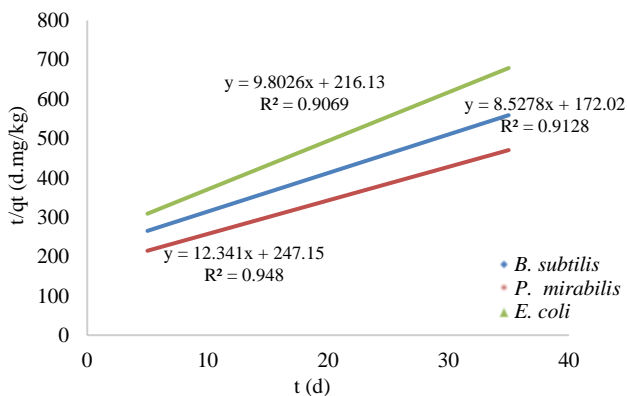


Figure 6. Pseudo-second order plot

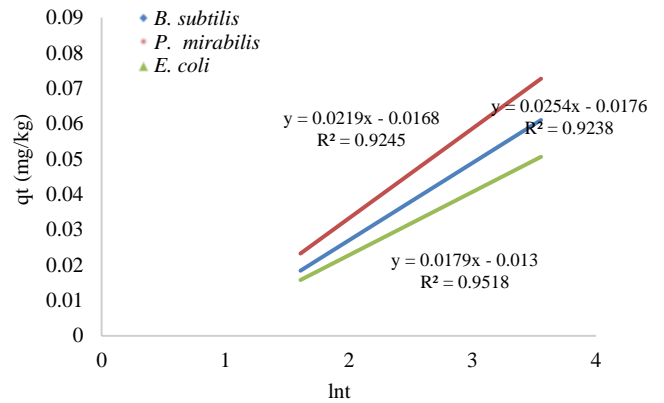


Figure 7. Elovich model for adsorption plot

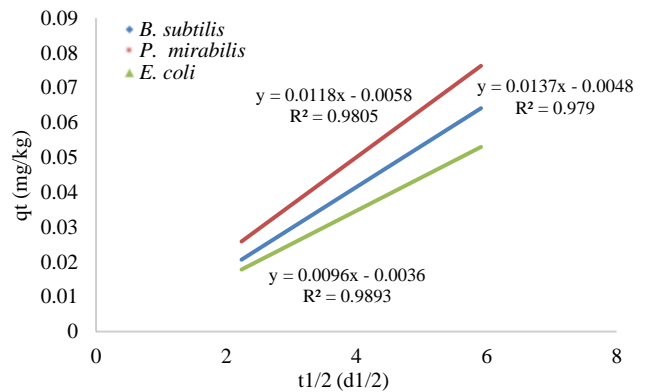


Figure 8. Intraparticle diffusion plot

removal processes as been affected by chemisorptive action. The fits between q_t and $t^{1/2}$ for intraparticle diffusion model are shown in Figure 8 with decreasing fit order as *E. coli*, *B. subtilis* and *P. mirabilis*; and respective fit R^2 of 0.9893, 0.9805 and 0.979. The R^2 values portrayed that the removal systems were affected by transport process, and the order of best fit is an indication of the order of removal magnitudes [23].

Models assessment showed that the batched experimental removal data for the organisms followed the tested kinetics models. However, careful examination and comparison of models' R^2 values in Table 2 showed that intraparticle diffusion model gave the best fit with R^2 of 0.9805 for removal by *B. subtilis*, 0.979 for *P. mirabilis* and 0.9893 for *E. coli*. The implication of these is that the diffusion process was the rate controlling step for cadmium removal by the organisms and the removals were transport controlled - the systems were dominated by physical process This is useful for optimized design of removal [38] and removal systems [37].

