

Batch isotherm and kinetic of cadmium ions biosorption by immobilised *Bacillus circulans* and *Saccharomyces cerevisiae* from aqueous solution

¹Luka, Y., ²Highina, B. K. and ²Zubairu, A.

¹Department of Chemical Engineering, Modibbo Adama University, Yola, Adamawa State, Nigeria

²Department of Chemical Engineering, University of Maiduguri, Borno State, Nigeria

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Abstract:

Heavy metals are very harmful to the environment and living organisms if the maximum permissible limit is exceeded. Cadmium is one of the known heavy metals that have effect on the environment and living organism. There is the need for an environmentally friendly and cost-effective technique such as biosorption for removal or controlling the excess of cadmium ions in aqueous solution. The isotherm and kinetic of lyophilised immobilised *Bacillus circulans* and *Saccharomyces cerevisiae* were investigated as biosorbents for biosorption of cadmium ions from aqueous solution in a batch process system. The biosorbents developed were characterised using Scanning electron microscope and Fourier transform infrared spectroscopy. The effects of environmental parameters on biosorption of cadmium ions onto immobilised biosorbents of *Bacillus circulans* and *Saccharomyces cerevisiae* in a batch process system were also compared and investigated. The optimum parametric values such as pH, contact time, biosorbent dosage and initial concentration for: Cadmium ions onto *Bacillus circulans* were obtained as 7, 100 min, 1.0 g and 100 mg/l, respectively; Cadmium ions onto *Saccharomyces cerevisiae* were recorded as 6, 80 min, 1.0 g and 100 mg/l, respectively. The biosorption mechanism shows better fitness of Freundlich isotherm model and the kinetics follows Pseudo second order for both immobilised *Bacillus circulans* and *Saccharomyces cerevisiae* for cadmium ions contacting. The results show that immobilised *Bacillus circulans* and *Saccharomyces cerevisiae* are highly efficient in the removal of cadmium ions from aqueous solution. The percentage removal for cadmium ions onto *Bacillus circulans* gives better optimum performance to *Saccharomyces Cerevisiae* contacting.

Corresponding author

Luka, Y.

sufuluk@mau.edu.ng

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1. Introduction

The wide spread of heavy metals into the water body and environment can be attributed to domestic, agricultural and industrial activities and is one of the major concerns of the ecosystems today [1, 2, 3, 4]. Treatment of water or wastewater using biosorption Techniques which is cost effective and environmentally friendly before use is the only solution to controlling water-borne diseases easily identified with contaminated or polluted water because of cadmium ions [2]. Cadmium ions if exceeded the maximum permissible limit in human beings may cause, bronchitis, cancer and kidney damage [5].

Several methods have been implemented for removal of heavy metals such as chemical precipitation, ion exchange, membrane filtration and reverse osmosis. These methods have high cost for energy and maintenance as well as the generation of toxic byproducts; and they are not environmentally friendly compared to biosorption [4, 6]. The use of freely suspended microbial biomass has its negative effects which includes small particle size, low mechanical strength and problems

associated with separation of immobilised *Bacillus circulans* and *Saccharomyces cerevisiae* (biomass) from the effluent [4]. Due to these reasons, immobilised *Bacillus circulans* and *Saccharomyces cerevisiae* were used as biosorbents for the biosorption in a Batch process System.

The aim of this study is to investigate Batch Biosorption isotherm and kinetic of immobilised *Bacillus circulans* and *Saccharomyces cerevisiae* to remediate cadmium ions from aqueous solution as well as investigating the environmental parameters affecting the biosorption.

2. Materials and methods

2.1 Preparation of Stock Solution and Serial Dilution

A stock solution (100 mg/l) of cadmium ions was prepared from cadmium nitrate ($Cd(NO_3)_2 \cdot 4H_2O$). To determine the potential of immobilised *Bacillus circulans* and *Saccharomyces cerevisiae* to remove Cadmium ions from aqueous solution and for optimisation of some

selected environmental factors in a batch process system. Synthetic wastewater was prepared by serial dilution of the stock solution of cadmium ions solution each into distilled water with the desired metal ions solution ranged between 20 – 100 mg/l and Nitrate salt were used as the counter ions for metal ions because of its low tendency to form complexes [7, 8, 9].

2.2 Immobilisation of Biosorbent

Entrapment method as adapted by Luka, et al. [8] was used for immobilisation of the beads. The resultant

beads after production were kept using Haier Thermocool Refrigerator HRD – 231SX at 4 °C (freezer) in $CaCl_2$ solution for 12 – 14 h for hardening as depicted in Plate 1. It was 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 °C for an hour and later adjusted to 40 °C till the whole beads were dried for avoidance of burning sensation to the bead produced as shown in Plate 2. After drying, each strain of immobilised biomass bead was kept inside tight bottle for future use as biosorbent.



Plate 1: Fresh and life immobilised biosorbent before heat killing



Plate 2: Washed and drained beads spread on a dryer tray ready for drying in an oven

2.3 Characterisation of Immobilised *Bacillus circulans*

The characterisation was done before and after biosorption of cadmium ions. PHENOM Prox Netherland Scanning electron microscope (SEM) gives information on surface morphology of each biosorbent of immobilised *Bacillus circulans* and *Saccharomyces cerevisiae*, respectively. While AGILENT TECHNOLOGIST USA – Cary 630 Fourier transform infrared spectroscopy (FTIR) gives information on functional groups presence and chemical composition of each biosorbent of immobilised

Bacillus circulans and *Saccharomyces cerevisiae*, respectively [4, 8, 9].

