BIOSORPTION OF LEAD IONS BY IMMOBILISED *BACILLUS CIRCULANS* FROM AQUEOUS SOLUTION IN A BATCH SYSTEM

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ABSTRACT

Lead is considered to pose great risks to living things and there is the need in providing an alternative environmental friendly and cost effective technique such as biosorption for the remediation of Lead ions in aqueous solution. This paper contains an analysis on biosorption of Lead ions from aqueous solution in a batch process system using a lyophilised immobilised biosorbent of Bacillus Circulans. *The optimal parametric factors affecting biosorption for Lead ions onto immobilised biosorbent of Bacillus* Circulans *such as pH, contact time, biosorbent dosage and initial concentration in a batch process system were investigated. The biosorbent developed was characterised using Scanning electron microscope with Energy dispersive X-ray spectroscopy as well as Fourier transformed infrared spectroscopy. The* optimum percentage removal *values for pH, contact time, biosorbent dosage and initial concentration in the batch process system were determined as* 3.0 *with 74.23, 100.0 min with 91.73%, 0.8g with 84.49% and 40.0 mg/l with 97.12%, respectively. The biosorption mechanism gives better fitness of Freundlich isotherm model compared to Langmuir isotherm model and the kinetics follows Pseudo- second-order for immobilised Bacillus circulans and lead ions contacting. The results show that immobilised Bacillus* Circulans *is efficient in the removal of Lead ions from aqueous solution.*

Keywords: Biosorption, Optimal parameters, Characterization of biosorbent, Isotherm and kinetic

1 INTRODUCTION

Among all the biological methods present, biosorption is an alternative process which makes use of one of the significant properties of both living and dead natural materials. Biosorption process mostly remediate the site faster and can remove higher amount of toxic metals than bioaccumulation [1]. This is due to its simplicity, analogous operation to conventional technology, apparent efficiency and availability of biomass and waste bio-products [2]. Biosorbents possess metalsequestering property which can remediate heavy metal ions in solution from ppm to ppb levels and can effectively sequester dissolved metal ions out of dilute complex solutions with high efficiency and quickly [3]. Lead ions approximated concentration ranges of discharge in the environment from battery industry is 5 to 66 mg/L, from mining industry is 0.02 to 2.5 mg/l and that from the oil industry is 125 to 150 mg/l. Lead is considered to pose great risks to animals and plants. It causes several illnesses for humans, such as anaemia, brain damage, kidney damage, loss of appetite, cognitive impairment, behavioural issues, vomiting and mental retardation [4, 5, 6]. In humans, binding of lead to those enzymes can change the cellular membrane permeability of organs and haemoglobin synthesis, it can also bioaccumulate in bones (its half-time is over 20 years). Thus, it is highly important to remove lead from wastewater before discharging it in the environment [6]. The aim of this work is to determine percentage removal of lead ions by optimisation of some vital parameters that affect biosorption using immobilised *Bacillus circulans* from aqueous solution in a Batch System.

2 MATERIALS AND METHODS

2.1 Preparation of Solution and Serial Dilution

A stock solution ($100\ mg/l$*) of lead ions was prepared from* Lead nitrate$(\mathrm{Pb(NO_3)_2})$. A synthetic wastewater was prepared by serial dilution of the stock solution of *Lead ions* solution into distilled water with the required amount and was used for analysis. The desired metal ions solution was ranged between$20 \text{ to } 100\ \mathrm{mg/l}$ and Nitrate salt was used as the counter ion for metal ions because of its low tendency to form complexes [7].

2.2 Immobilisation of Biosorbent

Entrapment method that involved drop wise addition of biomass suspended in polymer matrix of 2% (w/v) sodium alginate solution and 5% (w/v) biomass was adapted [8]. The beads were produced when the alginate biomass slurry was introduced drop wise into 250 ml of dissolve dehydrate 0.1 M $CaCl_2$ using 20 ml syringe for polymerisation. The beads after production was kept inside Haier Thermocool Refrigerator HRD – 231SX at 4°C (freezer) in $CaCl_2$ solution for 12 – 14 h for hardening. Then, washed with 500 ml distilled water twice. The drained beads were spread on a dryer tray using GENLAB ENGLAND – N535 oven and kept at $80 - 100$ ℃ for an hour and later adjusted to 40 ℃ till the whole beads were dried. After drying the bead were kept inside tight bottle for future use as biosorbent.

2.3 Characterisation of Immobilised *Bacillus circulans*

The characterisation was done before and after biosorption of Lead ions to test for the suitability of the biosorbent. PHENOM Prox Netherland Scanning electron microscope (SEM) with Energy dispersive X-ray spectroscopy (EDX) gives information on surface morphology and elemental composition of the biosorbent, respectively; while AGILENT TECHNOLOGIST USA – Cary 630 Fourier transformed infrared spectroscopy (FTIR) gives information on functional groups presence and chemical composition of the biosorbent.

2.4 Batch Process System Optimisation

Batch process system that were used in this study are Erlenmeyer conical flasks of$100\ ml$. Five flasks were incubated in a rotary shaker type at $150\ rpm$ and room temperature (32 ℃) for $100\ min$ to obtain equilibrium for each biosorbate and biosorbent used to monitor a particular parameter under investigation (Plate 1). The mixtures of lead and immobilised *Bacillus circulans* were decanted and the residual concentration of each lead ions solution after biosorption was determined using VGP210 - AAS, respectively. The removal percentage (R) of the metal ion by the immobilised *Bacillus circulans* was achieved using equation (1) [9, 10, 11].

$$R(\%) = \frac{C_i - C_e}{C_i} \times 100 \quad (1)$$

Where, $\mathrm{C_i}$ and $\mathrm{C_e}$ are the initial and equilibrium metal concentrations in the water ($\mathrm{mg/l}$), respectively.