TABLE 2. Kinetic parameters for kinetic models

Organisms	Kinetic models and their parameters							
	Pseudo - first order		Pseudo - second order		Elovich		Intraparticle diffusion	
	k (d ⁻¹)	R ²	k ₁ (kg.mg ⁻¹ .d ⁻¹)	R ²	α (mg.kg ⁻¹)	R ²	k ₂ (mg/kg.d ^{1/2})	R ²
<i>B. subtilis</i>	-1309	0.7991	0.4447	0.9069	0.0219	0.9245	0.0118	0.9805
<i>P. mirabilis</i>	-0.1114	0.8031	0.4225	0.9128	0.02539	0.9238	0.0137	0.979
<i>E. coli</i>	-0.1054	0.7626	61.6694	0.948	0.0179	0.9518	0.0096	0.9893

CONCLUSION

This work focused on laboratory study of the performances of indigenous *Bacillus subtilis*, *Proteus mirabilis*, and *Escherichia coli* for remediating cadmium contaminated soils collected from Agbabu Farm Settlement close to mining sites in Ondo state, Nigeria. It discovered the optimum values of the factors required for the optimum performances of these organisms for remediating the soil.

Attenuating the soil cadmium ion to below 3 mg/kg was hard for the organisms within the experiment times of 5 to 35 days. Only *B. subtilis* depleted Cd ion below the allowable level at time 35 days with an efficiency of 96.10 % at residual concentration of 2.74 mg/kg.

P. mirabilis and *E. coli* performed with high efficiencies of 85.05% and 79.35 % respectively; but these high efficiencies were inadequate to bring Cd concentration to below the safe level. The high efficiencies showed that the organisms have good potentials for cadmium removal; but the high concentration difference ($C_0 - C_A$) was the great limiting factor which inhibited the organisms in their performances.

The removal rate capacities were $-0.1309d^{-1}$ for *B. subtilis*; $-0.1114d^{-1}$ for *P. mirabilis*; $-0.1054d^{-1}$ for *E. coli*. The kinetics models fitted described well the experimental data. Models assessment revealed the removals as transport controlled; and diffusion process was the rate-controlling step. This knowledge is useful for optimized design of Cd removal process and systems using the selected organisms.

The information generated from this study will be helpful to researchers and decision makers in pursuit of the Millennium Development Goals in this sub-region. In addition, the information from this work will be useful to future researchers.

