Effects of pH and concentration on the capability of E. coli and S. epidermis with bentonite clay as biosorbent for the removal of Copper, Nickel and Lead from polluted water

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Abstract. This paper discusses the effects of pH and concentration on the capability of E. coli ATCC29522 and S. epidermis RP62A biofilm with bentonite in removing divalent copper, nickel and lead from wastewater. Batch adsorption study at laboratory scale was utilized to evaluate the potential use of bacterial biomass (E. coli ATCC 29522 and S. epidermidis RP62A) aided with geosynthetic clay (bentonite) for the removal of Cu$^{2+}$, Ni$^{2+}$and Pb$^{2+}$. Results revealed that removal of Cu$^{2+}$, Ni$^{2+}$and Pb$^{2+}$ by both types of organisms supported with bentonite were high in the first 4 hours of the experiment. This illustrates that the binding site on that particular time was abundant. Hence, the removal rate was evident at high concentration depicting the line adsorption equilibrium. It also revealed that S. epidermidis RP62A supported with bentonite had the highest affinity to Copper and Lead with Qm = 277.7 mg/g and 5.0075 mg/g, respectively. While E. coli ATCC 29522 had the highest affinity to Nickel (Qm= 58.82 mg/g). Hence, the sorption of Cu$^{2+}$, Ni$^{2+}$and Pb$^{2+}$ onto E. coli ATCC29522 and S. epidermidis RP62A biofilm supported with bentonite clay occurred through monolayer chemisorption on the homogeneous surface of E. coli ATCC29522 and S. epidermidis RP62A biofilm with bentonite clay. Batch kinetics studies revealed that the sorption of Cu$^{2+}$, Ni$^{2+}$and Pb$^{2+}$ onto E. coli ATCC29522 and S. epidermidis RP62A biofilm supported with bentonite clay was well described by a pseudo-second-order equation model of type 1 (R2 = 0.9999), which implies that chemisorption is the rate limiting step.

1 Introduction

Heavy metals pollution has become a global issue of great concern due to their higher toxicities, higher bioaccumulation in human body, food chain, nature of non-biodegradability and most likely carcinogenicities to human. Lead, mercury, chromium, arsenic, cadmium, zinc, copper and nickel are the most common contaminants found in contaminated surface water and groundwater as well as industrial wastewater.

The occurrence of these heavy metals in water causes great threats to humans and other living organisms [1]. According to Vijayaraghavan and Yun [2], there are many different sources of water pollutants and they were categorized into two: direct and indirect contaminants. Direct contaminants include those that were discharge by industries, refineries, mining vessels and waste treatment plants; whereas indirect contaminants are those that enter the water bodies which came from soil/ground water system and from the atmosphere via water rain. Heavy metals are regarded as toxic material due to their non-biodegradable characteristics and are very dangerous to living organism even in low concentration [3]. Thus, attention in removing these heavy metals requires effective removal methods.

Vast methods based on physicochemical such as extraction, ion exchange, chemical precipitation and membrane separation processes has been demonstrated for removal of heavy metals from aqueous solutions [3,4]. However, the mentioned methods do have some limitation when the concentration of metal in waste water is below 100 ppm. Hence, it is necessary to develop a cost effective and efficient separation method. Biosorption is a passive and metabolically-independent process such as the passive uptake of metals by microbial biomass that can be performed either by dead biomass, fragment of cells and tissues or live cells [5].

On other hand, removals of heavy metal using clay materials are also arising in remediation environment.
Example of the clay use in remediation process is bentonite. Natural bentonites are argillaceous materials that have been widely used to remove toxic metal ions, dyes, chlorophenols and drugs [6]. Due to their high specific surface area, high cation exchange capacity (CEC), their chemical and physical stability, they can be effectively employed as adsorbents for many wastewater pollutants [7]. Combination of biological microorganism and natural clay such as bentonite will form a new remediation system that will efficiently improve its capacity in removal of heavy metals.