Optimum values of pH, biosorbent doses, contact time and initial metal concentration was determined for lead ions biosorption in batch contactor. The optimum value of pH for each metal ion at an initial concentration of 100 mg/l and 1000 mg biosorbent in 50 ml of metal solution at room temperature (32°C) for 100 min at pH varying from 3.0 – 7.0 using HANNA INSTRUMENTS - $pHep^{(R)}$ pH meter was obtained by adding0.1 M NaOHorHCl. The effect of contact time was observed at room temperature, each metal ion initial concentration of $100\ \mathrm{mg/l}$ and 1000 mg biosorbent in 50 ml of metal solution at 32 ℃ for 100 min and an optimizedpH. The effect of contact time was investigated by varying at 20, 40, 60, 80 and 100 min, room temperature, initial concentration of 100 mg/l and 1000 mg biosorbent in 50 ml of metal solution. Moreover, effect of biosorbent dosage was optimised by using biosorbent amounts of 200, 400, 600, 800 and 1000 mg in 50 ml of an initial metal ions concentration of 100 mg/l each at room temperature and at optimised pH as well as contact time. For optimisation of initial metal concentration, biosorption investigation at optimised parametric values was achieved with

initial metal ions concentrations each of value $20, 40, 60, 80\ and\ 100\ mg/l$, respectively [12]. The concentrations of lead ions after iosorption were evaluated using standard method for examination of water and wastewater [13]. In batch system, the mixture of biosorbent and wastewater were decanted and the clear supernatant from aqueous sample were analysed using VGP210 - Atomic absorption spectroscopy for each lead ions concentration. The lead standards used for the calibration curve were: 5.0, 10.0, 15.0, 20.0 and 25.0 mg/l with their corresponding absorbance recorded as 0.026, 0.052, 0.078, 0.104 and 0.130 respectively.

Plate 1. Erlenmeyer Conical Flasks with Cork Containing Lead Ions and Immobilised *Bacillus circulans*

3.0 RESULTS AND DISCUSSION

3.1 Characterisation of Immobilised *Bacillus circulans*

3.1.1 SEM analysis

The results are presented in Plates 2 and 3, chronologically.

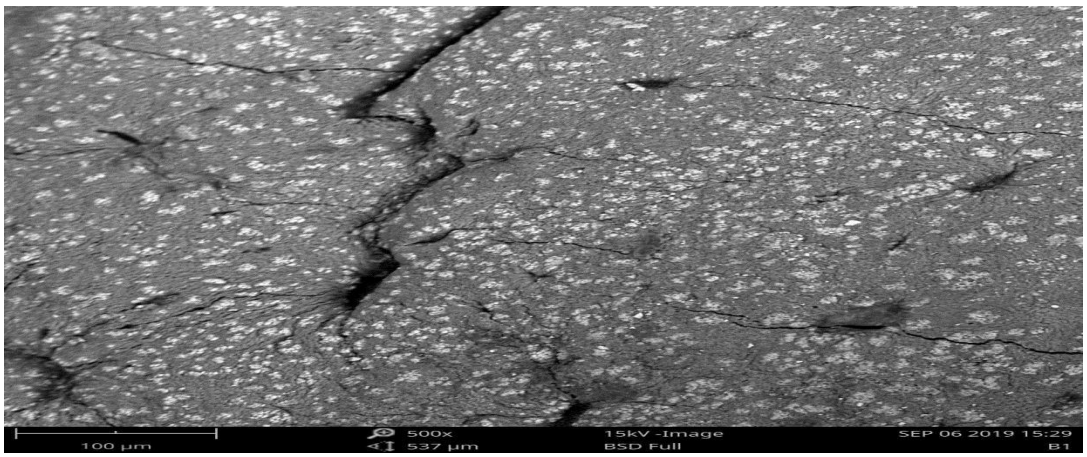


Plate 2. SEM Image of Immobilised *Bacillus Circulans* before Biosorption at 500x magnification

