Studies on biosorption equilibrium and kinetics of Pb(II) by a novel extracellular biopolymer produced by Pseudomonas fluorescence C-9

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Abstract—**In the present study we reported the feasibility of extracellular biopolymer(PF-9) produced by Pseudomonas fluorescence C-9 as an alternative biosorbent to remove Pb(II) metallic ions from environmental and industrial wastewater.The ability of PF-9 to remove the metallic ions was investigated by using batch biosorption procedure. The effects such as, pH, biosorbent dosage, sorbate concentrations and sorbate–sorbent contact time on the adsorption capacities of PF-9 were studied. Biosorption equilibriums were rapidly established in about 60 min and the adsorption kinetics followed the pseudo-second order kinetics model. The equilibrium adsorption data fitted well with Langmuir isotherm. The maximum Pb(II) adsorption capacity determined according to Langmuir isotherm were 92.66 mg /g PF-9 at pH 6.0 ,at 25°C and shaker speed 150 rpm, respectively. The carboxyl ,hydroxyl and amino groups of the PF-9 were involved in chemical interaction with the Pb(II) ions depicted by scanning electron microscopic (SEM) and Fourier transform infrared spectroscopic (FTIR) results. The study points to the potential of new use of extracellular biopolymer PF-9 as an effective biosorbent for the removal of Pb(II) from environmental and industrial wastewater.**

Keywords- extracellular biopolymer; biosorption; Pb(II); FTIR;SEM;adsorption kinetics; adsorption isotherm

I. INTRODUCTION

The increase in environmental pollution caused by toxic metals is of great concern because of their carcinogenic properties, non-biodegradability and bio-accumulation. Many industries, such as plating, paint and dyes, glass operations, and lead batteries industries, release various concentrations of heavy metals like plumbum. [1]. However, the presence of lead in water even at a very low level would be harmful to aquatic life and human health in terms of its toxicological, potential carcinogenic and neurotoxical effects [2]. The permissible limit of lead in drinking water and surface water intended for drinking, as set by EU, USEPA and WHO, are 0.010, 0.015 and 0.010 mg /L, respectively [3-4]. In this way, the removal of toxic heavy metal from aqueous effluents before being disposed in the environment is required [2]. Conventional methods of metal treatment such as precipitation, coagulation, evaporation, membrane process (electrodialysis and reverse osmosis), carbon adsorption, solvent extraction, chemical oxidation and reduction as well as ion exchange often involve high capital and operational costs and may also be associated with the generation of secondary wastes, which present treatment problems[5-6]. This has resulted a need for innovative treatment technologies for metals removal. Use of low cost adsorbents offers a potential alternative to existing methods for removal of metal ions from solutions. For the present work adsorptive properties of one such low cost adsorbent extracellular biopolymer, have been evaluated for Pb(II).

Recently, bioremediation using biopolymers derived from microorganisms has been emerging as an alternative secondary treatment for scavenging heavy metals from aqueous systems [7]. These exopolymers produced by microbes are mainly composed of polysaccharide, protein, humic substance, nucleic acid and lipid. Several charged surface functional groups in exopolymers like carboxyl, amino, phosphoric, carbonyl and hydroxyl groups are known to strongly bind metal cations in aqueous solution. While several studies focus on evaluating microbial biomasses as adsorbents in removing metals[8], polymers isolated from microorganisms still remain less investigated.As most exopolymers produced from bacteria are polar or charged, they can exist in different configurations depending on pH, temperature, ionic strength and background electrolyte in solutions [8]. These factors can change the overall conformation and the orientation of functional groups affecting the effective charge and cation binding property of the biopolymer.

Therefore, in our work, the effect of principal operational parameters on Pb(II) sorption, such as sorbent quantity, pH, initial Pb(II) concentrations and contact time, were monitored to optimize the sorption process for its possible use as a new biomaterial for wastewater treatment.The experimental data were correlated to different kinetic and adsorption models and the corresponding parameters were determined. Information regarding the binding sites and mechanism of sorption at molecular level has been explored employing Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM).

