

Adsorption of Cu (II) and Ni(II) in Aqueous Solution using Biofilm Supported with Kaolinite Clay

Ma. Evangeline Rey- Mellano^{1,2}, Delia B. Senoro², Lemmuel L. Tayo² and Meng-Wei Wan³

Abstract— Adsorption of each of the ions Cu (II) and Ni (II) on *Escherichia coli* ATCC25922 and *Staphylococcus epidermidis* RP62A supported by kaolinite clay in a batch system was investigated. Equilibrium adsorption capacity was determined to be a function of the solution pH, contact time, and initial metal concentrations. The results obtained from the adsorption experiments were used to understand the driving forces that govern the interaction between metal ions and the biosorbent. Minimum inhibitory concentration (MIC) values of *E. coli* ATCC 29522 for Cu (II) and Ni (II) are two times greater than *S. epidermidis* RP62A which means *E. coli* ATCC 29522 is very resistant to metals than that of *S. epidermidis* RP62A. The experimental data were analyzed by the Freundlich and Langmuir isotherms. The adsorption kinetics of metal ions best fits the pseudo-second order. The effect of pH on metal ion uptake was studied by performing equilibrium sorption experiments with varying the solution pH from 4 to 8. The result showed the optimum pH for the three metals is at pH 5 with initial concentration of 100 mg/L at contact time of 24 hours with constant temperature of 37°C.

Keywords— biosorbent, adsorption, *Escherichia coli* ATCC29522, *Staphylococcus epidermidis* RP62A

I. INTRODUCTION

COPPER (Cu) and nickel (Ni) are common involved in the redox and metabolism activities as parts of enzyme participators in the electron transfer in microbial respiration [1]. Toxic metals can displace the necessary metals in the enzymes, which have the similar structure, resulting in the enzymes inactivation [2]. This contamination has disturbing outcomes on the ecological balance and poses challenges to local experts and available technologies. Copper is discharged from the electrical industry, pulp, paper mills and petroleum refineries. Moreover, lead is toxic to human as it replaces calcium and accumulates in skeletal system [3]. It is consumed in a big number of industries like electroplating and

Ma. Evangeline Rey- Mellano¹ is with Cagayan State University, Tuguegarao City, Cagayan 3500 Philippines

Delia B. Senoro², is with the School of Civil, Environmental and Geological Engineering, Mapua Institute of Technology, Intramuros, 1002 Manila.

Lemmuel L. Tayo² is also with School of Chemical Engineering and Chemistry of Mapua Institute of Technology, Intramuros, 1002 Manila.

Meng-Wei Wan³ is with Department of Environmental Resources Management, Chia Nan University of Pharmacy and Sciences. He is the currently General Secretary as well as Professor of the said school.

batteries manufacturing. Further, there is evidence of nickel carcinogenicity [4].

The use of biological material for the removal or recovery of precious and toxic metals was observed during recent years which includes living and non-living microorganisms due to its effective performance, minimization of chemical and/ or biological sludge and its low cost [5, 6]. Microorganisms like algae, bacteria, fungi and yeasts have proven to be good metal biosorbents due to its metal sequestering properties and adsorption capacity [7] to reduce heavy metal concentration. Bacteria provide a large contact interface due to its high surface area-to-volume ratio which accepts the interaction with metals from the surrounding environment [8]. Bacteria produce macromolecules that are called extracellular polymeric substances (EPSs) which derive from lysis or hydrolysis. Proteins, humic substances, polysaccharides and uronic acid generally constitute EPSs that contains different functional groups like phosphoric, carboxyl, hydroxyl and amine groups [10].

Use of adsorbents like zeolites and clays, as support to biofilm, is also popular due to their availability, minimum cost, easiness of extraction, and retention capability [11]. Large surface area of natural clays also assisted by the edges and the faces of clay particles, accounts for the best capacity of the clay minerals to adsorb heavy metals [12]. A method that uses clays covered with a microorganism allows combining the features of both for an even more competent heavy metals removal [13]. The main objective was to determine the adsorption capacity of *Escherichia coli* ATC 25922 and *Staphylococcus epidermidis* RP62A biofilm with kaolinite clay as a support for the removal of Cu (II) and Ni (II) from aqueous solution. The result of the study is significant in the endeavor of removing heavy metals from contaminated aqueous solution thus also help future researchers to improve process in removing of heavy metals.