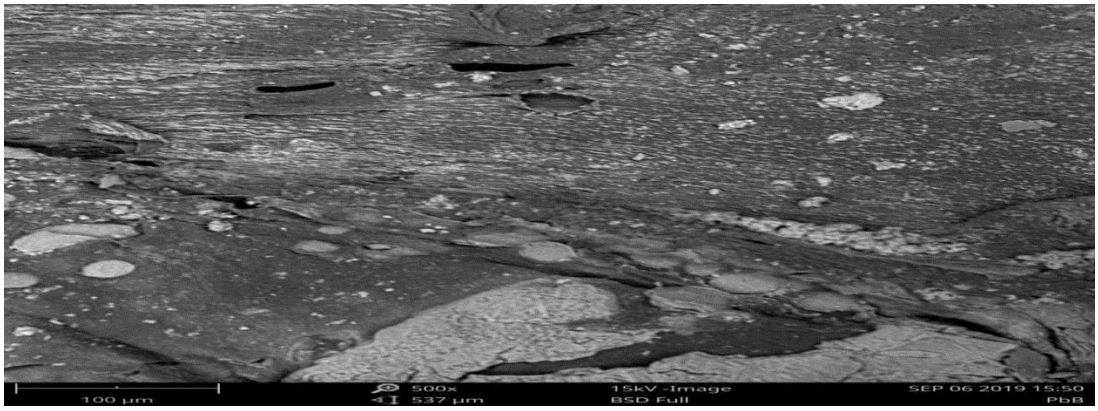
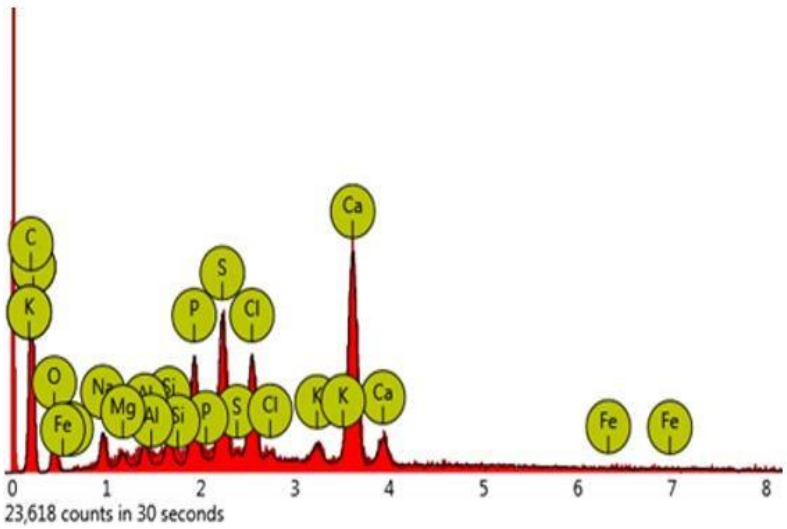


Plate 3. SEM Image of Immobilised Bacillus Circulans for Lead ions after biosorption at 500x magnification

The results shown by SEM images of immobilised Bacillus circulans biosorbent for lead ions clearly displayed a change in the surface morphology of the biosorbents after exposure to biosorbates. The surface of biosorbents were observed to be made up of heterogeneous structure and micropores which were known for more biosorption and internally bioaccumulation of biosorbates. The results of this investigation showed similarity to that of Ririhena et al. [14], which was reported that SEM of Saccharomyces cerevisiae after biosorption showed changes and have more holes than before biosorption which look more hydrated and plumper. This morphological change occurred due to Cu^{2+} ions biosorption at the cell wall.

3.1.2 EDX analysis

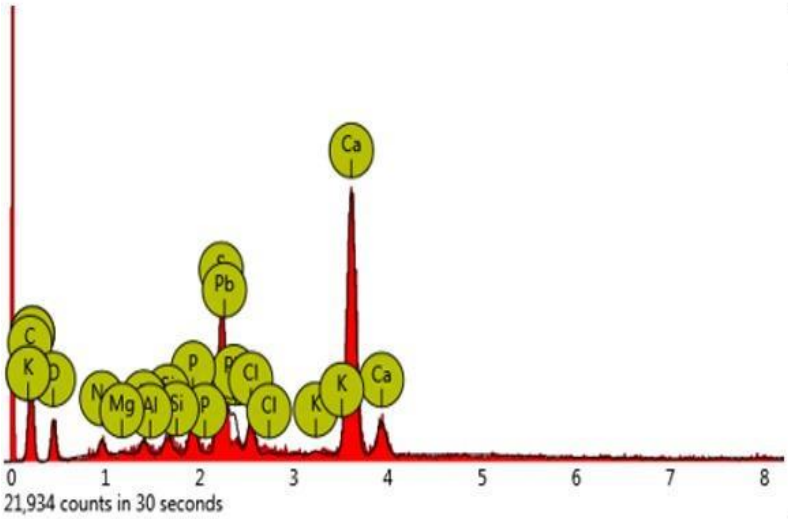
The results are demonstrated in Figure 1 for biosorbent before biosorption while Figure 2 display that of the biosorbent after biosorption, respectively.



Element	Atomic %	Weight %
Carbon (C)	68.57	48.22
Calcium (Ca)	7.65	17.94
Oxygen (O)	11.09	10.39
Sulfur (S)	3.77	7.08
Chlorine (Cl)	2.86	5.94
Phosphorus (P)	2.55	4.63
Sodium (Na)	1.26	1.7
Potassium (K)	0.56	1.29
Silicon (Si)	0.61	1
Aluminium (Al)	0.59	0.92
Magnesium (Mg)	0.39	0.56
Iron (Fe)	0.09	0.31
Totals	100	100

Figure 1. EDX images of immobilised *Bacillus circulans* before biosorption

No amount of lead ions was observed on immobilised *Bacillus circulans* as displayed in Figure 1 before biosorption.



Element	Atomic %	Weight %
Calcium (Ca)	22.02	30.39
Lead (Pb)	3.88	27.69
Carbon (C)	45.08	18.64
Oxygen (O)	15.00	8.26
Sulfur (S)	6.19	6.83
Phosphorus (P)	2.39	2.55
Chlorine (Cl)	2.07	2.53
Silicon (Si)	1.01	0.98
Sodium (Na)	1.11	0.88
Aluminium (Al)	0.85	0.79
Potassium (K)	0.24	0.33
Magnesium (Mg)	0.16	0.13
Totals	100	100

Figure 2. EDX images of immobilised *Bacillus circulans* after biosorption

Energy dispersive X-ray spectroscopy (EDX) was used to show that lead ions (Pb^{2+}) have been adsorbed on the surface of the immobilised *Bacillus circulans* as shown in Figure 2 after biosorption due to changes observed in the elemental composition of the biosorbent [15].