2.4 Batch Process System

Batch process system that was used in this study are Erlenmeyer conical flasks of 100 ml. Five flasks for each metal ions were incubated in a rotary shaker type at 150 rpm and room temperature of 32°C for 100 min to obtain equilibrium for each biosorbate and biosorbent used to monitor a particular parameter under investigation. The mixtures were decanted and the residual concentrations of each cadmium ions solution after biosorption were

determined using VGP210 - AAS, respectively. The removal percentage (R) of the metal ion by the biosorbent was achieved using equation 1 [8, 10, 11]:

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

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

2.5 Optimisation of environmental Parameters

Optimum values of pH, biosorbent doses, contact time and initial metal concentration [4] were determined for Cadmium ions biosorption in batch mode. The optimum value of pH for each metal ions at an initial concentration of $100 \frac{mg}{l}$ and 1.0 g 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 were obtained by adding 0.1 M NaOH or HCl. The effect of contact time was investigated at room temperature, each metal ion initial concentration of $100 \frac{mg}{l}$ and 1.0g biosorbent in 50 ml of metal solution at 32 °C for 100 min and an optimised pH. The effect of contact time was studied by varying at 20, 40, 60, 80 and 100 min room temperature, initial concentration of 100 mg/l and 1.0g biosorbent in 50 ml of metal

solution. Furthermore, effects of biosorbent dosage were optimised by using biosorbents amounts of 0.2, 0.4, 0.6, 0.8 and 0.1 g 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 investigations at optimised parametric values were achieved with initial metal ions concentrations each of value 20, 40, 60, 80 and 100 mg/l respectively [12]. In batch system, the mixture of biosorbent and wastewater were decanted and the clear supernatants from aqueous sample were analysed using VGP210 - Atomic absorption spectroscopy for each cadmium ions concentration. The final concentration of each metal ions was measured using Standard method for examination of water and wastewater as adapted by Luka, et al. [8].

3. Results and discussion

3.1 Characterisation of biosorbents

3.1.1 SEM analysis of immobilised *Bacillus circulans*

The SEM images results for biosorbents before and after biosorption of cadmium are presented in Plates 3 - 6. Plates 3 - 4 depict the change in morphology displayed by *Bacillus circulans* before and after biosorption with cadmium ions, respectively.

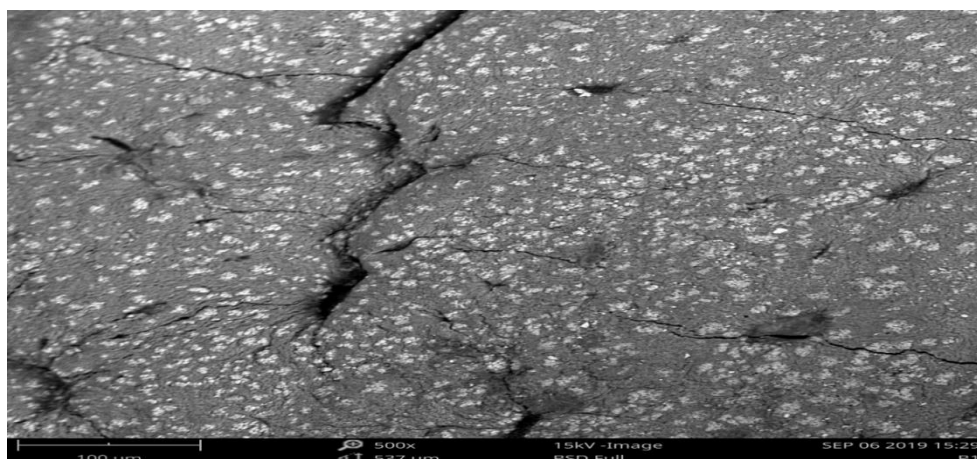


Plate 3: SEM image of immobilised *Bacillus Circulans* before biosorption at 500x magnification

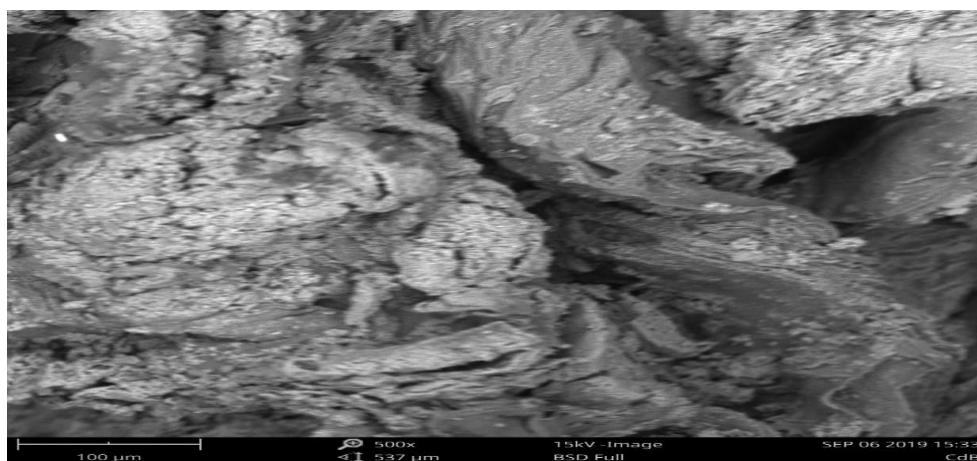


Plate 4: SEM image of immobilised *Bacillus Circulans* for Cadmium ions after biosorption at 500x magnification

The results shown by SEM images of immobilised *Bacillus circulans* biosorbent for cadmium ions for each analysis clearly show a change in the surface morphology of the biosorbents after exposure to cadmium ions. The surface of biosorbents was observed to be made up of heterogeneous structure and micropores which were known for more biosorption and internally bioaccumulation of biosorbate. The results of this studies show similarity to that of Luka, et al., [8] and Ririhena, et al., [13] which were reported that SEM of *Saccharomyces cerevisiae* after biosorption show changes and have more holes than

before biosorption which look more hydrated and plumper. This morphological change occurs due to cadmium ions biosorption at the cell wall.

