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Biosorption Kinetics of *vetiveria zizanioides* Rhizobacter on Heavy Metals Contaminated Wastewater

Aremu M. O.*, Agarry S. E., and Oyebamiji T. E
Department of Chemical Engineering, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.
*Corresponding author e-mail: moaremu@lautech.edu.ng

Abstract
The burden of heavy metals pollution in the environment has increased over the last century. Consequently, concerted efforts towards addressing this menace in the environment and subsequently on health has being on the increase. A number of plants and microorganisms are currently being experimented for their potential to uptake heavy metals from both soil and water. However, the kinetics of uptake of heavy metals in wastewater which are necessary for the design of the treatment system have been largely neglected, this paper therefore investigates the kinetics of biosorption of heavy metals in contaminated wastewater using two microorganism isolated from rhizospheric soil of *Vetiveria zizanioides* (*vetiver*) plant. The result of bioaccumulation studies showed that *Bacillus cereus* shows the maximum bioaccumulation capacity of 96.75% for Lead, 23% for Cadmium and 16.98% for Zinc while *Bacillus subtilis* accumulated 95.2% of the Lead, 41.3% of Cadmium and 32.2% of Zinc from solution. Also, the result of kinetic studies revealed that the kinetic data agrees with pseudo-second order kinetic model.
Keywords: Biosorption, Heavy metals, Rhizospheric, Wastewater, *Vetiveria zizanioides.*

1. Introduction

The state of human environment in recent time is an issue of great concern since the quality of the environment is a function of the concentration of pollutants in such environment and severe environmental degradation has being a prevailing situation in the major cities recently. Due to increase in the industrialization, urbanization and technological advancement of the globe, solid and liquid pollutants are discharged daily uncontrollably into the environment resulting in imbalance in the ecological system of the world [2; 4; 13]. In the field of water pollution, removal of toxic metals from sewage and industrial waste water is a matter of great interest [19; 16; 4]. Some of the major effects of environmental pollution is that it renders it less economically useable. Long time discharge of wastewater or irrigation water with heavy metals may result in the accumulation of heavy metals like; Pb, Cd, Zn, As, Cr, Ni and a lot more in the soil, which may in turn result in the contamination of human food chain [18; 3]. Human exposure to heavy metals occur either through ingestion, inhalation or skin contact and the chronic problems associated with heavy metal exposure may range from intermediate poisoning to death. Heavy metals like Cd affects mineral assimilation, physiological and biochemical characteristics of plant, it also retards plant growth [15]. According to Pinto [12], Cd is highly toxic and soluble in water. In some plant species, the interactions of Cd and metal nutrients have shown changes in the plant nutrient concentration and composition [10].

Lead is a prominent soil and water contaminant [17]. Lead poisoning was reported to be a causative factor for the death of more than 800,000 children not more than the age of five in the united State. Likewise, high level of inhalation and ingestion of lead-laden aerosol and dust is as a result of socio-ecological and climatic factors [9; 14].

Before the eras of bioremediation, several methods such as sedimentation and filtration, flocculation, neutralization, electrodialysis, reverse osmosis and adsorption are the techniques conventionally explored in water treatment [5]. The disadvantages of these techniques include incomplete metal removal, high reagent cost and energy requirements and generation of toxic sludge or other waste products [1]. None the less, these techniques are costly, less efficient, time consuming and there is always an associated problem of secondary disposal. Furthermore, applications of some of these techniques are restricted due to economic and technological constraints [11].

Bioremediation is a biological process in which microorganism is used to remove pollutant from the contaminated environment (water and soil), it can either be in-situ or ex-situ process. Some examples of bioremediation technology are bioventing, landfarming, bioreactor, composting, rhizofiltration, bioargumentation and biostimulation [7]. Phytoremediation as an aspect of bioremediation has proven satisfactorily in various ways both for contaminated soil and water.

Vetiveria zizanioides (vetiver) is one of the hyperaccumulator of some heavy metals. According to [2], vetiver has distinctive morphological features, for instance its root is like an underground curtain or wall, enabling the plant to retain water and moisture and therefore creating a favorable environment to a diversity of microorganisms in the soil. A number of microorganisms (Algae, Fungi and Bacteria) are being experimented for their potential as biosorbent in bioremediation studies. Nonetheless, specific microbial species are used to oxidize, precipitate and reduce heavy metals toxicity in the environment. The objective of this paper therefore is to investigate the potential and kinetics of *vetiver rhizobacter* in ex-situ bioremediation of water contaminated with Pb, Zn and Cd.