3.1.3 FTIR analysis

The FTIR results are presented in Figures 3 and 4, respectively.

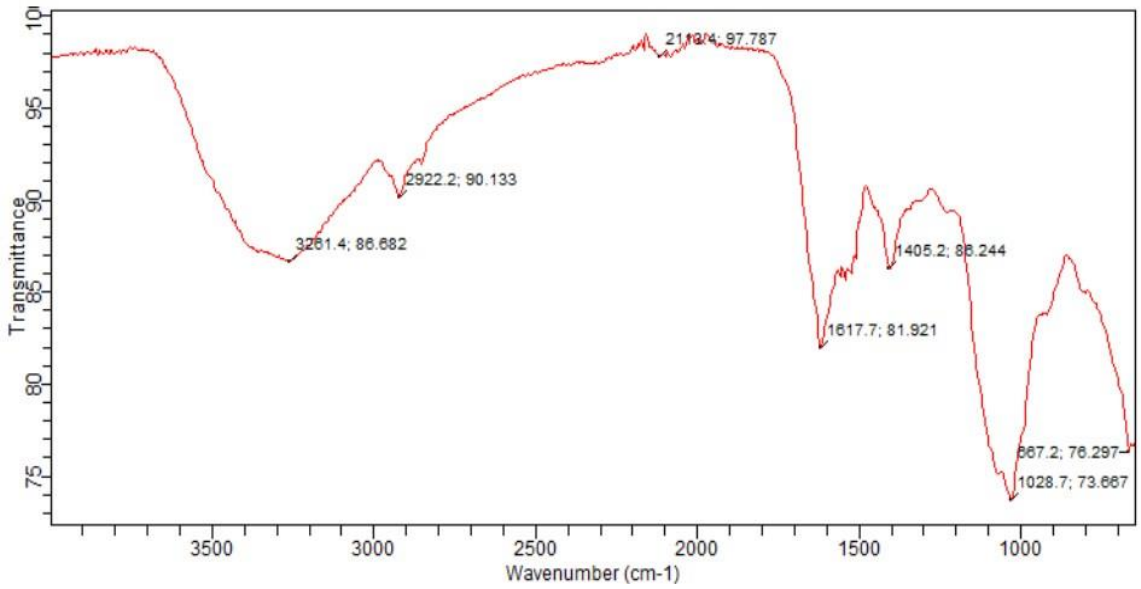


Figure 3. FTIR Spectra of Immobilised *Bacillus Circulans* before Biosorption

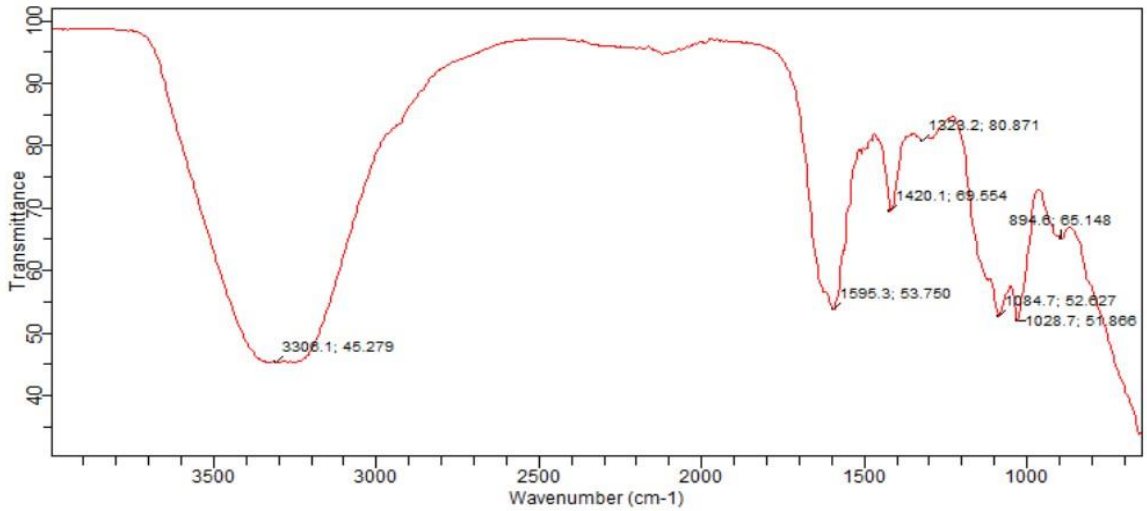


Figure 4. FTIR Spectra of Immobilised *Bacillus Circulans* for Lead ions after biosorption

The FTIR spectra for immobilised Bacillus circulans (Figure 3) showed the functional groups that are present in the biosorbent before biosorption. The major peaks were depicted at 3261.4, 2922.2, 2110.4, 1617.7, 1405.2, 1028.7 and 667.2 cm^{-1}; this implies the assigned functional group are $-OH$ stretch for carboxylic acid, $C-H$ stretch for aldehyde, $C \equiv C$ stretch for alkyne, $N-H$ bend for amine, $-OH$ bend for phenol/ tertiary alcohol, $C-N$ stretch for primary amine and $-OH$ for alcohol out of phase bend, respectively [12, 16, 17]. This showed interaction exist between the lead ions and the immobilised Bacillus circulans because the peaks were shifted after biosorption as depicted by Figures 3 and 4 which is similar to what was observed by Kariuki et al. [17], where the FTIR spectrum profiles for roger mushroom free and loaded with copper and lead ions recorded a shift in the functional group which reveals that chemical interaction between the biosorbate and biosorbent exist.