3.1.2 SEM analysis of immobilised *Saccharomyces cerevisiae*

Plates 5 - 6 show the images of immobilised *Saccharomyces cerevisiae* biosorbent. The plates display the change in morphology before and after biosorption with cadmium ions respectively.

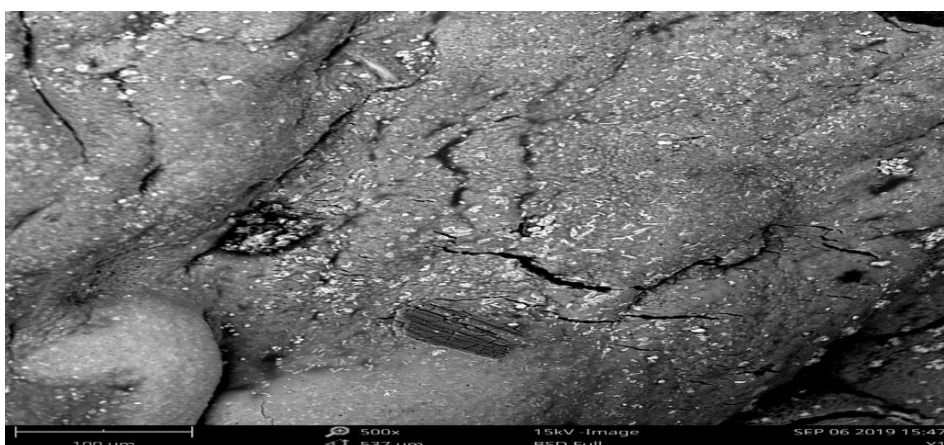


Plate 5: SEM image of immobilised *Saccharomyces Cerevisiae* before biosorption at 500x magnification

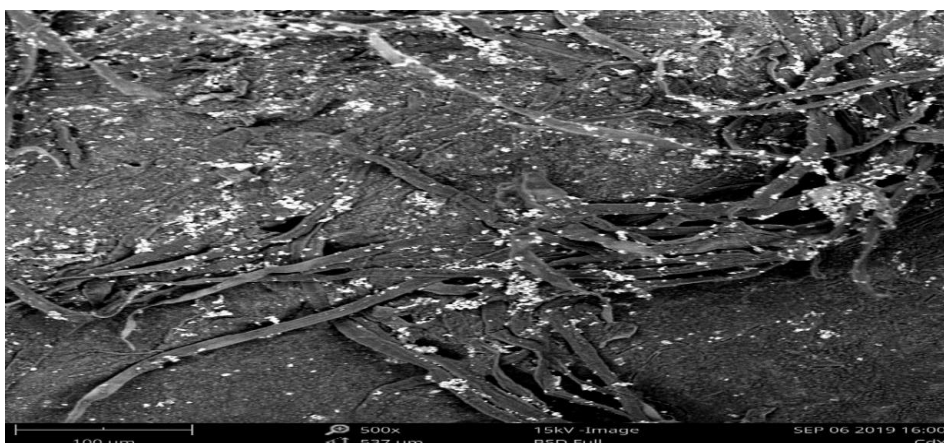


Plate 6: SEM image of immobilised *Saccharomyces Cerevisiae* for Cadmium ions after biosorption at 500xmagnification

The surface of *Saccharomyces cerevisiae* before biosorption in Plate 5 contains many traps but shorter than that of *Bacillus circulans* in Plate 3. The micrograph of *Bacillus circulans* before biosorption has tinier micropores on the surface to that of *Saccharomyces cerevisiae*. After biosorption of cadmium ions onto *Bacillus circulans* and cadmium ions onto *Saccharomyces cerevisiae*, the surface structure of both biosorbents were transformed significantly as shown in Plates 2 and 4 for *Bacillus circulans* and *Saccharomyces cerevisiae* biosorbents, respectively [14, 15].

3.1.3 FTIR of immobilised *Bacillus circulans*

The results of the FTIR Spectra for biosorbents before and after biosorption with cadmium are presented in Figures 1 - 4. Figures 1 – 2 demonstrate the trend obtained

for *Bacillus circulans*. The FTIR spectra for *Bacillus circulans* in Figure 1 showed the functional groups that are present in the biosorbent before biosorption. The major peaks were noted 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 [8, 16, 17].

After biosorption of cadmium onto *Bacillus circulans* biosorbent, there were shift in the peaks. The peaks were shifted to 3254.0, 1595.3, 1416.4, 1297.1, 1084.7, 1028.7 and 890.8 cm^{-1} this is attributed to interaction between Cd^{2+} and the functional groups present in *Bacillus circulans* biosorbent surface.