II. METHODOLOGY

Materials Preparation

The bacterium *Escherichia coli* ATCC25922 and *Staphylococcus epidermidis* RP62A were obtained at Chia Nan University of Pharmacy and Sciences, Tainan Taiwan. Stock heavy metals concentrations were 10 mg/L, 50 mg/L, 100 mg/L and 200 mg/L which were prepared by dissolving Ni (NO₃)₂ · 6H₂O (Ferah, Berlin), Cu (NO₃)₂ · 2.5H₂O (JT Baker,

USA) in deionized water (18.2 Ω), and kaolin (JT. Baker, USA). Glass wares used for experimental purposes were washed in 10% nitric acid and rinsed with deionized water to remove any possible interference by other metals.

Agar and Broth Medium Preparation

A 1.5g of agar (Bacto Agar, BD, USA) and 3.70g of BHI (Brain Heart Infusion, BD, USA) was dissolved in 100 mL DI water, then adjusted to a pH of 4.6. The medium was sterilized in autoclave (Speedy Autoclave, TOMIN) at 121 °C for 45 minutes, cool to room temperature. Prepared medium was used for bacterial growth. Bacto Brain Heart Infusion (BHI, BD, USA) was dissolved in 100 mL DI water, was adjusted to 4.6 pH. The medium was sterilized in an autoclave (Speed Autoclave, TOMIN) at 121.0 °C for 45 minutes, cool to room temperature. Prepared medium was used to enhance bacterial growth.

TABLE 1
COMPONENTS OF BACTERIAL BIOFILM AS BIOSORBENTS

Metal Conc'n (mg/L)	Composition of Biosorbent
0	Add 0.5 g of Kaolinite clay + 7.5 mL bacterial suspension + 75 mL BHI broth
10	Add 0.5 g of Kaolinite clay + 7.5 mL bacterial suspension + 74.925 mL BHI broth
50	Add 0.5 g of Kaolinite clay + 7.5 mL bacterial suspension + 74.625 mL BHI broth
100	Add 0.5 g of Kaolinite clay + 7.5 mL bacterial suspension + 74.25 mL BHI broth
200	Add 0.5 g of Kaolinite clay + 7.5 mL bacterial suspension + 73.5 mL BHI broth

Bacterial Growth

Streak plating was used to culture bacteria from bacterial tube stored at -20°C to agar (Bacto Agar, BD, USA) plate, and was incubated for 16-18 hours at 37°C. After overnight incubation, single colony was selected from the agar plate and transferred in test tubes with 3 mL Brain Heart Infusion broth for its cultivation. Bacterial growth was enhanced by diluting 1.0 mL (from tubes with 3 mL BHI broth + single colony) with 20 mL BHI broth that is 1:20 proportion after 24-hour incubation. Incubated for 24 hours at 37°C and absorbance was read using UV-VIS Spectrophotometer (2800 Series, UNICO, Taiwan) at a wavelength of 600 nm. Procedure of McFarland was also used.

Minimum Inhibitory Concentration

Bacteria were cultured overnight at 37 °C in LB agar and bacterial concentration was adjusted to 10⁶ CFU/mL in Mueller-Hinton (MH) broth. The 100 μ L bacterial suspensions were added to 96-well micro titer plates and incubated with 100 μ L various concentrations of metal solutions (4000-62.5 ppm). The absorbance was read with a microplate reader at a wavelength of 600 nm after 20 hours.

Biosorbent Formation with Kaolinite Clay as Support

Batch experiments were performed by agitating 0.5 g of kaolinite clay with 7.5 mL of bacterial suspension and 75 mL of 10, 50, 100 and 200 mg/L heavy metal solutions in 250 mL Erlenmeyer flasks. Experiments without heavy metal was prepared for comparison purposes. The Erlenmeyer flask was

kept at constant temperature of 37 °C for 1 day with moderate agitating at 150 rpm and 4 days without agitating in orbital shaker incubator (721 SR HIPOINT, Taiwan).