II. METHODS

A. Preparation of Biosorbent

Pseudomonas fluorescence C-9 obtained from activated sludges in Zhenjiang Dagang sewage plant was cultivated aerobically in 500 ml conical flasks containing sterile nutrient broth on a rotary shaker (150 rpm) at 30℃. Cells were harvested at the end of exponential phase, i.e. after 48 h incubation. After cultivation, the bacterial culture producing the most extracellular biopolymer was centrifuged at 10,000g for 20 min at room temperature and the supernatant liquid was then decanted into three volumes of cold ethanol, shaken vigorously and held at 4℃ for 24 h. Precipitated

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extracellular biopolymer was freeze-dried to obtain a crude biopolymer preparation , named PF-9.

B. Characterizations of the Biosorbent

The FTIR spectra was recorded using an infrared spectrometer (NEXUS870 America-Nicolet) between wavenumbers of 4000 and 400 cm^{-1}. A scanning electron microscope (Hitachi S4800, Japan) was used to visualize the surface morphology and structure of the biosorbent PS-2.

C. Chemicals

All chemicals and reagents used were of analytical grade and were obtained from Shanghai Chemical Reagent Co., China. Stock solutions of Pb(II) containing 1000 mg/L of lead element were prepared from lead nitrate in deionised water. All working solutions were prepared by diluting the stock solutions with deionised water to keep constant ionic strength. APHS-2 C pH meter (Shanghai Kangyi Instrument Co., China) was used to measure the pH values of the solutions. A constant temperature shaker (Shanghai Scientific Instrument Co. Ltd., China) was used for adsorption experiments. The concentrations of Pb(II) were determined by TAS-986 Flame Atomic Absorption Spectrometer(FAAS) (Beijing Purkinje General Instrument Co., Ltd), and average values of three replicates were taken for each determination. Solutions of 0.1 mol/ L NaOH and 0.1 mol / L HNO_3 were used for pH adjustment.

D. Batch Biosorption Experiments

In batch adsorption experiments, different doses of biosorbent (1.0 and 8.0 g/L) of the PF-9 was added into several 150 ml Erlenmeyer flasks, each containing 36 ml metal solution. The flasks were then shaken at 150 rpm in a constant temperature shaker at a pre-settled temperature for 24 h. The effect of contact time (0–120 min), concentration (50–300 mg/L), and solution pH (2.0–8.0), biosorbent dose (1.0 and 8.0 g/L) was studied at 25 °C. Isotherms were obtained by adsorbing different concentrations of Pb(II) ions. After prescribed contact time, the solutions were centrifugated at 5000 rpm for 5 min to separate PF-9 from the metal solution. Residual concentrations of Pb(II) ions in the supernatant solutions was determined by flame atomic absorption spectrometer(FAAS) analysis. All the experiments were carried out in duplicates and the average values were used for further calculations. The adsorbent phase concentrations of Pb(II) (q_e) were calculated according to the following equation:

$$q_e = V(C_0 - C_e) / W \qquad (1)$$

Where C_0 and C_e are the initial and equilibrium concentrations of Pb(II) (mg/L), respectively, V is the volume of the solution (L), and W is the mass of biosorbent (g).

III. RESULTS AND DISCUSSION

A. Adsorption Mechanism Determined by FTIR and SEM Analysis

In order to understand the surface binding mechanism, it was essential to identify the functional groups present on the biosorbent involved in this process. The main effective binding sites could be identified by FTIR spectral comparison of the biosorbent PF-9 and Pb(II) adsorbed PF-9. As seen in Table 1, it was obtained the FTIR spectra that PF-9 represents the eight adsorption bands, indicating the adsorptive nature of the biosorbent. The spectral analysis before and after Pb(II) adsorption indicated that, among eight adsorption bands, there were six significant band decreases of the functional groups on the biomass were detected at the bands of 3415, 2928, 1440, 1432, 1260, and 468 cm^{-1}, these bands were especially involved in Pb(II) adsorption.The broad and intense peak at 3415cm^{-1} was assigned to the stretching of O—H group due to inter- and intramolecular hydrogen bonding of polymeric compounds, the O — H stretching vibrations occur within a broad range of frequencies indicating the presence of "free" hydroxyl groups and bonded O—H bands of carboxylic acids [9]. The band at 2928 cm^{-1} indicated symmetric or asymmetric C—H stretching vibration of aliphatic acids. Peak observed at 1638cm^{-1} was the stretching vibration of C=O bond.Asymmetric and symmetric stretching vibrations of ionic carboxylic groups (–COO−) appeared at 1440 cm^{-1} and 1432 cm^{-1}. The peaks at 1260cm^{-1} may be assigned to -SO_3 stretching, the peaks at 1145 cm^{-1} may be assigned to C-O stretching, the peaks at 468 cm^{-1} is attributed to amine groups. Consequently, the FTIR studies showed that the functional groups like bonded -OH groups ,aliphatic C-H, secondary amine, carboxyl, –SO_3 stretching and amine groups were involved in Pb(II) adsorption.