2. Materials and Methods

2.1 Microorganisms

Bacillus cereus, Lactobacillus species and *Bacillus subtilis* were isolated from rhizospheric soil of *Vetiveria zizanioides* plant.

2.2 Isolation of Microorganisms

It has been established that soil surrounding *Vetiveria zizanioides* root has associated microorganisms which complement the plant in its bioremediation activities (Brian, 2007). Serial dilution of the rhizospheric soil sample collected was carried out as follows: 1g of sample was measured separately into conical flasks and 10ml of distilled water added. The solution was subjected to vigorously shaking and allowed to settle for 2hrs. Thereafter, 1ml of the suspended solution was inoculated into Nutrient Agar (NA) media and incubated at 37^0C for 48 hours. Pure microbial strains were then obtained by series of sub-culturing. The fittest strains were then preserved and maintained in agar slants containing nutrient broth. The microbial strains were then characterized on the basis of Colonial morphology, cellular morphology and biochemical characteristics of the bacterial isolates.

2.3 Preparation of wastewater samples

Simulated wastewater samples containing Zn (II), Cd (II) and Pb (II) ions were prepared from $ZnCl_2$, $CdCl_2$ and $PbCl_2$ so that 120mg/L of Zn, 30mg/L of Pb and 30mg/L of Cd were analytically obtained in the wastewater. The pH of the simulated wastewater was maintained at 5.5 to prevent hydrolysis. The concentration of metal ions in simulated wastewater was analyzed by Atomic Absorption Spectrophotometer.

2.4 Nutrient medium

The nutrient growth medium contains the following salts in 1 liter of distilled water ; KH_2PO_4 (2.72 g/L), K_2HPO_4 (5.22 g/L), $(NH_4)_2SO_4$ (2.0 g/L), $MgSO_4$ $7H_2O$ (0.5g/L), $FeSO_4{\cdot}7H_2O$ (0.0022 g/L), $ZnSO_4{\cdot}7H_2O$ (0.004g/L), $MnSO_4$ $4H_2O$ (0.004g/L), $CuSO_4{\cdot}5H_2O$ (0.004g/L), D-Glucose (20g/L), Yeast Extract (1g/L) .

2.5 Heavy Metal Uptake Experiment

To study the kinetics of uptake of heavy metals from wastewater, all the experiments were performed in triplicates. Pure strains of the microorganisms obtained from serial dilution of rhizospheric soil were inoculated into nutrient agar (NA) media on petri-dishes and allowed to grow for 48 hrs after which the microorganisms were scraped to innoculate the conical flasks containing solutions of mineral salt medium and heavy metal salts each. Inoculated flasks were incubated for 48 hrs at 30°C on a rotary shaker (Gallenkamp, England) at 120 rpm. 5 ml samples were aseptically withdrawn at 12 hrs intervals and used to assay for microbial growth and heavy metals concentrations in the media. These were achieved by dry weight analysis of the biomass and Atomic Absorption Spectroscopy Analysis of the withdrawn samples in the air -acetylene (reducing) flame mode samples respectively.

2.6 Assay (Analytical) Method

2.6.1 Dry Weight Analysis of the Biomass

The biomass growth was determined by centrifuging and filtering the withdrawn samples (culture medium) through weighed Whatmann filter paper no. (44). The precipitated biomass were thoroughly washed with distilled water, dried at 105ºC in oven, cooled and then re-weighed. The biomass dry weight was determined by subtracting the weight of empty filter paper from the weight of filter paper with biomass when the weight is constant. [5].

2.7 Kinetic Modelling

Series of contact time experiments was carried out for Lead, Zinc and Cadmium at temperature of 30^oC and pH 5.5 to obtain data for $\boldsymbol{q_e}$, the amount of adsorption at equilibrium.

q_e (mg/g), was calculated by

$$\boldsymbol{q_e} = \frac{(Co - Ce)V}{W} \qquad (1)$$

where V is the volume of the solution, C_O and C_e (mg) are the initial and equilibrium concentrations of the heavy metals respectively and W is the biomass weight.