Adsorption Experiments

The batch kinetic study was conducted by mixing the composition of biosorbent with heavy metal solution, components illustrated in Table 1, in 250 mL Erlenmeyer flasks and incubate in different contact time of 4, 12 and 24 hours with constant temperature of 37 °C. The pH of the initial solutions was measured using pH meter (HACH Sension3, Taiwan). Adjustments in the solution pH were done by adding 0.1 M NaOH and 0.1 N HCl. After every contact time, the bacterial solution with heavy metal was centrifuge at 3000 rpm for 5 minutes and filtered by 0.20 μ m micro pore filter to separate aqueous phase from solid phase for elemental analysis.

Batch Adsorption Studies

Batch experiments were performed by agitating the sample solution in orbital shaker incubator (721 SR HIPOINT, Taiwan). The effect of pH on metal ions uptake was studied by performing equilibrium sorption experiments at pH range of 4 to 8. Adjustments in the solution pH were done by adding 0.1 N NaOH and 0.1 N HCl while metal ion concentration of 100 mg/L and temperature of 37 °C. Amount of biosorbent and contact time of 24 hours was kept constant. Adsorption equilibrium study was conducted using 0.5 g of kaolinite clay with 7.5 mL of bacterial suspension and 75 mL of 10, 50, 100 and 200 mg/L heavy metal solutions with contact time of 4, 12 and 24 hours contact time and optimum pH of 5.0. After attaining equilibrium, biosorbent was centrifuge and filtered using 3000 rpm at 5 mins and 0.20 μ m micro pore filter respectively. The aqueous-phase concentration of metal is determined using ICP-OES. The equilibrium uptake capacity and percent removal of biofilm with kaolinite clay for Cu (II) and Ni(II) was calculated by

$$q_e = \frac{(C_0 - C_e) \times V}{m} \quad (1)$$

$$\% \text{ removal} = \frac{C_0 - C_{eq}}{C_0} \times 100 \quad (2)$$

respectively.

III. RESULTS AND DISCUSSION

Minimum Inhibitory Concentration tests

The results showed that minimum inhibitory concentration values of *E. coli* ATCC 29522 for Cu (II) and Ni (II) is two times greater than *S. epidermidis* RP62A. This demonstrates that *E. coli* ATCC 29522 has higher resistance or tolerance to Cu (II) and Ni (II) compared with *S. epidermidis* RP62A. This results illustrate that *E. coli* ATCC 29522 performs better than *S. epidermidis* RP 62A in terms of removing metals from aqueous solution.

TABLE II
RESULTS OF MIC TEST OF *E. coli* ATC 25922 AND *S. epidermidis* RP62A

Metal	Bacteria	MIC (mg/L)
Cu	<i>E. coli</i> 25922	250
	<i>S. epidermidis</i> RP62A	125
Ni	<i>E. coli</i> 25922	125
	<i>S. epidermidis</i> RP62A	62.5

Batch Adsorption Tests

Following sections show the result of the batch adsorption tests.

Effect of pH on Metal ions uptake

The effect of pH on metal ion uptake was studied by performing equilibrium sorption experiments by varying the solution pH from 4 to 8. The amount of Cu ions adsorbed by both *E. coli* ATC 29522 and *S. epidermidis* RP62A bacterial solution supported by kaolinite clay is shown in Figure 1. *E. coli* ATC 29522 removed 99.74% at pH 5 while *S. epidermidis* RP62A removed Cu (II) by 99.98% at pH 5 and 8. This result is comparable to the work Yilmaz et al. (2010) by which highest metal uptake values were obtained at pH 5 and 6. It was found that Cu^{2+} started precipitating as $\text{Cu}(\text{OH})_2$ above pH 5.5. Hydrogen (H^+) cations has been considered as competitive ones in ion exchange process. Consequently, ion exchange of metal is favoured by high pH values; however, lower than the minimum pH at the start of precipitation event.