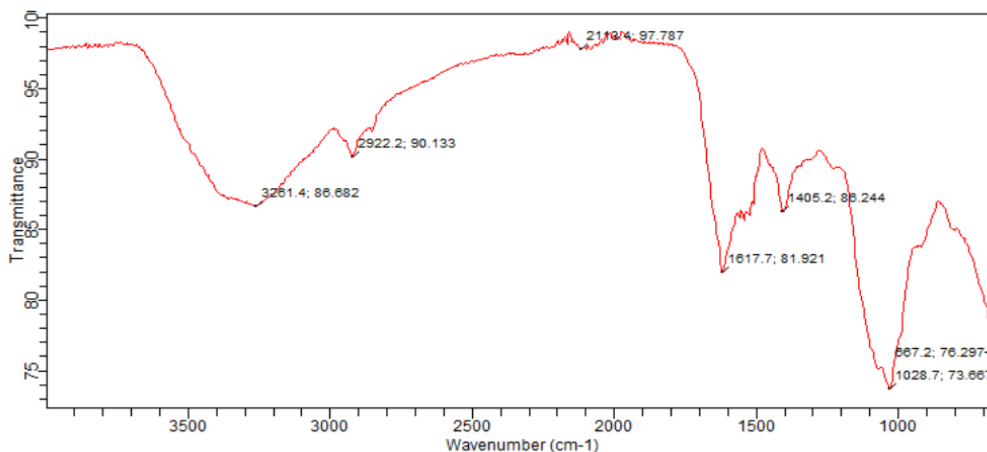


Figure 1: FTIR spectra of immobilised *Bacillus Circulans* before biosorption

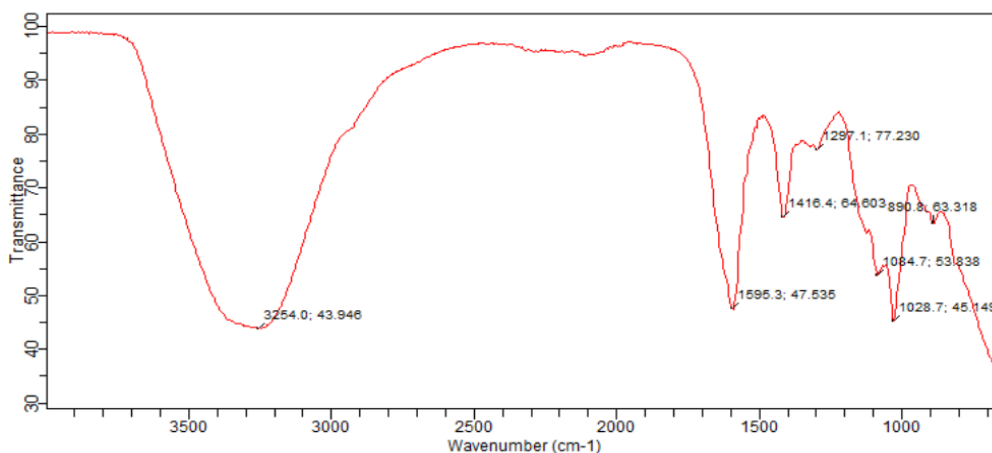


Figure 2: FTIR spectra of immobilised *Bacillus Circulans* for Cadmium ions after biosorption

3.1.4 FTIR of immobilised *Saccharomyces Cerevisiae*

Figures 3 – 4 show the outcome of using *Saccharomyces cerevisiae* as biosorbent. The FTIR spectra of *Saccharomyces cerevisiae* biosorbent before biosorption (Figure 3) were recorded at 3272.6, 2922.2, 2102.2, 1621.4, 1405.2, 1233.7, 1028.7 and 805.1 cm^{-1} ; the corresponding functional groups 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, $C-O$ stretch for alcohol/carboxylic acids/esters/ethers, $C-N$ stretch for primary amine and $C-H$ for aromatic out of plane bend, respectively. Peaks shift were recorded after biosorption of cadmium ions by *Saccharomyces cerevisiae* biosorbents in Figure 4 at 3208.9, 1636.3, 1457.4 and 1039.9 cm^{-1} due to the interaction that occur between the biosorbent and the biosorbate [15, 18, 19].

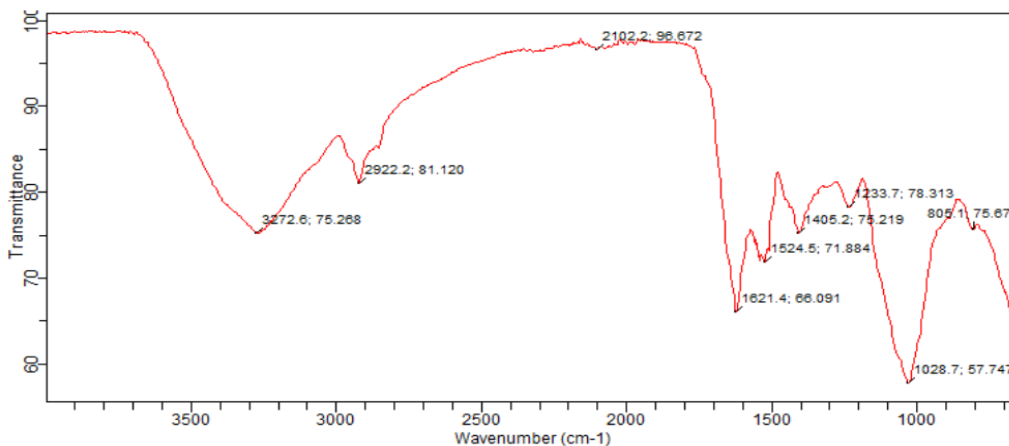


Figure 3: FTIR spectra of immobilised *Saccharomyces Cerevisiae* before biosorption

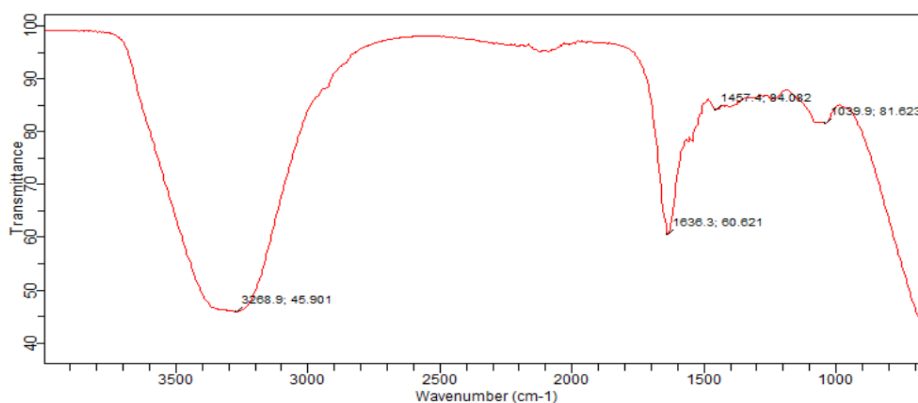


Figure 4: FTIR spectra of immobilised *Saccharomyces Cerevisiae* for Cadmium ions after biosorption

3.2 Optimisation of environmental parameters

The results for optimisation of environmental parameters affecting biosorption of cadmium ions onto immobilised *Bacillus circulans* and *Saccharomyces cerevisiae* which include pH of biosorbate, contact time, biosorbent dosage and initial concentration of cadmium ions are presented in Figures 5 - 8, respectively. Figure 5 present optimum values of pH for cadmium ions.