TABLE 1 THE FTIR SPECTRAL CHARACTERISTICS OF PF-9

IR peak	**Assig-nment**			
	Before Adsor-ption	*After Pb (II) Adsorption*	*Differences*	*Functional groups*
1	3415	3407	-8	Hydroxyl groups
2	2928	2918	-10	Aliphatic C-H group
3	1638	1639	+1	C=O stretching
4	1440	1433	-7	Carboxyl groups
5	1432	1422	-10	Carboxyl groups
6	1260	1251	-9	-SO_3 stretching
7	1145	1150	+5	C-O stretching
8	468	462	-6	Amine groups

The surface morphology of PF-9 was studied with the use of SEM. A microporous structure (Fig. 1) was observed at a resolution of 1500× while the image of PF-9 was taken with a particle size of 30.0 μm. The surface of PF-9 was rough and irregular with large area for metal-surface interaction, the surfaces of PF-9 after adsorption indicated that the pores and surfaces of adsorbent were covered and became smooth by adsorbate.

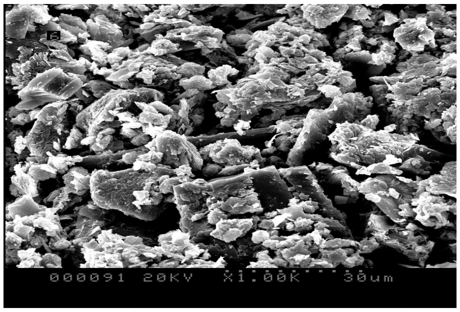

Figure 1. Scanning electron microscopic (SEM) analysis of PF-9

B. *Effect of pH*

The pH of the aqueous solution is an important operational parameter in the adsorption process, because it affects the speciation of lead, the surface charge, concentration of the counter ions on the functional groups of the adsorbent, and the degree of ionization of the adsorbate during reaction. Fig. 2 illustrated the effect of pH on on Pb(II) adsorption onto PF-9. The results indicated that the maximum uptake of Pb(II) occured at pH 6.0 for PF-9. It was evident that the adsorption capacity of Pb(II) increases sharply as the pH increasing from 2.0–5.0 and then increases slowly at pH in 5.0–6.0. At pH in 7.0–8.0, the adsorption capacity of Pb(II) decreases remarkably with increasing pH. This phenomenon may relate to pH_{ZPC} and the surface charge of the particles. Beyond pH 8.0, precipitate formation was observed in Pb(II) solution, and therefore the tests were terminated at this pH level. Consequently, Pb(II) adsorption showed maximum removal efficiency at the pH of 6.0.

This sorption behavior can be explained on the basis of surface change and proton-competitive sorption. The surface charge on the adsorbents was dependent on pH and provides a better understanding of the type of bond formed between Pb(II) and the surface. In this study the surface charge of PF-9 was studied by determining the point of zero charge (PZC) value of PF-9 using standard potentiometric method. And the PZC for PF-9 was found to 4.5. Namely, the surface charge is zero at the pH of 4.5. Thereby, when pH values were below the isoelectric point, the overall surface charge on the PF-9 became positive, which will inhibit the approach of positively charged metal ions. And then, as the pH was lowered, an enhancement of positive potential in metal removal resulted in the increase in competition between protons and metal cations for the same functional groups and the increase in positive surface charge, as well as resulted in a higher electrostatic repulsion between the surface and the metal ions. Thus, at lower pH values, the adsorption capacity of Pb(II) decreased with decreasing pH. When pH values were above the isoelectric point, there was a net negative charge on the surface, and the active group such as carboxyl and hydroxyl were free so as to promote interaction with the metal cations [10]. Therefore, the metal uptake increased with the increase in pH. Namely, at higher pH values, the surface of PF-9 had more negative charges which results in higher attraction of Pb(II) ions.