3.2 Process Optimisation

The results for batch process system optimisation of lead onto immobilised *Bacillus circulans* which include pH of Lead ions, contact time, biosorbent dosage and initial concentration of Lead ions are illustrated in Figures 5 - 8, respectively. Figure 5, presents optimum value of pH of biosorbate.

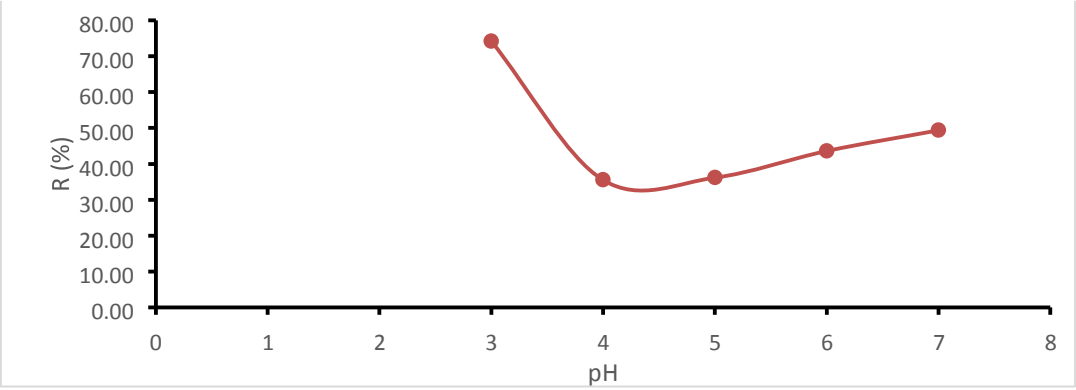


Figure 5. Effect of pH on Removal Percentage of Lead onto *Bacillus Circulans*

Lead ions onto immobilised Bacillus circulans as biosorbent measured optimum percentage removal (Figure 5) at pH of 3 with value of 74.23%. The results obtained are in line with Jones et al., [10] which shows fluctuations in biosorption efficiency with a constant increase in pH of the biosorbates of Cd^{2+}, Fe^{2+}, Ni^{2+} and Zn^{2+} using Mucilage from Dicerocaryum Eriocarpum Plant as biosorbent. The batch investigation on effect of contact time are presented in Figure 6.

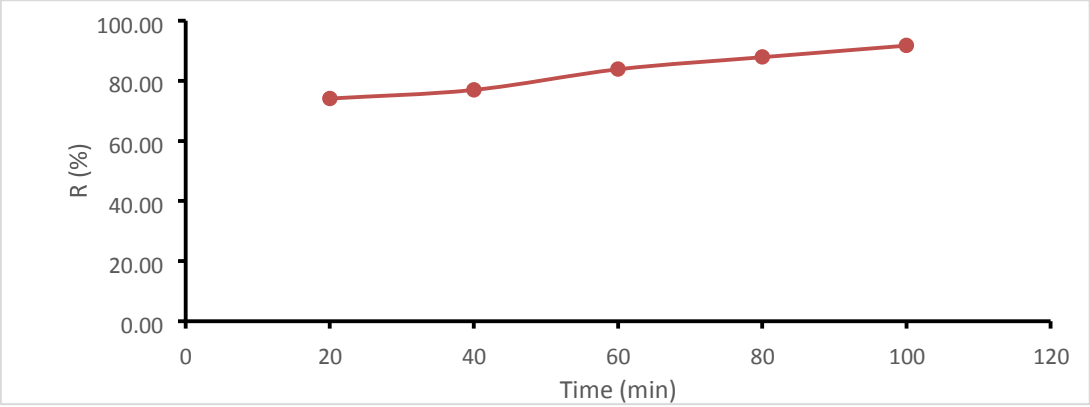


Figure 6. Effect of Contact Time on Removal Percentage of Lead ions onto *Bacillus Circulans*

Lead ions onto *Bacillus circulans*, shows a gradual increase in percentage removal (Figure 6) and an optimal value was recorded at contact time $100\ min$ with value of 91.73 %. The results of this study show similarity to those of Colak *et al.*, [18], where Biosorption of lead from aqueous solutions by *Bacillus strains* possessing heavy-metal resistance as biosorbent show that larger number of vacant surface sites are available for biosorption in the initial stage and virtually remain constant with increase in contact time. The batch process system study on effect of Biosorbent dosage are illustrated in Figure 7.