REFERENCES

- Singh, J.S., Abhilash, P.C., Singh, H.B., Singh, R.P. and Singh, D.P., 2011. Genetically engineered bacteria: an emerging tool for environmental remediation and future research perspectives. *Gene*, 480(1-2): 1-9.
- Galiulin, R.V., Bashkin, V.N., Galiulina, R.A. and Kucharski, R., 2002. Airborne soil contamination by heavy metals in Russia and Poland, and its remediation. *Land Contamination & Reclamation*, 10(3): 179-187.
- Girma, G., 2015. Microbial bioremediation of some heavy metals in soils: an updated review. *Egyptian Academic Journal of Biological Sciences, G. Microbiology*, 7(1): 29-45.
- Dixit, R., Malaviya, D., Pandiyan, K., Singh, U.B., Sahu, A., Shukla, R., Singh, B.P., Rai, J.P., Sharma, P.K., Lade, H. and Paul, D., 2015. Bioremediation of heavy metals from soil and aquatic environment: an overview of principles and criteria of fundamental processes. *Sustainability*, 7(2): 2189-2212.
- Athar, M. and Vohora, S.B., 1995. Heavy metals and environment. New Age International (P) Limited, New Delhi.
- Hussein, H., Farag, S. and Moawad, H., 2003. Isolation and characterization of *Pseudomonas* resistant to heavy metals contaminants. *Arab Journal of Biotechnology*, 7: 13-22.
- Perelo, L.W., 2010. In situ and bioremediation of organic pollutants in aquatic sediments. *Journal of Hazardous Materials*, 177(1-3): 81-89.
- Deepali, A., 2011. Bioremediation of Chromium (VI) from textile industry's effluent and contaminated soil using *Pseudomonas putida*. *Iranian (Iranica) Journal of Energy and Environment*, 2(1): 24-31.
- Atikpo, E., 2016. Spatial Distribution and Attenuation of Heavy Metals Pollution in Amaoye Ishiagu Forest Soils. A PhD Thesis Submitted to the Department of Civil Engineering, University of Benin, Benin City, Nigeria.
- Murthy, S., Bali, G. and Sarangi, S.K., 2012. Biosorption of lead by *Bacillus cereus* isolated from industrial effluents. *British Biotechnology Journal*, 2(2): 73-84.
- Samarth, D.P., Chandekar, C.J. and Bhadekar, R.K., 2012. Biosorption of heavy metals from aqueous solution using *Bacillus licheniformis*. *International Journal of Pure and Applied Sciences and Technology*, 10(2): 12-19.
- Chiroma, T.M., Ebewele, R.O. and Hymore, F.K., 2014. Comparative assessment of heavy metal levels in soil, vegetables and urban grey waste water used for irrigation in Yola and Kano. *International Refereed Journal of Engineering and Science*, 3(2): 1-9.
- Cheesbrough, M., 2006. *District Laboratory Practice in Tropical Countries Part 2*, Cambridge University Press. New York.
- Cowan, S.T., 2004. *Cowan and Steel's manual for the identification of medical bacteria*. Cambridge university press. New York.
- Baron, E.J., Peterson, L.R. and Finegold, S.M., 1994. *Bailey and Scott's diagnostic microbiology*, 9th edition, Mosby company, Baltimore.
- Holt, J.G., 1977. *The shorter Bergey's manual of determinative bacteriology*. The shorter Bergey's manual of determinative bacteriology. 8th edition. Williams and Willkins Company, Baltimore.
- Lima, E.C., Royer, B., Vagheti, J.C., Brasil, J.L., Simon, N.M., dos Santos Jr, A.A., Pavan, F.A., Dias, S.L., Benvenuti, E.V. and da Silva, E.A., 2007. Adsorption of Cu (II) on *Araucaria angustifolia* wastes: determination of the optimal conditions by statistic design of experiments. *Journal of Hazardous Materials*, 140: 211-220.
- Atikpo, E. and Micheal, A., 2018. Performance Evaluation of Six Microorganisms Utilized for the Treatment of Lead Contaminated Agricultural Soil. *Journal of Applied Sciences and Environmental Management*, 22(7): 1105-1109.
- Augustine, A.A., Orike, B.D. and Edidiong, A.D., 2007. Adsorption kinetics and modeling of Cu (II) ion sorption from aqueous solution by mercaptoacetic acid modified cassava (*manihot sculenta cranz*) wastes. *Journal of Environmental, Agricultural and Food Chemistry*, 6(4): 2221-2234.
- Igwe, J.C. and Abia, A.A., 2007. Adsorption kinetics and intraparticulate diffusivities for bioremediation of Co (II), Fe (II) and Cu (II) ions from waste water using modified and unmodified maize cob. *International Journal of Physical Sciences*, 2(5): 119-127.
- McKay, G. and Poots, V.J., 1980. Kinetics and diffusion processes in colour removal from effluent using wood as an adsorbent. *Journal of Chemical Technology and Biotechnology*, 30(1): 279-292.
- Chien, S.H. and Clayton, W.R., 1980. Application of Elovich equation to the kinetics of phosphate release and sorption in soils. *Soil Science Society of America Journal*, 44(2): 265-268.
- Ho, Y.S., McKay, G., Wase, D.A.J. and Forster, C.F., 2000. Study of the sorption of divalent metal ions on peat. *Adsorption Science & Technology*, 18(7): 639-650.
- Lopez, A., Lazaro, N., Priego, J.M. and Marques, A.M., 2000. Effect of pH on the biosorption of nickel and other heavy metals by *Pseudomonas fluorescens* 4F39. *Journal of Industrial Microbiology and Biotechnology*, 24(2): 146-151.
- Gourdon, R., Bhende, S., Rus, E. and Sofer, S.S., 1990. Comparison of cadmium biosorption by Gram-positive and Gram-negative bacteria from activated sludge. *Biotechnology Letters*, 12(11): 839-842.
- Korenevskii, A.A., Khamidova, K.H., Avakyan, Z.A. and Karavaiko, G.I., 1999. Silver biosorption by micromycetes. *Microbiology*, 68(2): 139-145.
- McLean, R.J.C. and Beveridge, T.J., 1990. Metal-binding capacity of bacterial surfaces and their ability to form mineralized aggregates. In: *Microbial Mineral Recovery*, pp.185-222.
- Brady, D. and Duncan, J.R., 1994. Bioaccumulation of metal cations by *Saccharomyces cerevisiae*. *Applied Microbiology and Biotechnology*, 41(1): 149-154.
- AjayKumar, A.V., Darwish, N.A. and Hilal, N., 2009. Study of various parameters in the biosorption of heavy metals on activated sludge. *World Applied Sciences Journal*, 5(5): 32-40.