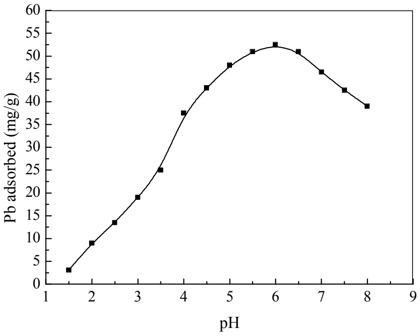

Figure 2. Effect of pH on the biosorption of Pb(II) from 100 mg /L solutions by 4.0 g/L PF-9 during orbital shaking at 150rpm at 25 ℃ for 60 min.

C. *Effect of Biosorbent Dosage*

One of the parameters that strongly affects the sorption capacity is the concentration of the sorbent in the liquid phase. The influence of the solid to liquid ratio on the sorption capacity of PF-9 was investigated for the constant initial concentration of Pb (II) ions of 100 mg/L (liquid phase) and the PF-9 mass (solid phase) varied between 1.0 and 8.0 g/ L. The results were presented in Fig. 3. Increase in the solid phase mass from 1.0 to 4.0 g/L resulted in a rapid increase in the uptake of Pb(II) ions. Further increase in the solid phase mass, however, did not result in sufficient increase in the sorption capacity of PF-9. As the difference in metal sorption capacity of PF-9 between 4.0 and 8.0 g /L solid phase mass was noted to be insignificant, the solid phase mass of 4.0 g/L was selected for further studies.

It may be concluded from these results that at lower PF-9 dosage (i.e., below 4.0 g/ L), the metal ions were competing for sorption at limiting sorption sites. However, as the dosage of PF-9 was increased, the availability of sorption sites eased resulting in greater percentage removal of the Pb(II) ions. The insignificant increase in uptake of Pb(II) ions at PF-9 dosage higher than 4.0 g/ L may be attributed to the presence of excess/surplus metal-binding sites on PF-9 than the available Pb(II) ions present in solution at the fixed concentration of 100 mg /L. These observations were in agreement with others reported in literature for the sorption of metal ions by different biological materials [11].

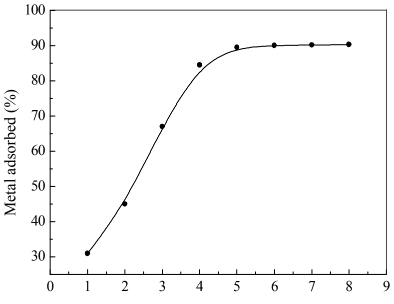

Figure 3 Effect of dosage of PF-9 on the sorption of 100 mg/L Pb(II) , solution pH 6.0, during 60 min orbital shaking at 150 rpm at 25 ℃.

D. *Adsorption Kinetics*

The time required to achieve adsorption-equilibrium for differrent initial concentrations of Pb(II) metal ions from aqueous solution was determined.The adsorption efficiency measured was plotted as a function of time and shown in Fig. 4. As seen in this figure, the initial retention profile of Pb(II) ions to the composite adsorbents was very fast and steep. After this initial retention period, the adsorption-equilibrium was gradually established within 60 min for all Pb(II) ions. The mechanism of Pb(II) ions adsorption process can occur in three following aspects. Firstly, the Pb(II) ions were transferred from the solution to the biosorbent surface. Secondly, diffusion of ions into pores of adsorbent and the last stage was related to the diffusion of the metal ions on the internal surface of the material, binding the pores spaces. The first step of adsorption may be affected by metal ion concentration, agitation period and rate. The last step of the adsorption was considered as a rate-limiting step and as a relatively rapid process. It was also clear from Fig.4 that efficiency of adsorption increased with increase in initial concentration of Pb(II). The equilibrium adsorption amount of Pb(II) was found to increase from 36.80 to 80.40 mg /g as the initial concentration increased from 50 to 200 mg /L .

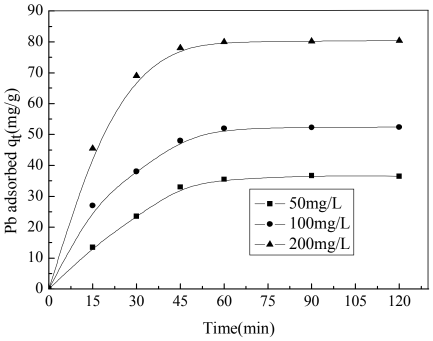

Figure 4. Effect of contact time on Pb(II) adsorption ,solution pH 6.0, during 60 min orbital shaking at 150 rpm at 25 °C.