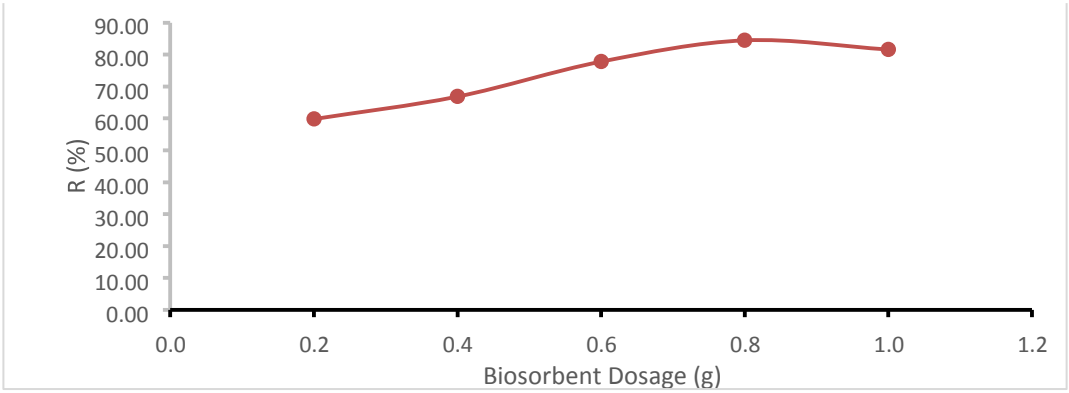


Figure 7. Effect of Biosorbent Dosage on Removal Percentage of Lead ions onto *Bacillus Circulans*

The maximum percentage removal for lead ions onto *Bacillus circulans* was measured at $0.8\ g$ with value of 84.49 % as shown in Figure 7. This is in agreement with the explanation given by Kariuki *et al.* [17], where biosorption of Lead and Copper onto rogers mushroom biomass as biosorbent increases rapidly with increase in biosorption site of biomass dosage by varying the biomass from $0.2\ g$ to$3.0\ g$. For effect of initial concentration of Lead ions as biosorbate onto immobilised *Bacillus circulans* in a batch process system study are illustrated in Figure 8.

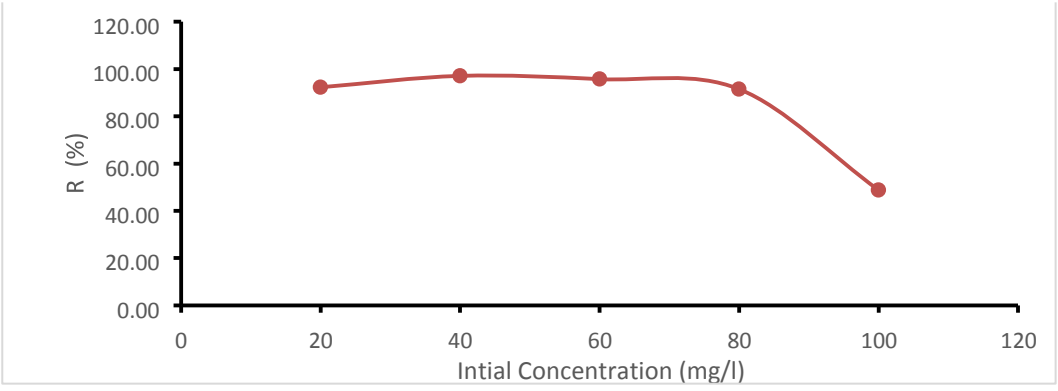


Figure 8. Effect of Initial Concentration on Removal Percentage of Lead onto *Bacillus Circulans*

The optimum percentage removal for lead ions onto *Bacillus circulans* (Pb B) was measured at 40 mg/l with value 97.12% as shown in Figure 8. This shows that an increase in initial concentration of lead ions above 40 mg/l affects the activities of the functional groups at the surface of immobilised *Bacillus circulans* which resulted in an increase in mass transfer resistance between the aqueous biosorbate and the solid biosorbent. Below 40 mg/l the results agree with Kariuki *et al.* [17], where biosorption of lead and copper ions using rogers mushroom biomass as biosorbent increase with increase in the initial concentration of each biosorbate.

3.3 Biosorption Isotherm and Kinetics Testing

3.3.1 Isotherm

The results of biosorption isotherm testing for Langmuir isotherm model are shown in Figure 9 and that of the Langmuir model isotherm parameters are presented in Table 1.

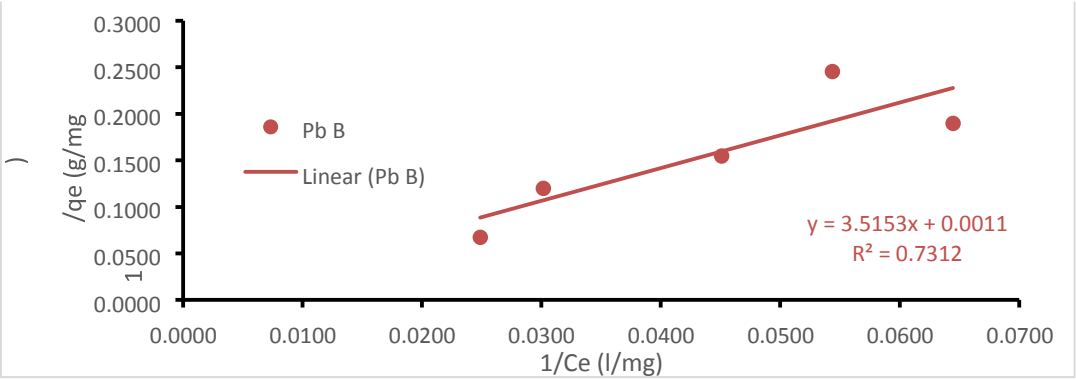


Figure 9. Langmuir Model Fitness for Lead ions onto immobilised *Bacillus Circulans* Table

1. Langmuir Isotherm Model Parameters

Sample	(mg/g)	K_L (l/mg)	R_L	R^2
	909.091	0.000313	0.9697	0.7312

The monolayer saturated capacity (q_{max}) *value was recorded as* $909.0910\ mg/g$ *for* lead ions onto *Bacillus circulans*$(Pb\ B)$ and R^2 value at 0.9697 for lead ions onto *Bacillus circulans* $(Pb\ B)$. Therefore, a higher value of q_{max} *recorded as* $909.0910\ mg/g$ *which is above the initial concentration* implies that the biosorption data do not give better fit of Langmuir isotherm model for Lead ions onto immobilised *Bacillus circulans* [16, 17]. The results of biosorption isotherm testing for Freundlich isotherm model are presented in Figure 10 and Freundlich model isotherm parameters are shown in Table 2.