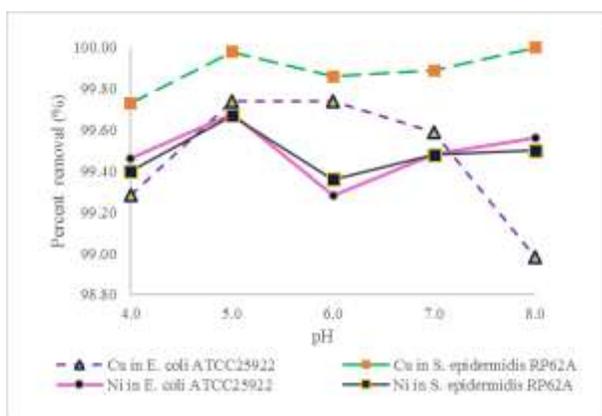


Fig. 1. Effect of pH on the adsorption efficiency of Cu (II) and Ni (II) @ $C_0=100$ mg/L; temperature = 37°C

The low adsorption at lower pH (acidity) by *E. coli* ATC 29522 was attributed to high solubility and ionization of copper salt in the acidic medium. The maximum adsorption of Cu^{2+} which range from pH 5 to 6 is associated to partial hydrolysis of Cu^{2+} which resulted to the formation of $\text{Cu}(\text{OH})^+$ and $\text{Cu}(\text{OH})_2$ by which the adsorption capacities are higher than that of Cu^{2+} . It was recorded that lower adsorption of Cu happened at pH higher than 6. This was attributed to the precipitation [13] of Cu ions as $\text{Cu}(\text{OH})_2$ and availability of the biofilm.

Both *E. coli* ATCC29522 and *S. epidermidis* RP62A biofilm performed well at pH 5 which recorded a Ni (II) removal of 99.68 % and 99.67%, respectively. This was due

to the effect of pH on metal binding, sites of the biomass surface and on metal speciation in aqueous solutions. This result is similar to [15] which recorded a maximum adsorption for Ni (II) at pH 5 – 6. However, [13] reported that Ni ions precipitated at pH 8 due to the formation of nickel hydroxide hence avoided the pH beyond 8.

In general, pH 3.0–6.0 has been found favorable for the adsorption of metal ions by microbial biomass [19]. The favorable pH for the adsorption of Cu (II) and Ni (II) ions by *E. coli* ATCC29522 and *S. epidermidis* RP62A biofilm supported by kaolinite clay was found to be 5.0. These results are similar to the results of the work of [18].

Effect of Initial Metal Concentration

The initial concentration of ions in solution has a key role as a driving force to overcome the mass transfer resistance between the liquid phase and solid phase. Therefore, it is expected that with an increase in metal ions the metal uptake will increase.

Batch experiments were carried out at different initial metal concentrations of 10,50, 100 and 200 mg/L with contact time at 4, 12 and 24 hours at pH 5 with constant temperature of 37 °C. Figures 4 to 7 showed that Cu and Ni uptake rate were relatively high at the first four hours of the process due to abundance of available adsorption sites. However, the adsorption rate gradually decreased with an increase in process time leading to the adsorption equilibrium. At low metal concentrations, adsorption sites are available and could easily be occupied since the ratio between the number of metal moles in solution and available surface area is low. Therefore, adsorption is independent of the initial concentration. Nevertheless, the removal efficiency is dependent on the initial concentration. This is due to the number of available sites was less than the number of metal moles. At higher concentration, the number of ions competing for the available binding sites on the biomass surface increases thus reduced the number of binding sites [20].

In Figure 4 and 5, shows that *E. coli* ATCC29522 has a higher sorption capacity (19.512 mg/g) than *S. epidermidis* RP62A (19.227 mg/g) on the removal of 100 mg/L of Cu. It also shows that sorption equilibrium has reached after four hours. This is due to the availability of sorption sites. The adsorption of copper ion on adsorbent depends on initial concentration. Copper ions have smaller hydrated radii (8.38 Å); hence, enters into smaller pores on the surface of the adsorbent. Copper has high electro negativity (1.9) and standard reduction potential (0.341) which show a trend with sorption capacity [21].