The percentage removal of cadmium ions was recorded to be higher onto *Bacillus circulans* (Cd B) to *Saccharomyces cerevisiae* (Cd Y) for batch contactor Figures 5. Maximum Percentage removal (Figure 5) was obtained at pH of 6 with values 92.28 % for

Saccharomyces cerevisiae (Cd Y). While, optimum percentage removal for *Bacillus circulans* (Cd B) was recorded at pH of 7 with values 93.11 %. The percentage removal is less at lower pH because majority of hydrogen ions with the metal ions compete for the active binding sites of both *Bacillus circulans* and *Saccharomyces cerevisiae*. The results obtained are in line with Jones, et al., [2] 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 is presented in Figure 6.

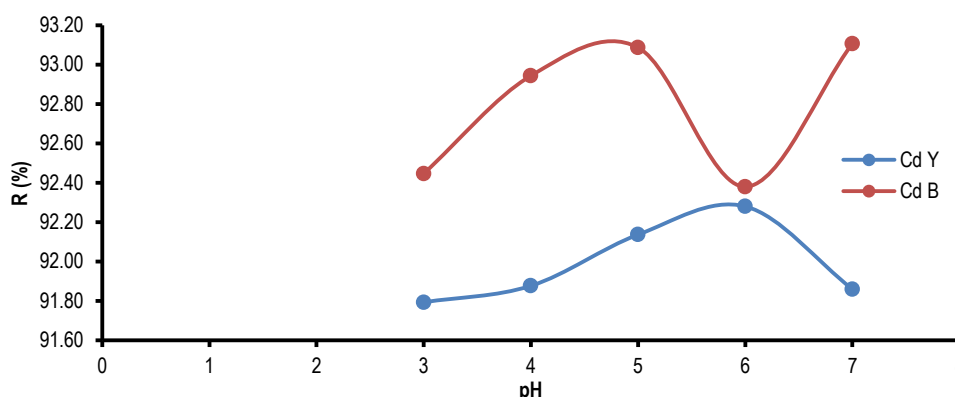


Figure 5: Effect of pH on removal percentage of Cadmium onto *Bacillus Circulans* and *Saccharomyces Cerevisiae*

Maximum percentage removal as can be seen from Figure 6 was recorded at contact time of 80 min with values 92.11 % for cadmium ions onto *Saccharomyces cerevisiae* (Cd Y). These results show an increase of time from 20 min through 80 min and later decreased at 100 min. While, optimum percentage removal of cadmium ions for *Bacillus circulans* was recorded at contact time of 100 min with value of 93.17 %. In the beginning for cadmium biosorption as shown in Figures 5 - 6, percentage removal was faster due to large number of vacant surface site presence or empty active site on both biosorbents. This is in agreements with the explanation given by Jones et al., [2] for Cd^{2+} , Fe^{2+} , Ni^{2+} and Zn^{2+} ions using Mucilage from *Dicerocaryum Eriocarpum* Plant as biosorbent, where biosorption increased rapidly at the beginning and became very slow or steady with time.

The optimum percentage removal for cadmium ions onto *Bacillus circulans* (Cd B) and cadmium ions onto *Saccharomyces cerevisiae* (Cd Y) increases with increase in biosorbent dosage from 0.2 g to 1.0 g as shown in Figure 7. Reason may be due to availability of more active site for the biosorbate at higher biosorbent dosage. The optimum percentage removal for cadmium ions onto *Bacillus circulans* (Cd B) and cadmium ions onto *Saccharomyces cerevisiae* (Cd Y) were measured at 1.0 g as shown in Figure 7 with value 93.52 % and 92.08 %, respectively [2, 17]. The illustrations for effect of initial concentration of percentage removal are presented in Figure 8.

Percentage removal of biosorbate increases with increase in initial concentration from 20 $\frac{mg}{l}$ to 100 $\frac{mg}{l}$. This may be due to availability of more active site which is

not saturated with biosorbate at higher initial concentration of biosorbate. The maximum percentage removal for cadmium ions onto *Bacillus circulans* (Cd B) and cadmium

ions onto *Saccharomyces cerevisiae* (Cd Y) were measured at 100 mg/l as shown in Figure 8 with value 93.69 % and 92.25 %, respectively.