The main goal of this study focuses on investigating the potential removal of Cu$^{2+}$, Ni$^{2+}$ and Pb$^{2+}$ from Simulated Wastewater using Biofilms of Escherichia coli ATCC 25922 (E.coli) and Staphylococcus epidermidis RP62A (S. epidermidis) supported with bentonite in a single-component system, with focus on evaluating the effects of pH and concentration on the removal. Significantly, this study attempt to augment results that are helpful in removing heavy metals from polluted water specially contaminants coming from industrial facilities. Furthermore, this is an avenue for future researchers to thoroughly develop and ameliorate biosorption technology for the removal of heavy metals. This study will be limited to the analysis of heavy metals concerned, which are mentioned above with the concentration of 10, 50, 100 and 200 ppm (mg/L), pH ranging from 4-8 with constant temperature of 37°C. Agitation rate will be considered at constant speed of 150 rpm and bentonite as a support.

2 Methodology

This study was conducted at Innovation Green Technology Laboratory of Chia Nan University of Pharmacy and Science, Taiwan in collaboration with Sustainable Development Research of Mapua Institute of Technology, Philippines.

2.1 Materials

Bentonite was obtain from Choneye Pure Chemicals (Taipei, Taiwan). The bacterium Escherichia coli ATCC25922 and Staphylococcus epidermidis RP62A was obtained at Chia Nan University of Pharmacy and Sciences, Tainan Taiwan. Sodium hydroxide (NaOH) and Hydrochloric acid (HCl) were used to vary the pH of the system. NaOH (0.1 M), was prepared by dissolving 2.0g of NaOH crystals in 500 mL deionized water. Hydrochloric acid (0.1 M) was prepared by diluting 4.229 mL concentrated HCl (36.5%) with 500 mL deionized water. Glassware utilized were eroded in 10% nitric acid and rinsed with deionized water. Stock of heavy metals concentrations were 10 mg/L, 50 mg/L, 100 mg/L and 200 mg/L which was prepared by dissolving Ni (NO$_3$)$_2$, 6H$_2$O (Ferak, Berlin), Cu (NO$_3$)$_2$, 2.5H$_2$O (JT Baker, USA), Pb (NO$_3$)$_2$, 6H$_2$O (Darmstadt, Germany) in deionized (DI) water (18. 2 Ω, ELGA Purelab Ultra).

2.3 Agar Medium Preparation

An agar (Bacto Agar, BD, USA) amounting to 1.50 g and 3.70 g of BH (Bacto Brain Heart Infusion, BD, USA) with 7.70 g/L calf brain extract, 9.80 g/L beef heart extract, 10.0 g/L of proteose peptone, 2.0 g/L dextrose, 5.0 g/L of Sodium Chloride, and 2.5 Disodium Phosphate were dissolved in 100 ml DI water. The medium was autoclave (Speedy Autoclave, TOMIN) at 121 °C for 45 min, cool to room temperature. Prepared medium was used for bacterial growth.

2.4 BHI Broth Medium Preparation

BHI (Bacto Brain Heart Infusion, BD, USA) with 7.70 g/L calf brain extract, 9.80 g/L beef heart extract, 10.0 g/L of proteose peptone, 2.0 g/L dextrose, 5.0 g/L of Sodium Chloride, and 2.5 Disodium Phosphate amounting to 3.70 g was dissolved in 100 ml DI water, was then adjusted to a pH of 4.6. The medium was sterilized in an autoclave (Speedy Autoclave, TOMIN) at 121.0 °C for 45 min, cool to room temperature. Prepared medium was used to enhance bacterial growth.

2.5 Bacterial Cultivation

Streak plating was used to culture bacteria from bacterial tube stored at -20.0°C to the prepared agar medium by drawing a three-zoned area in the plate with an sterilized loop in a laminar flow and was incubated for 16-18 hours at 37.0°C. After incubation overnight, single colony was selected from the agar plate and transferred in test tubes with 3.0 mL BHI broth medium for its cultivation.

2.6 Enhance Bacterial Suspension

Bacterial growth was enhanced by diluting 1.0 ml of incubated bacterial suspension (from 3.0 ml BHI broth + single colony) with 20.0 ml BHI broth (1:20 ratio). Bacterial suspension was incubated for 24 hours at 37.0°C. So that the number of bacteria will be within a given range, bacterial suspension absorbance was read using UV-Vis Spectrophotometer (2800 Series, UNICO, Taiwan) at a wavelength of 600 nm.