The amount biosorbed at time *t*, q_t, was calculated from the kinetic experiment by:

$$q_t = \frac{(Co - Ct)V}{W} \qquad (2)$$

C_o and C_t are the concentrations at initial and any time t respectively, V is the volume of the solution and W is the biomass weight.

3. Results and Discussions

The results of series of contact time experiments for Lead, Zinc and Cadmium was used to plot Figure 1. Figure 1 shows the contact time for the three heavy metals to reach equilibrium with initial concentrations; Pb 30mg, Zn 120mg and Cd 30mg. The plot shows that initially there is an increase in the rate of biosorption with time but a point is reached when dynamic equilibrium is attained. At this point, biosorption rate becomes constant and the time required to attain this point is called equilibrium time. it is also obvious that the adsorption capacity increased from.

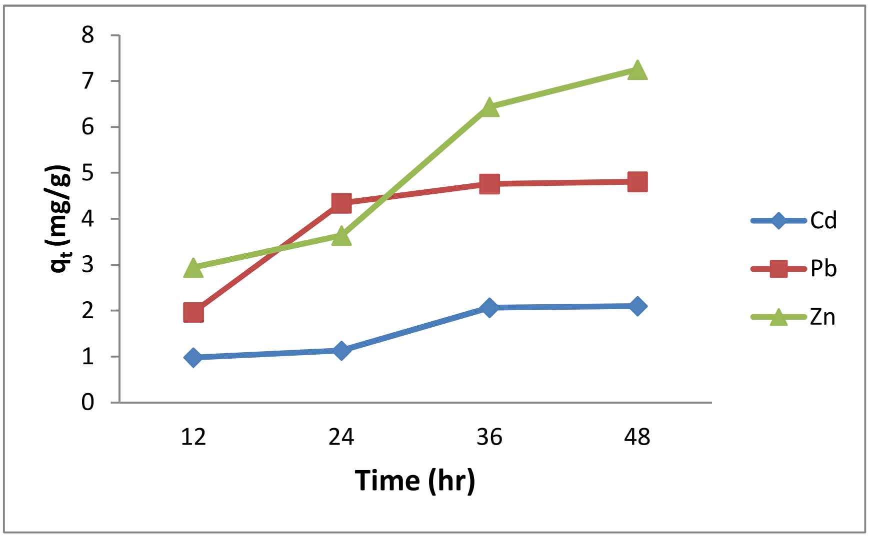

Figure 1: Biosorption - Time graph for adsorption of Lead, Zinc and Cadmium by *Bacillus subtilis* (at 30 ^{0}C and pH 5.5)

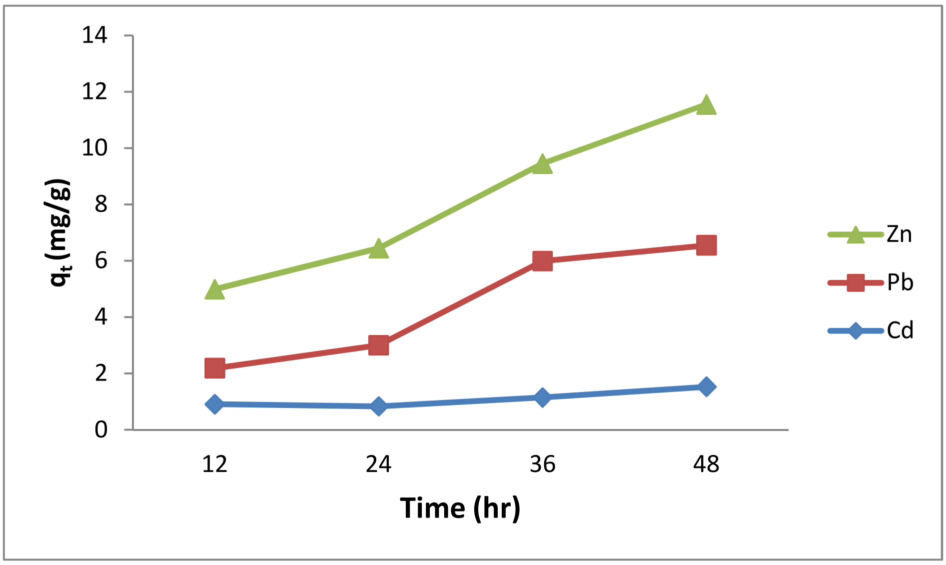

Figure 2: Biosorption - Time graph for adsorption of Lead, Zinc and Cadmium by *Bacillus cereus* (at 30 ^{0}C and pH 5.5)