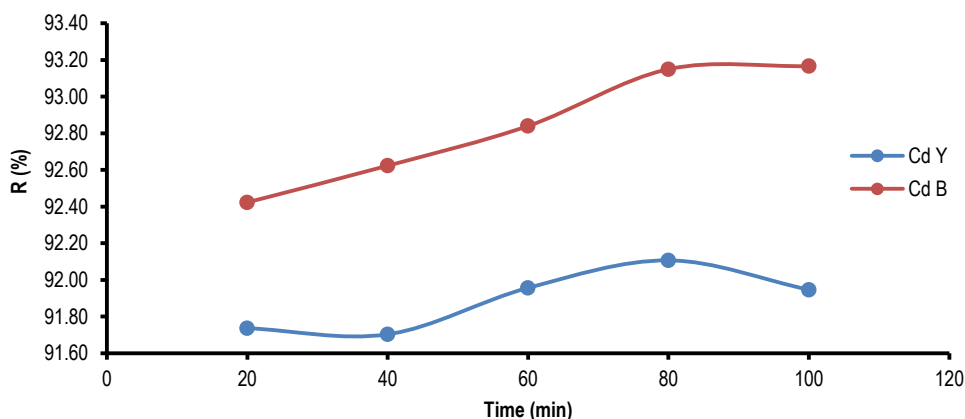


Figure 6: Effect of contact time on removal percentage of Cadmium onto *Bacillus Circulans* and *Saccharomyces Cerevisiae*

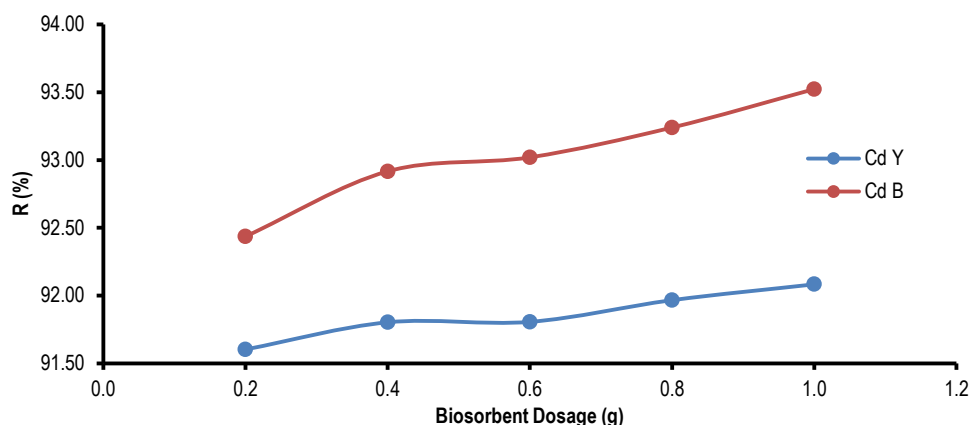


Figure 7: Effect of biosorbent dosage on removal percentage of Cadmium onto *Bacillus Circulans* and *Saccharomyces Cerevisiae*

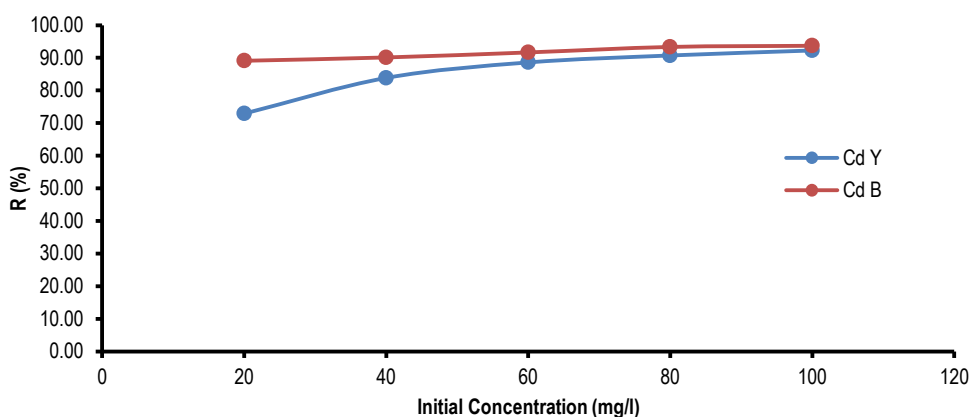


Figure 8: Effect of initial concentration on removal percentage of Cadmium onto *Bacillus Circulans* and *Saccharomyces Cerevisiae*

3.3 Biosorption Isotherm and Kinetics Testing

3.3.1 Biosorption Isotherm

The results of biosorption isotherm testing for Langmuir isotherm model and Freundlich isotherm are presented in Figures 9 and 10, respectively. In addition, Langmuir model isotherm parameters and Freundlich

isotherm parameters are presented in Tables 1 and 2, respectively.

3.3.1.1 Langmuir isotherm model

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

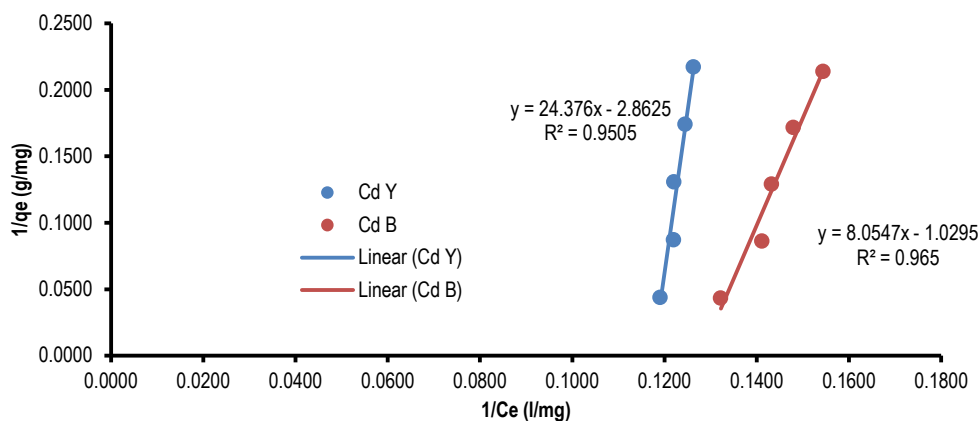


Figure 9: Langmuir model fitness for Cadmium ions onto *Bacillus Circulans* and *Saccharomyces Cerevisiae*

Table 1: Langmuir isotherm model parameters

Sample	q_{max} (mg/g)	K_L (l/mg)	R_L	R^2
<i>Cd Y</i>	-0.3493	-0.117	-0.0931	0.9505
<i>Cd B</i>	-0.9713	-0.128	-0.0849	0.965

The Langmuir isotherm model gives maximum value of coefficient of determination (R^2) for cadmium ions onto *Bacillus circulans* (*Cd B*) as 0.9650 and shown in Table 1. The monolayer saturated capacity (q_{max}), Langmuir constant (K_L) and separation factor (R_L) give negative values for all the two contacting processes. This shows

that the biosorption data do not give better fit of Langmuir isotherm model [8, 16, 17].