Figure 6 and 7 also shows the rapid uptake of Nickel after hours. And at a higher contact time, adsorption followed at a slower rate until 24 hours. At higher concentration, the average distance between the adsorbed species was reduced affecting the distribution of surface charge.

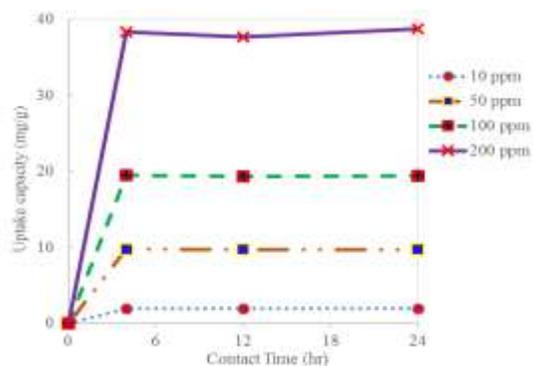


Fig. 4 Effect of initial metal concentration on equilibrium adsorption capacity of Cu using *E. coli* ATCC29522 biofilm supported by kaolinite clay

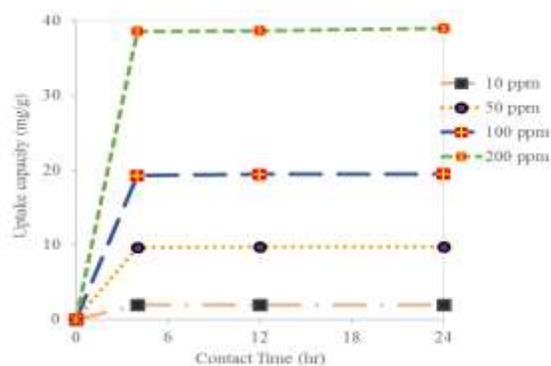


Fig. 5 Effect of initial metal concentration on equilibrium adsorption capacity of Cu using *S. epidermidis* RP62A biofilm supported by kaolinite clay

Therefore, the ability of adsorbate to migrate to the adsorbent surface changed and fixation reduced [14]. Finally, at very high concentrations of metal ions, solid-liquid equilibrium is limited by diffusion of metal ions into the microbial mass.

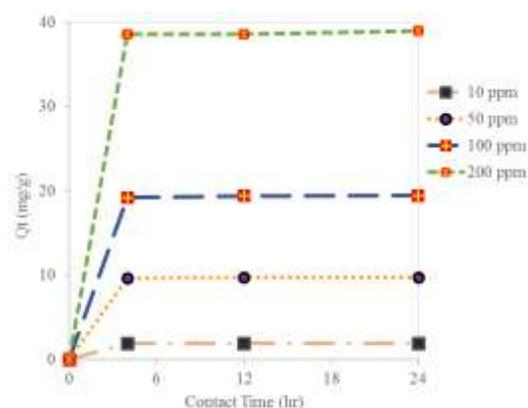


Fig. 6 Effect of initial metal concentration on equilibrium adsorption capacity of Ni using *E. coli* ATCC29522 biofilm supported by kaolinite clay

In other words, the metal ions diffused through intraparticle diffusion into the biofilm surface; however, this mechanism was slow [14].

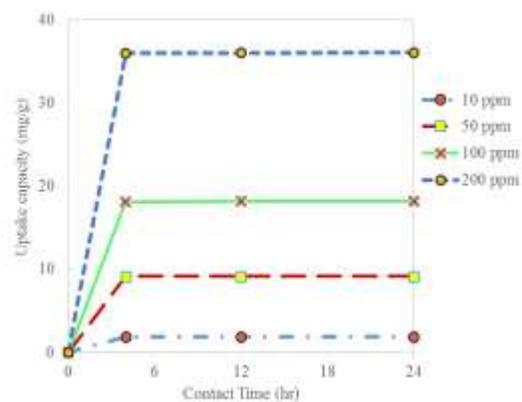


Fig. 7 Effect of initial metal concentration on equilibrium adsorption capacity of Ni using *S. epidermidis* RP62A biofilm supported by kaolinite clay

The equilibrium capacity of Cu and Ni reached its equilibrium after four hours. This is due to the repulsive force being exerted by occupied binding sites on biosorbent's surface on metal ions.