3.1 Bioaccumulation of the Heavy Metals by Bacillus cereus and Bacillus subtilis.

Figure 3 shows the potential of the microorganisms in bioaccumulation of the heavy metals. The plot shows that bioaccumulation of Lead, Zinc and Cadmium increases with time. The highest level of Lead bioaccumulation was achieved in medium containing *Bacillus cereus* (96.75%) followed by 95.2% in the medium with *Bacillus subtilis.* There was inconsistency in the trend of bioaccumulation in the medium with *lactobacillus species,* the result of *lactobacillus species* was therefore neglected. The result of bioaccumulation studies showed that *Bacillus cereus* shows the maximum bioaccumulation capacity of 96.75% for Lead, 23% for Cadmium and 16.98% for Zinc while *Bacillus subtilis* accumulated 95.2% of the Lead, 41.3% of Cadmium and 32.2% of Zinc from solution.

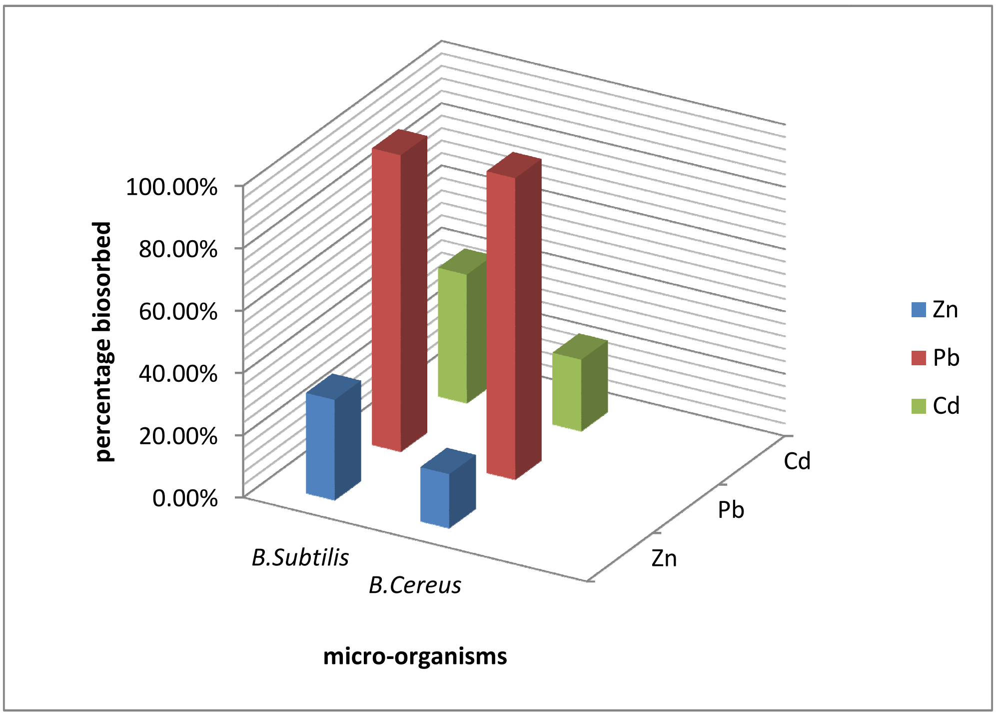

Figure 3: Variation in bioaccumulation capacity of *Bacillus subtilis and Bacillus cereus* (at 30°C and pH 5.5)

3.2 Biosorption Kinetic Studies

For biosorption kinetic studies, various kinetic equations were tested on the obtained data to determine the fitness of the kinetic models on the obtained data. Pseudo first-order equation was used to test the rate constant of adsorption. According to [6], the equation is given by:

$\ln (q_e - q_t) = \ln q_e - k_1 t$ (3)

q_e and q_t are the amounts of heavy metal ions biosorbed (mg/g) at equilibrium and at time t. The plots of $\ln(q_e - q_t)$ versus t was used to calculate K_1 (h^{-1}) for different concentrations of Lead, Zinc and Cadmium ions. The plot of log (q_e-q_t) against time (t) suppose to give a straight line from which q_e is the intercept and K_1 is the slope of the plot but the relation in Figures 4 and 5 do not show a linear relation as supposed by Lagegergren if pseudo first order relation will be befitting.