30. Thieman, W.J., 2009. Introduction to biotechnology. Pearson Education India.
31. Chatain, V., Bayard, R., Sanchez, F., Moszkowicz, P. and Gourdon, R., 2005. Effect of indigenous bacterial activity on arsenic mobilization under anaerobic conditions. *Environment International*, 31(2): 221-226.
32. Guo, H., Luo, S., Chen, L., Xiao, X., Xi, Q., Wei, W., Zeng, G., Liu, C., Wan, Y., Chen, J. and He, Y., 2010. Bioremediation of heavy metals by growing hyperaccumulaor endophytic bacterium *Bacillus* sp. L14. *Bioresource technology*, 101(22): 8599-8605.
33. Norris, P.R. and Kelly, D.P., 1977. Accumulation of cadmium and cobalt by *Saccharomyces cerevisiae*. *Microbiology*, 99(2): 317-324.
34. Damodaran, D., Suresh, G. and Mohan, R., 2011. Bioremediation of soil by removing heavy metals using *Saccharomyces cerevisiae*. In 2nd international conference on environmental science and technology. Singapore. pp.22-27.
35. Lin, C.C. and Lin, H.L., 2005. Remediation of soil contaminated with the heavy metal (Cd²⁺). *Journal of hazardous materials*, 122(1-2): 7-15.
36. Kang, C.H., Kwon, Y.J. and So, J.S., 2016. Bioremediation of heavy metals by using bacterial mixtures. *Ecological Engineering*, 89: 64-69.
37. Owamah, H.I., 2014. Biosorptive removal of Pb (II) and Cu (II) from wastewater using activated carbon from cassava peels. *Journal of Material Cycles and Waste Management*, 16(2): 347-358.
38. Boparai, H.K., Joseph, M. and O'Carroll, D.M., 2011. Kinetics and thermodynamics of cadmium ion removal by adsorption onto nano zerovalent iron particles. *Journal of Hazardous Materials*, 186(1): 458-465.

Persian Abstract

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چکیده

توانایی‌های سه باکتری جدا شده بومی از خاک‌های آلوده به کادمیوم جمع‌آوری شده از محل مزرعه آگابو نزدیک به معدنی در ایالت اوندو، نیجریه به منظور ارائه اطلاعات مفید برای تصفیه خاک و مدیریت بهداشت خاک در این منطقه فرعی مورد بررسی قرار گرفت. باسیلوس سوبتیلیس، پروتئوس میرابیلیس و اشرشیا کولای جدا شده از خاک‌ها به نمونه‌های مختلف خاک تلقیح گردیده و شرایط مطابق با فاکتورهای بهینه‌سازی شده که از آزمایشات مرحله اول تعیین شدند تنظیم شد. نمونه‌های مورد بررسی برای غلظت کادمیوم باقیمانده با زمان (روز) با استفاده از اسپکتروفتومتر جذب اتمی اندازه‌گیری گردید. کاهش کادمیوم خاک از غلظت اولیه ۷۰/۲۱ میلی گرم در کیلوگرم تا کمتر از حداکثر مجاز ۳ میلی گرم در کیلوگرم برای ارگانسیم‌ها دوره طولانی بود. باسیلوس سابتیلیس در زمان ۳۵ روز با بازده ۹۶/۱۰ درصد و غلظت باقیمانده ۲/۷۴ میلی گرم بر کیلوگرم حذف را انجام داد. پروتئوس میرابیلیس، و اشرشیا کولای به ترتیب راندمان بالاتر از ۸۵/۰۵ درصد و ۷۹/۳۵ درصد را ارائه نکردند. نرخ ظرفیت‌های حذف d^{-1} ۰/۱۳۱- برای باسیلوس سابتیلیس، d^{-1} ۰/۱۱۱- برای پروتئوس میرابیلیس، d^{-1} ۰/۱۰۵- برای اشرشیا کولای به دست آمد. چهار مدل سینتیکی به خوبی با داده‌های تجربی منطبق شدند. ارزیابی مدل نشان داد که فرایند نفوذ در حذف به عنوان یکی از پدیده‌های انتقال مرحله کنترل‌کننده سرعت است.