2.7 Minimum Inhibitory Concentration

Minimum inhibitory concentration (MIC) was conducted at the Department of Medical Laboratory Science and Biotechnology, Kaohsiung Medical University, Kaohsiung Taiwan.Bacteria were cultured overnight at 37 °C in Lactose Broth agar and bacterial concentration was adjusted to 106 CFU/mL in Mueller- Hinton broth. The 100 μL bacterial suspensions were added to 96- well micro titer plates and incubated with 100 μL various concentrations of metal solutions (4000- 62.5 ppm). After incubation at 37 °C for 20 hours, the absorbance was read with a microplate reader at a wavelength of 600 nm.
2.8 Biosorbent Formation with Bentonite as Support
Agitation 0.50 g of bentonite clay with 7.50 mL of bacterial suspension and 75 mL of 10, 50, 100 and 200 mg/L heavy metal solutions in 250 mL Erlenmeyer flasks. The Erlenmeyer flask was kept at constant temperature of 37 °C for 1 day with moderate agitating at 150 rpm and 4 days without agitation in orbital shaker incubator (721 SR HIPOINT, Taiwan).

2.9 Particle Separation Process
After every contact time, the bacterial solution with heavy metal was centrifuge (KUBOTA 2420, Japan) at 3000 rpm for 5 minutes and filtered by 0.20 µm micro pore syringe filter (Whatman, USA) to separate aqueous phase from solid phase for Physical and Chemical analysis.

2.10 Metal Ion Analysis using ICP- OES
To quantitatively determine the metal ion concentration, inductively coupled plasma optical emission spectrometry (ICP-OES) (DV2000 Series Perkin Elmer, USA) was used. Standard solutions for the instrument’s calibration curve were prepared. Samples were diluted to 10 mg/L of ICP standards [8].

2.11 Biosorption Optimization
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 M NaOH or 0.1 M HCl while metal ion concentration of 100 mg/L, temperature of 37 °C, amount of biosorbent and contact time of 24 hours were kept constant.

2.12 Batch Biosorption Studies
Study was conducted by agitating sample solution with 0.5 g of bentonite, 7.5 mL of bacterial suspension and 75 mL of 10, 50, 100 and 200 mg/L heavy metal solutions in an orbital shaker incubator (721 SR HIPOINT, Taiwan) at 150 rpm with contact time of 4, 12 and 24 hours and optimum pH of 5.0. After attaining equilibrium, biosorbent was centrifuge and filtered using 3000 rpm at 5 minute and 0.20 µm micro pore syringe filter respectively. The aqueous-phase concentration of metal is determined using ICP- OES.

3 Result and Discussion
3.1 Minimum inhibitory concentration (MIC)
Results of the MIC diagnostic showed that E.coli ATCC 25922 and S. epidermidis RP62A can survive and resist in lead solution with concentration greater than 2000 mg/L. On the other hand, E.coli ATC 25922 is resistant to Copper and Nickel solution at 250 mg/L and 125 mg /L respectively. However, S. epidermidis RP62A was resistant to copper and nickel up to 125 mg/L and 62.5 mg/L, respectively.

3.2 Effect of pH
The effect of pH on Cu²⁺, Ni²⁺ and Pb²⁺ uptake were investigated by performing equilibrium sorption experiments by varying the solution pH from 4 to 8. The effect of pH in the solution (Figure 1) at initial concentration of 100 mg/L and temperature of 37 °C showed that E. coli ATCC 29522 removed 99.94% at pH 5 while S. epidermidis RP62A removed Cu²⁺ by 100% at pH 5. The increase in sorption was attributed to fewer hydrogen ions in solution that may compete with the copper for adsorption to negatively-charged surfaces [9]. Ni²⁺ provide an slight increase of biosorption from pH 5 to 6 as showed in Figure 2. Sari [10], reported a maximum biosorption for nickel at pH 5 – 6 with low Ni²⁺ adsorption at lower pH values. Both E. coli ATCC29522 and S. epidermidis RP62A supported with bentonite clay in Figure 3 showed a sharp increase of Pb²⁺ removal as pH rose from 4.0-5.0 due to absorbent binding sites were not yet occupied. At this pH, both E. coli ATCC29522 and S. epidermidis RP62A removed 99.21% -99.89 % and 99.92 -100.0% respectively. In the present study, the pH = 5.0 had an average percent removal of 99.71% and 99.81% in both E. coli ATCC29522 and S. epidermidis RP62A respectively as shown in Figure 1 to 3. This pH was chosen for subsequent studies of Cu²⁺, Ni²⁺ and Pb²⁺ removal.
3.3 Batch Biosorption Studies

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 ºC. Uptake rates on copper (Fig. 3 & 4), nickel (Fig. 5 & 6), and lead (Fig. 7 & 8) were recorded sharp increase of biosorption at the first four hours. This was attributed to the abundance of available adsorption sites. 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. However, the adsorption rate was expected to gradually decrease with an increase in process time leading to the adsorption equilibrium [11]. The equilibrium time is defined as the time required to attain this state of equilibrium and the adsorbent capacity is determined based on the concentration of the copper remaining in the solution under the stated operating conditions [12].