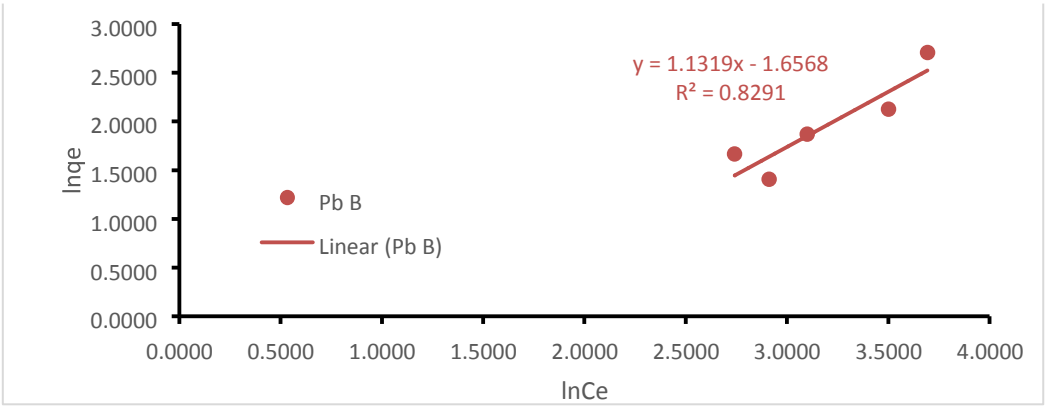


Figure 10. Freundlich Model Fitness for Lead ions onto *Bacillus Circulans*

Table 2. Freundlich Isotherm Model Parameters

Sample	1/n	n	K_F (mg/g l)	R^2
	1.1319	0.8835	0.191	0.8291

The Freundlich isotherm model parameters shown in Table 2 presented the coefficient of determination (R^2) for the biosorption of Lead ions onto immobilised Bacillus Circulans with the value recorded as 0.8291. The value of intensity of heterogeneity $(\frac{1}{n})$ of lead ions onto immobilised Bacillus circulans$(Pb\ B)$ was recorded as 1.1319which is above unity and is an indicative that the biosorption process is cooperative adsorption. The value of intensity of biosorbate biosorption onto biosorbent (n) is 0.8835 which is less than one an indicates that biosorption is favourable. The equivalent maximum biosorption capacity for Freundlich constant (K_F) *gives value measured as* $1.91X\ 10^{-1}mg/g\ l$. Therefore, Freundlich isotherm parameters are indicative of better fitness of Freundlich isotherm model to Langmuir isotherm model for Lead ions biosorption onto immobilised Bacillus Circulans [16].

3.3.2 Kinetics

Results of biosorption kinetic testing are presented in Figures 11 - 13.

Pseudo first order model

Pseudo first order model plot is given in Figure 11 and model parameters are shown in Table 3.

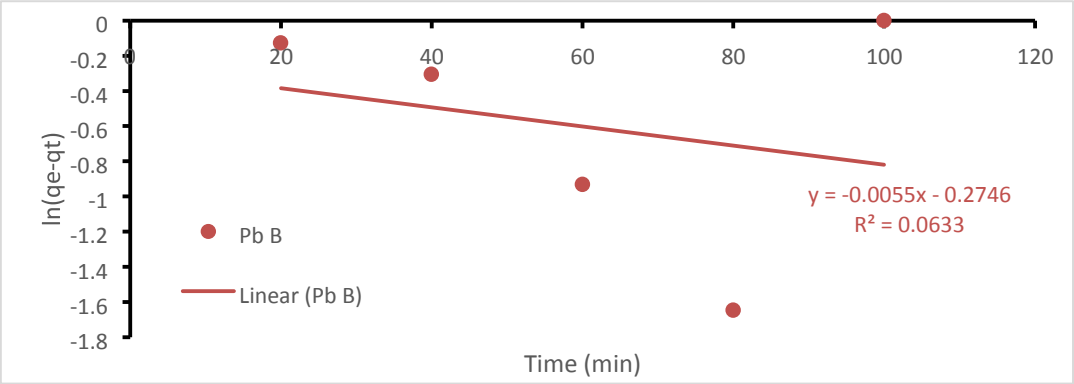


Figure 11. Pseudo first order Model Fit of Lead ions onto *Bacillus Circulans*

Table 3. Pseudo first order Model Parameters

Sample	(1/min)	(mg/g)	R^2
Pb B	0.0055	2.444	0.0633

Figure 11 and Table 3 show that Pseudo first order Kinetics Model plots and Parameters obtained recorded lower values of coefficient of determination (R^2) and the experimental q_e values are not in agreement with the model's values for biosorbate biosorption onto biosorbent. This is an indication that Pseudo first order Kinetics Model is not fit for biosorption of lead ions onto *Bacillus circulans* [11, 19].