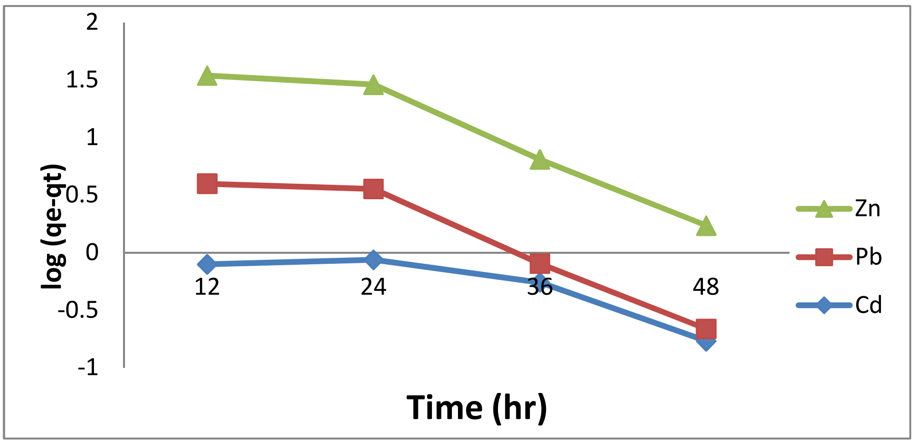

Figure 4: Plot of Pseudo-first order kinetics for the biosorption of Lead, Zinc and Cadmium ion by *Bacillus subtilis.*

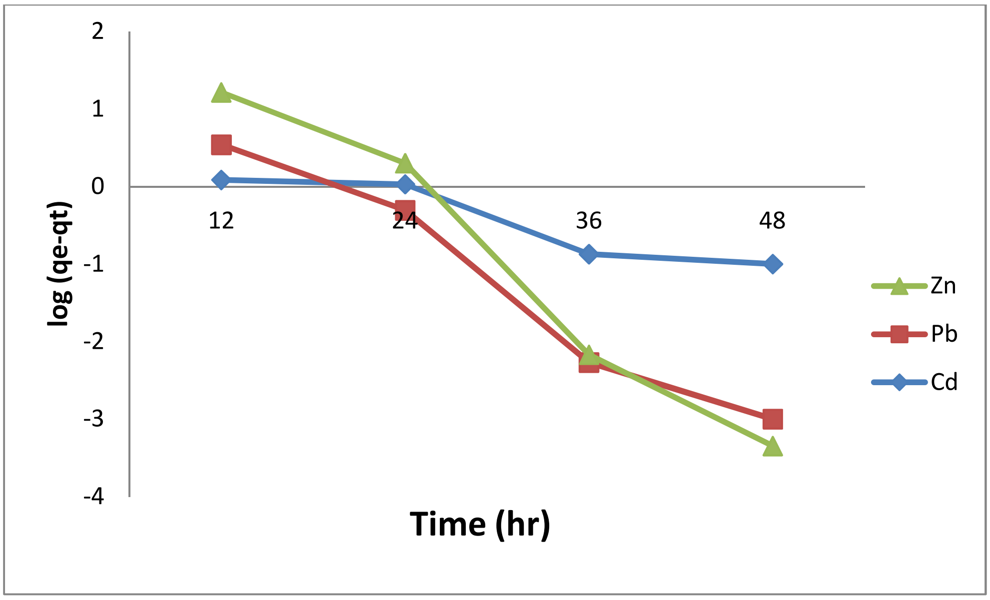

Figure 5: Plot of Pseudo-first order kinetics for the biosorption of Lead, Zinc and Cadmium ion by *Bacillus cereus.*

Since the pseudo first order equation do not best describe the data, Pseudo second order equation was tested. Malik, [8] opined a pseudo second order equation based on equilibrium adsorption. The equation is expressed as shown below

$$\frac{t}{qt} = (K_2 q_e^{2})^{-1} + (q_t)^{-1} t \qquad (4)$$

where K_2 (g/mg s) is the rate constant of second-order adsorption. The plot of t/q_e versus t shown in figures 6 and 7 show a good agreement between experimental and calculated q_e values. This means that the second order kinetic model is valid in describing the nature of biosorption of Lead, Zinc and Cadmium by *Bacillus cereus* and *Bacillus subtilis*. q_e is the slope and K_2 is the intercept of the plot.