Nevertheless, the removal efficiency is dependent on the initial concentration. At higher concentration, the number of ions competing for the available binding sites on the biomass surface increases thus reduces the number of binding sites. The sorption rate gradually declined with time until equilibrium was achieved starting from 4 hours up to 24 hours. The intensification in removal efficiency of Ni²⁺ ions is due to the augmentation surface area resulting from the increase in adsorbent mass [13]. The initial rapid phase in Fig. 7 & 8 was due to the availability of an initial large number of vacant sites which later became difficult due to the repulsive forces between Pb²⁺ adsorbed on the microsphere surface and Pb²⁺ in bulk solution [14] and slowed down as the sites are gradually filled up. Therefore, the kinetics will be more dependent on where Pb²⁺ are transported from the liquid phase to the sorption sites [15].
Further, the kinetic parameters, and correlation coefficients are listed in Table 1. The correlation coefficient ($R^2$) for the pseudo-second order model is 0.9999 [13]. The calculated $Q_e$ value is found to be much closer to the experimental $Q_e$ value [13]. These results confirm that the adsorption kinetics of Cu$^{2+}$, Ni$^{2+}$, and Pb$^{2+}$ ions onto the *E. coli* ATCC 29522 and *S. epidermidis* RP62A biofilm supported by bentonite is mainly governed by pseudo-second order equation and hence the rate-limiting of the biofilm is chemisorption.

### Table 1. Kinetic Data for the Biosorption of Cu$^{2+}$, Ni$^{2+}$ and Pb$^{2+}$ Ions

<table>
<thead>
<tr>
<th>Biosorbent</th>
<th>Heavy Metal</th>
<th>Pseudo-First Model</th>
<th>Pseudo Second Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$Q_e$ (mg/g)</td>
<td>$K_1$ (min$^{-1}$)</td>
</tr>
<tr>
<td><em>E. coli</em> ATCC 29522</td>
<td>Cu</td>
<td>4.27</td>
<td>0.16</td>
</tr>
<tr>
<td>Ni</td>
<td>4.36</td>
<td>0.15</td>
<td>0.57</td>
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<tr>
<td>Pb</td>
<td>5.09</td>
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<tr>
<td><em>S. epidermidis</em> RP62A</td>
<td>Cu</td>
<td>3.93</td>
<td>0.19</td>
</tr>
<tr>
<td>Ni</td>
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</tr>
<tr>
<td>Pb</td>
<td>4.45</td>
<td>0.31</td>
<td>0.84</td>
</tr>
</tbody>
</table>

### 4 Conclusion

The results revealed that removal of Cu$^{2+}$, Ni$^{2+}$ and Pb$^{2+}$ by *E. coli* ATCC 29522 and *S. epidermidis* RP62A biofilm with bentonite were 99.94% and 100%, 99.32% and 99.43%, 99.89 and 100% respectively, at pH 5.0 with the temperature of 37°C and at concentrations of 10, 50, 100, 200 ppm. The data from the minimum inhibitory concentration (MIC) showed that *E. coli* ATCC 25922 and *S. epidermidis* RP62A can live and resist in lead solution with concentration higher than 2000 mg/L. Additionally, *E. coli* ATCC 25922 is resistant to Cu$^{2+}$ and Ni$^{2+}$ at 250 mg/L and 125 mg/L, respectively, as opposed to *S. epidermidis* RP62A which was at 125 mg/L and 62.5 mg/L, respectively. The uptake performances of both organisms supported with bentonite were high in the first 4 hours of the experiment. It was associated with the high availability of adsorption sites that were spontaneously available and easily occupied. Hence, the removal rate was very evident at high concentration. Further, it could be concluded that *E. coli* ATCC 29522 and *S. epidermidis* RP62A biofilm with bentonite is a potential sorbent for the removal of Cu$^{2+}$, Ni$^{2+}$ and Pb$^{2+}$ from polluted water.

### References