Adsorption isotherms Study

Sorption isotherms were experimentally determined for the biosorbent used (biofilm + Kaolin). Two different models Freundlich and Langmuir isotherm models were fitted to the experimental data and constants calculated for the best fit are presented in Table 3. Based on the correlation coefficient (R^2) values, the adsorption of Cu (II), Ni(II) and Pb(Ii) ions onto both *E. coli* ATCC29522 and *S. epidermidis* RP62A with kaolinite clay are best described by Langmuir model.

$$q_e = \frac{Q_0 K_L C_e}{1 + K_L C_e} \quad (3)$$

The R_L obtained for Cu(II) and Ni(II) with *E. coli* ATCC29522 and *S. epidermidis* RP62A as biosorbents are 0.8408 and 0.9960, 0.7540 and 0.7343, 0.5763 and 0.3827, respectively. Langmuir dimensionless parameter (R_L) determine favorable condition and its tendency to reverse.

In addition, it shows that the experimental points were in good agreement with the linear model generated by the Langmuir isotherm. This implies monolayer distribution of metal ions onto homogeneous active sites on biosorbent surface. The n values obtained is greater than 1, which signifies favorable adsorption conditions which then implies that biofilm supported by kaolin has high affinity for metal ions in the solution. Langmuir is best fit for the study.

Adsorption Kinetics of Metal Ions

The adsorption rate constants were determined from the pseudo- first and second order model listed in Table 4. It was observed that the is dynamic behavior is well described by the second- order kinetic model with correlation coefficients almost equal to 1 ($R^2 > 0.99$). This implies the chemisorption is the leading step of the adsorption process of Cu(II) and Ni(II) using bacterial biofilm supported with kaolinite clay. The correlation coefficients for the first- order model are

relatively low in all metal ions which describes that first-order kinetic model was unlikely and not appropriate.

IV. CONCLUSIONS

Results of this study showed that *Escherichia coli* ATCC29522 and *Staphylococcus epidermidis* RP62A supported with kaolinite clay were both able to remove the Cu(II) and Ni(II) from aqueous solution. The percentage removal for *E. coli* ATCC29522 and *S. epidermidis* RP62A biofilm of Cu (II) and Ni (II) are extremely high at pH 5.0 having 99.74% and 99.98%, 99.68 % and 99.67% respectively. Minimum inhibitory concentration values of *E. coli* ATCC29522 for Cu (II) and Ni (II) were two times greater than *S. epidermidis* RP62A. This means that *E. coli* ATCC29522 has tolerance to metals compared with of *S. epidermidis* RP62A. The results of MIC affirm that the adsorption process for Cu (II) at 200 mg/L was attributed to the adsorption capacity of kaolin support only. The *S. epidermidis* RP62A was inhibited at 125 mg/L. On Nickel, *E. coli* ATCC29522 and *S. epidermidis* RP62A inhibited up to 125 mg/L and 62.5 mg/L, respectively. Hence removal of Ni(II) ions at 200 mg/L was also attributed to kaolinite support. It was observed that the adsorption experiment is more effective than the study made by [14] which did not use biofilm support.

Cu and Ni uptake rate was relatively high at the beginning of the process due to abundance adsorption sites available. However, the number of active sites for the adsorption gradually decreases with an increase in time. At low metal concentrations, adsorption sites are available and easily occupied due to the ratio between the number of metal moles in solution and less available surface area. Nevertheless, at high concentrations of metal the removal efficiency is dependent on the initial concentration. This was associated to

the number of available sites relative to the number of metal moles. The adsorption rate slightly decreased leading to the formation of the adsorption equilibrium.