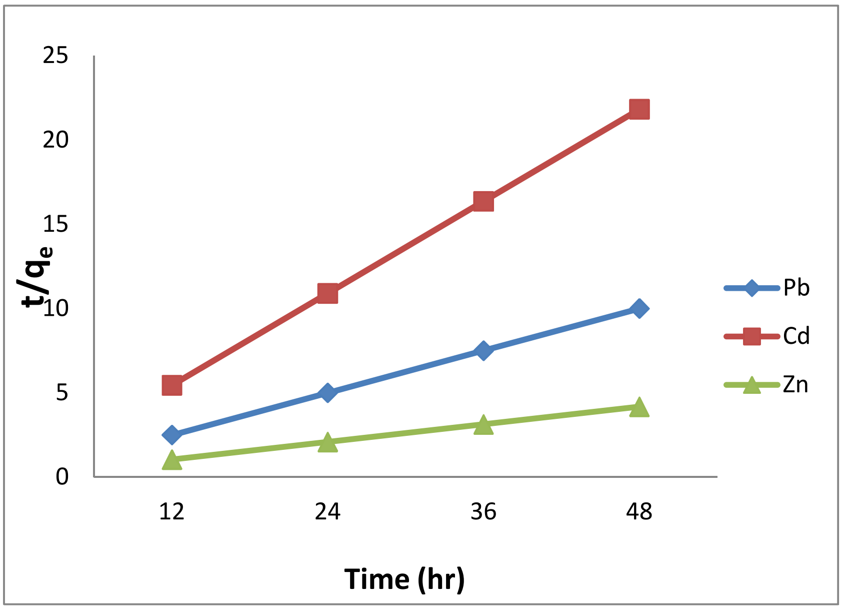

Figure 6: The plot of Pseudo-second order kinetics for the biosorption of Lead, Zinc and Cadmium ion by *Bacillus cereus.*

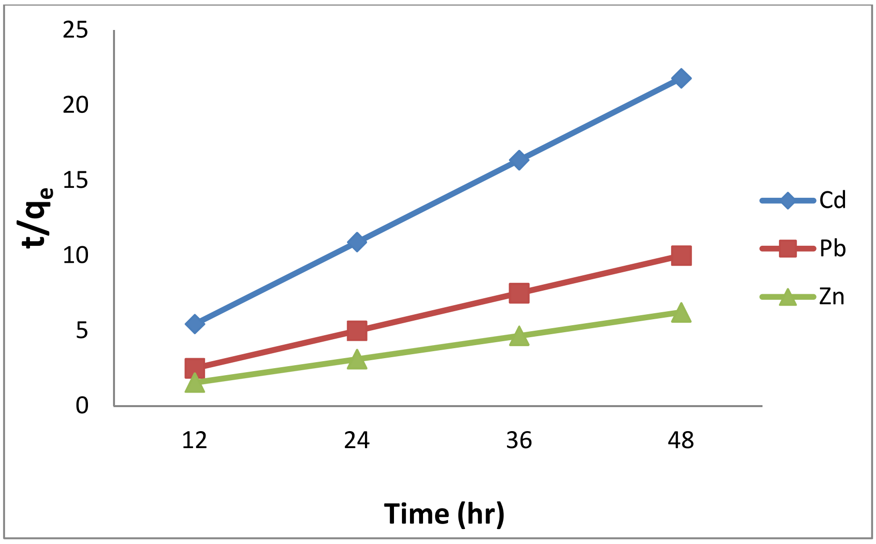

Figure 7: The plot of Pseudo-second order kinetics for the biosorption of Lead, Zinc and Cadmium ion by *Bacillus subtilis.*

4. Conclusion

The microbial biomass employed for this experiment are *Bacillus subtilis and Bacillus cereus* The study showed that *Bacillus subtilis and Bacillus cereus* are effective in removing Lead, Zinc and Cadmium from wastewater. The result shows that Lead was the most bioaccumulated metal followed by Cadmium while Zinc was the least bioaccumulated metal from media by *Bacillus subtilis* and *Bacillus cereus.* Also, the result of kinetic studies revealed that the kinetic data agrees with pseudo-second order kinetic model.

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