3.3.1.2 Freundlich model

Figure 10 illustrated Freundlich isotherm model fitness testing and Freundlich model isotherm parameters are presented in Table 2.

Table 2: Freundlich isotherm model parameters

Sample	$1/n$	n	K_F (mg/g l)	R^2
<i>Cd B</i>	10.731	0.0932	8.06×10^{-9}	0.9577
<i>Cd Y</i>	27.125	0.0369	1.72×10^{-24}	0.9163

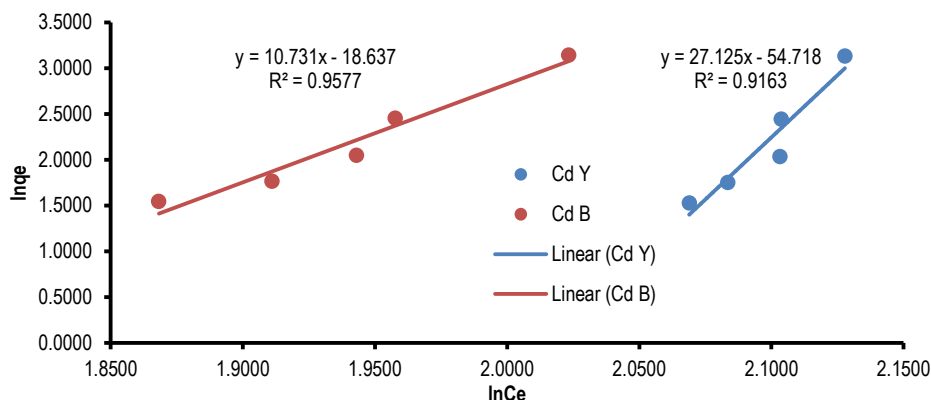


Figure 10: Freundlich model fitness for Cadmium ions onto *Bacillus Circulans* and *Saccharomyces Cerevisiae*

The Freundlich isotherm model parameters shown in Table 2 presented the coefficient of determination (R^2) for the biosorption of all biosorbate onto biosorbent with the maximum value recorded as 0.9577 for cadmium ions onto *Bacillus circulans* (*Cd B*). The values of intensity of heterogeneity ($\frac{1}{n}$) were recorded to be more than one which indicate biosorption process is cooperative. Furthermore, values of intensity of biosorbate biosorption onto biosorbent (n) are less than one for all biosorption process which indicates biosorption of all biosorbate onto biosorbent are favourable. The equivalent maximum biosorption capacity for Freundlich constant (K_F) gives value measured in the ranged of 1.72×10^{-24} – $8.06 \times 10^{-9} \text{ mg/g l}$. The higher the value of K_F implies higher maximum biosorption capacity of biosorbate onto biosorbent. Therefore, Freundlich isotherm

parameters are indicative of favourable fitness of Freundlich isotherm model for cadmium ions biosorption onto the biosorbents investigated [8, 11, 16, 17, 20].

3.3.2 Batch Biosorption kinetic models testing

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

3.3.2.1 Pseudo first order model

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

Table 3: Pseudo first order model parameters

Sample	K_1 (1/min)	q_e (mg/g)	R^2
<i>Cd Y</i>	-0.0111	-1.473	0.0303
<i>Cd B</i>	-0.0513	-2.5856	0.6254

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 cadmium ions biosorption onto the different biosorbents. This is an indication that Pseudo first order Kinetics Model is not fit for biosorption of cadmium ions onto *Bacillus circulans* and *Saccharomyces cerevisiae* [8, 11, 20].

3.3.2.2 Pseudo second order model

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

Table 4: Pseudo second order model parameters

Sample	K_2 (g/mg min)	q_e (mg/g)	h (mg/g min)	R^2
Cd Y	2.125	4.60	45.045	1.0000
Cd B	0.744	4.67	16.233	1.0000

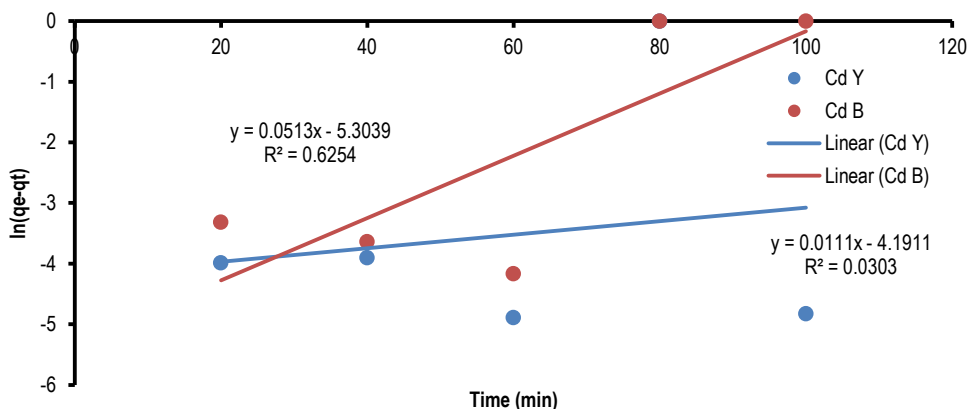


Figure 11: Pseudo first order Model Fit of Cadmium ions onto *Bacillus Circulans* and *Saccharomyces Cerevisiae*