Langmuir best describes the experimental data that indicates the adsorption of Cu(II) and Ni(II). The experimental points were in good agreement with the linear model generated by the Langmuir isotherm. This implies monolayer distribution of metal ions onto homogeneous active sites on biosorbent surface. The n values (1.998 to 2.5) obtained were greater than 1 which signifies favorable adsorption conditions which then implies that biofilm supported by kaolin has high affinity to metal ions in the solution. The kinetics equilibrium is well described by the second-order kinetic model with almost correlation coefficients equal to ($R^2 > 0.99$). Based on the results of the study, it can be concluded that both *E. coli* ATCC29522 and *S. epidermidis* RP62A with kaolinite clay are biosorbent materials favorable for the removal of divalent ions of Cu and Ni. Best performing biofilm is *E. coli* ATCC29522 at pH 5 with temperature of 37° C. It was also noted that pH of the solution shall not go beyond 8.

TABLE III
LANGMUIR AND FREUNDLICH ISOTHERM MODEL CONSTANTS AND
CORRELATION COEFFICIENTS

Heavy Metal	Biosorbent	Langmuir	Freundlich	
		R ²	n	R ²
Cu	<i>E. coli</i> ATC 29522	0.997	2.567	0.9723
Ni		0.997	2.207	0.9917
Cu	<i>S. epidermidis</i> RP62A	0.9986	2.611	0.9922
Ni		0.9993	1.998	0.9908

TABLE IV
KINETICS DATA FOR THE ADSORPTION OF Cu(II) AND Ni(II) IONS

Biosorbent	Heavy Metal	Pseudo- First Model			Pseudo Second Model		
		Q _e (mg/g)	K ₁ (min ⁻¹)	R ²	Q _e (mg/g)	K ₂ (min ⁻¹)	R ²
<i>E. coli</i>	Cu	2.9792	0.0034	0.55	0.728	0.4951	0.99
ATCC29522	Ni	0.9251	-0.5671	0.64	-6.00E-05	0.5516	0.99
<i>S. epidermidis</i>	Cu	1.9557	-0.4879	0.92	0.0027	0.1513	0.99
RP62A	Ni	1.5251	-0.5343	0.76	0.0009	0.0551	0.99

ACKNOWLEDGMENT

This study was supported by the sandwich program of Mapua Institute of Technology facilitated by its office of International Linkages for Research and Development and conducted at Innovation Green Technology Laboratory, Chia Nan University of Pharmacy with the funding number MOST: 104-2221-E-041-002.

REFERENCES

- [1] Zandvoort M, Hullebusch V, Feroso FG, and Lens PNL. Traces metals in anaerobic granular sludge reactors: Bioavailability and dosing strategies. *Engineering in Life Sciences* 2006;6:293-301. <http://dx.doi.org/10.1002/elsc.200620129>
- [2] Bruins, MR, Kapil S, Oehme FW. 2000. Microbial resistance to metals in the environment. *Ecotoxicology and Environmental Safety*,2000; 45:198-207. <http://dx.doi.org/10.1006/eesa.1999.1860>
- [3] Anielak AM, and R Schmidt. Sorption of Lead and Cadmium Cations on Natural and Manganese- Modified Zeolite. *Polish Journal on Environmental Studies*, 2011;20:43.
- [4] Volesky B. *Adsorption of Heavy Metals*. Boca Raton, Florida: CRC Press Inc; 1990.
- [5] Sahin Y and Ozturk A. Adsorption of chromium(VI) ions from aqueous solution by the bacterium *Bacillus thuringiensis*. *Process Biochem*, 2005;40:1895-1901. <http://dx.doi.org/10.1016/j.procbio.2004.07.002>
- [6] Alluri HK, Ronda SR, Settalluri VS, Bondili JS, Suryanarayana V, and Venteshwar P. 2007. Adsorption: an eco-friendly alternative for heavy metal removal. *Afr. J. Biotechnol*, 2007; 6 (25): 2924–2931.