Pseudo second order model

Pseudo second order model graphs are illustrated in Figure 12 and model parameters are presented in Table 4.

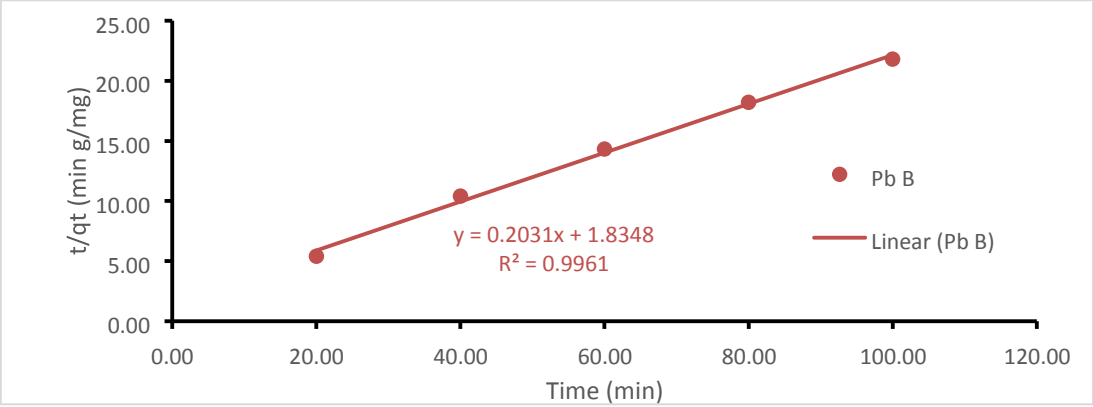


Figure 12. Pseudo second order Model Fit of Lead ions onto *Bacillus Circulans* Table

4. Pseudo second order Model Parameters

Sample	(g/mg min)	(mg/g)	h (mg/g min)	R^2
	0.0225	4.92	0.5448	0.9961

The experimental values obtained were tested using Pseudo second order kinetics model and Parameters as depicted in Figure 12 and Table 4. Higher values of coefficient of determination (R^2) was recorded as 0.9961 and the experimental q_e value is in agreement with the model's value for biosorbate biosorption onto biosorbent illustrating that Lead ions gives better fitness of Pseudo second order kinetics model to Pseudo first order kinetics model. This also indicate that all the three biosorbates onto different biosorbents used in this research agree with Pseudo second order kinetics model and the rate-limiting step may be chemisorption that involves valance forces through exchange or sharing of electrons [11, 16, 19]. The values of (K_2) which suggest the biosorption process in two reactions. The first one is the fast reaction and equilibrium is attained quickly while the second is slower reaction that can proceed for long time.

Intra particle diffusion model

Intra particle diffusion model results are demonstrated in Figure 13 and model parameters are presented in Table 5.

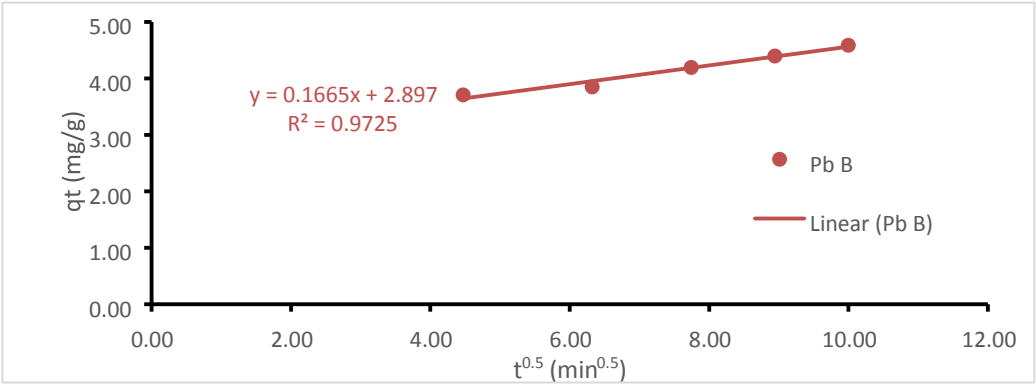


Figure 13. Intra particle Diffusion Model Fit of lead ions onto *Bacillus Circulans*

Table 5. Intra Particle Diffusion Model Parameters

Sample	(mg/g $min^{0.5}$)	C (mg/g)	R^2
	0.1665	2.897	0.9725

Furthermore, Figure 13 and Table 5 depicted that an intercept (C) was established for biosorbate biosorption onto biosorbent. The value of intercept was recorded as 2.897 mg/g illustrating that intra

particle diffusion is not the rate controlling mechanism for biosorption [5, 7]. The Pseudo second order kinetics model is the model that fit the experimental data.

4 CONCLUSIONS

The immobilised *Bacillus circulans* biosorbent developed was suitable for biosorption of Lead ions in aqueous environment in the batch process system at room temperature of 32 ℃.The Scanning electron microscope with Energy dispersive X-ray spectroscopy as well as Fourier transformed infrared spectroscopy depicted some change in surface morphology, elemental composition and peaks respectively, which were confirmation for binding of lead ions on the immobilised *Bacillus circulans* biosorbent. The Freundlich isotherm model and Pseudo second order kinetic model gave better performance of biosorption isotherm model and kinetic model tested, respectively.

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