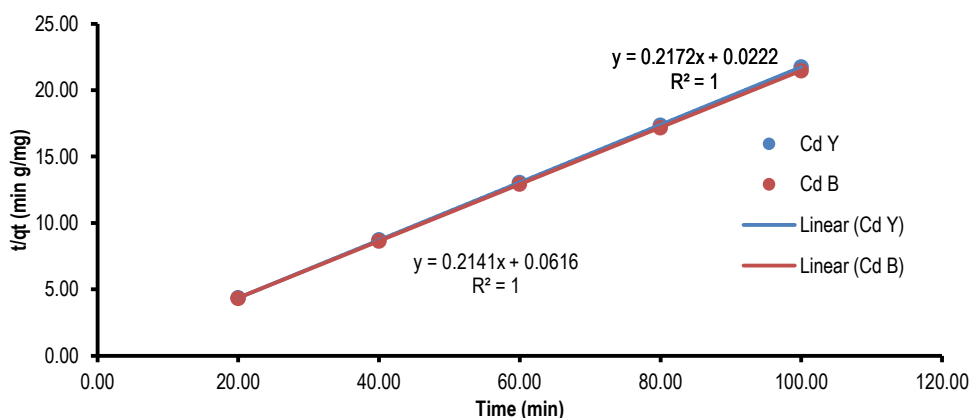


Figure 12: Pseudo second order Model Fit of Cadmium ions onto *Bacillus Circulans* and *Saccharomyces Cerevisiae*

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) for both biosorbents were recorded as 1.0000 and the experimental q_e values agree with the model's values for all cadmium ions biosorption onto biosorbents. This also indicate that cadmium ions onto the 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, 20]. The values of (K_2) which suggest the biosorption process in two reactions. The first one is the fast reaction, and equilibrium is attaining quickly while the second is slower reaction that can proceed for long time. The K_2 values for cadmium ions biosorption onto *Bacillus circulans* biosorbent is less than one which indicate the mechanism is not chemisorption except for cadmium ions onto *Saccharomyces cerevisiae* (Cd Y) that recorded a K_2 of

2.1250 g/mg min only. The initial biosorption rate of cadmium ions (h) recorded the highest value as 45.0450 mg/gmin for cadmium ions onto *Saccharomyces cerevisiae* (Cd Y).

3.3.2.3 Intra particle diffusion model

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

Table 5: Intra particle diffusion model parameters

Sample	K_{tp} (mg/g min ^{0.5})	C (mg/g)	R^2
Cd Y	0.0030	4.5717	0.0623
Cd B	0.0073	4.5870	0.9640

Furthermore, experimental results obtained from batch contactors were also tested for Intra particle diffusion kinetic model and parameters as presented in Figure 13 as well as Table 5.

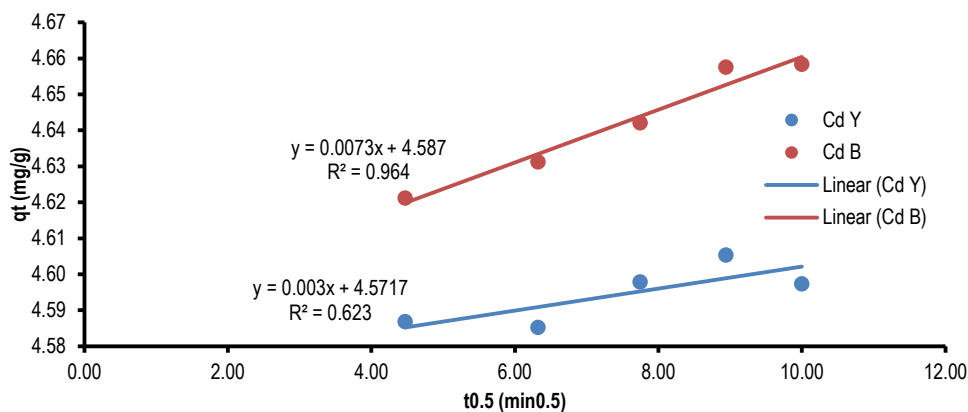


Figure 13: Intra particle Diffusion Model Fit of Cadmium ions onto *Bacillus Circulans* and *Saccharomyces Cerevisiae*

There are three steps involved in biosorption processes of substance [7] from liquid phase onto solid phase biosorbent. The steps are bulk diffusion, external diffusion through liquid layer around the biosorbent particles and intra particle diffusion. From Figure 13 it is clearly observed that for cadmium ions biosorption onto biosorbents an intercept (C) was established. The values of intercept ranged from 4.5717 – 4.5870 *mg/g* illustrating that the line does not pass through the origin and describing the boundary layer effects. Therefore, intra particle diffusion is not the rate controlling mechanism for biosorption [7, 8, 15, 20].

4. Conclusions

The following conclusions can be drawn from the results of this investigation:

- Bacillus circulans* and *Saccharomyces cerevisiae* biosorbents were able to remove Cadmium ions from aqueous solution; displaying suitability of both biosorbents for biosorption as confirmed by Scanning electron microscope and Fourier transform infrared spectroscopy.
- Immobilised *Bacillus circulans* gives better performance to *Saccharomyces cerevisiae* as biosorbents for the biosorption of cadmium ions from wastewater in a Batch process system.
- Concerning batch system models investigated, Freundlich isotherm model parameters shown best fit for cadmium ions onto the biosorbents. The kinetics obeyed Pseudo second order kinetics model with R^2 of 1.0000 for both biosorbents investigated.

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