- <http://dx.doi.org/10.5897/AJB2007.000-2461>
- [7] Volesky B. Biosorbent materials. *Biotechnology Bioengineering Symposium Journal*; 1986:121-126.
- [8] Zouboulis AI, Loukidou MX, and Matis KA. Adsorption of toxic metals from aqueous solutions by bacteria strains isolated from metal-polluted soils. *Process Biochemistry*, 2004;39: 909- 916.
[http://dx.doi.org/10.1016/S0032-9592\(03\)00200-0](http://dx.doi.org/10.1016/S0032-9592(03)00200-0)
- [9] Comte S, Guibaud G, and Baudu M. 2008. Adsorption properties of extracellular polymeric substances (EPS) towards Cd, Cu and Pb for different pH values. *Journal of Hazardous Materials*, 2008; 151: 185-193
<http://dx.doi.org/10.1016/j.jhazmat.2007.05.070>
- [10] Hullebusch, Van ED, Zandvoort MH, and Lens PNL. 2003. Metal immobilization by biofilms: mechanisms and analytical tools. *Rev. Environ. Sci. Bio/Technol*, 2003;2:9-33.
<http://dx.doi.org/10.1023/B:RESB.0000022995.48330.55>
- [11] Comte S, Guibaud G, and Baudu M. Adsorption properties of extracellular polymeric substances (EPS) resulting from activated sludge according to their type: soluble or bound. *Process Biochemistry*, 2006;41: 308-315.
<http://dx.doi.org/10.1016/j.procbio.2005.10.014>
- [12] Carvalho WA, Vignado C, and Fontana J. 2008. Ni(II) removal from aqueous effluents by silylated clays. *Journal of Hazardous Materials*, 2008;153: 1240- 1247.
<http://dx.doi.org/10.1016/j.jhazmat.2007.09.083>
- [13] Bhattacharyya KG, and Gupta S. Influence of acid activation on adsorption of Ni(II) and Cu(II) on kaolinite and montmorillonite: kinetic and thermodynamic study. *Chemical Engineering Journal*, 2008a; 136: 1-13.
<http://dx.doi.org/10.1016/j.cej.2007.03.005>
- [14] Quintelas C, Rocha Z, Silva B, Fonseca B, Figueiredo H, and Tavares T. 2009. Removal of Cd(II), Cr(VI), Fe(III) and Ni(II) from aqueous solutions by an E. coli biofilm supported on kaolin. *Chemical Engineering Journal*;2009: 319- 324.
<http://dx.doi.org/10.1016/j.cej.2008.11.025>
- [15] Sari A, Tuzen M, Citak D, and Soylak M. 2007. Equilibrium, kinetic and thermodynamic studies of adsorption of Pb(II) from aqueous solution onto Turkish kaolinite clay. *Journal of Hazardous Materials*,2007;149:283-291.
<http://dx.doi.org/10.1016/j.jhazmat.2007.03.078>
- [16] Melichova S, and Hromada L. Adsorption of Lead and Copper ions from Aqueous Solution using Natural Bentonite. *Pol. J. Environ. Study*, 2013; 22: 457-464.
- [17] Anis, M, Sajjad H, and Bari A. Adsorption of Lead and Copper from Aqueous Solution Using Unmodified Wheat Straw. *Environmental and Engineering Management Journal*,2013;12: 2117-2124.
- [18] Leung WC, Wong MF, Chua H, Lo W, Yu PHF, and Leung CK. Removal and recovery of heavy metals by bacteria isolated from activated sludge treating industrial effluents and municipal wastewater. *Water Sci. Technol*,2000; 4:233-240.
- [19] Vijayaraghavan K, and Yun KS. Bacterial biosorbents and adsorption. *Biotechnology Advances, Biotechnol. Bioeng*, 2008; 26: 266- 291.
- [20] Horsfall M, Ogban F, and Akporhonor EE. Sorption of chromium (VI) from aqueous solution by cassava (*Manihot sculenta* Cranz.) waste biomass. *Chem Biodivers*, 2006; 3(2): 161-174.
<http://dx.doi.org/10.1002/cbdv.200690019>
- [21] Mohan Jr D, Pittman C, and Steele P. Single, binary and multicomponent adsorption of copper and cadmium from aqueous solutions on kraft lignin- a biosorbent. *J. Colloid. Interf. Sci*,2006; 297 :489-504.
<http://dx.doi.org/10.1016/j.jcis.2005.11.023>
- [22]