The controlling mechanism of Pb(II) uptake was investigated by fitting the experimental sorption data to pseudo-first order and pseudo-second order kinetics models. The pseudo-first order rate equation of Lagergren is represented as:

$$\log(q_e - q_t) = \log(q_e) - \frac{k_1}{2.303}t \qquad (2)$$

Where q_e and q_t are the amounts of adsorbed Pb(II) ions on the biosorbent at equilibrium and at time t (respectively mg/g) and k_1 is the first-order biosorption rate constant (min^{-1}).

The pseudo second-order equation is also based on the sorption capacity of the solid phase and is given as:

$$\frac{t}{q_t} = \frac{1}{k_2 {q_e}^2} + \frac{1}{q_e}t \qquad (3)$$

Where k_2 is the second-order biosorption rate constant (g/mg.min); q_e is the biosorption capacity at the equilibrium (mg/g).

The results of the kinetics parameters for Pb(II) ions, calculated from the linear plots of pseudo-first order and pseudo-second order kinetics models were presented in Table 2. The low correlation coefficient values obtained for the pseudo-first order model indicated that sorption of metal ions did not follow the pseudo-first order reaction. The insufficiency of the pseudo-first order model to fit the kinetics data could possibly be due to the limitations of boundary layer controlling the sorption process. The experimental data were observed to fit well to the pseudo-second order equation.

TABLE 2 KINETIC CONSTANTS USING PSEUDO FIRST-ORDER AND PSEUDO SECOND-ORDER MODELS FOR THE BIOSORPTION OF PB (II) IONS ONTO PF-9 AT VARIOUS INITIAL PB(II) CONCENTRATIONS

C_0 (mg/L)	**Pseudo-first order constants**			**Pseudo-second order constants**		
	q_e* *(mg/ g)	***K_1 (min^{-1})***	***R^2***	***q_e* *(mg /g)***	***K_2* *(g /mg·min)***	***R^2***
50	27.0	-0.015	0.790	36.79	0.0258	0.999
100	41.2	-0.036	0.887	51.39	0.1634	0.997
200	70.1	-0.008	0.909	94.60	0.2949	0.994

E. Adsorption Isotherm

The successful representation of the dynamic adsorptive separation of solute from solution onto an adsorbent depends upon a good description of the equilibrium separation between the two phases. An adsorption isotherm is characterized by certain constant values, which express the surface properties and affinity of the adsorbent and can also be used to compare the adsorptive capacities of the adsorbent for different pollutants.The Langmuir and Freundlich isotherm model are often used to describe equilibrium sorption isotherms. The Langmuir model originally developed for adsorption of gases onto solids assumes that adsorption occurs in a monolayer or that adsorption may only occur at a fixed number of localized sites on the surface with all adsorption sites identical and energetically equivalent. Therefore, the Langmuir equation is based on the assumptions of a structurally homogeneous adsorbent, and is described by the following equation:

$$\frac{C_e}{q_e} = \frac{1}{q_{\max} K_c} + \frac{C_e}{q_{\max}} \qquad (4)$$

Where q_e is the amount of Pb(II) adsorbed per unit weight of adsorbents at equilibrium (mg/g), C_e the equilibrium concentration in the solution (mg/L), q_{max} the maximum adsorption at monolayer coverage (mg/g), and K_c is the Langmuir constant related to the affinity of binding sites (L/mg), and is a measure of the energy of adsorption.

Alternatively, the Freundlich equation is an empirical equation based on adsorption on a heterogeneous surface. The equation is commonly represented by

$$\ln q_e = 1/n(\ln C_e) + \ln K_f \qquad (5)$$

Where q_e and C_e have the same definitions as in Eq. (4), k_f is a Freundlich constant representing the adsorption capacity, and n is a constant depicting the adsorption intensity (dimensionless).

The equilibrium adsorptions of Pb(II) by PF-9 as a function of the initial Pb(II) ions concentration were shown in Fig. 5. From Fig. 5, it was seen that the adsorption capacity increases with the increasing of initial Pb(II) ions concentrations for PF-9 until it reached the maximum capacity of 92.66 mg/g (Fig.5).

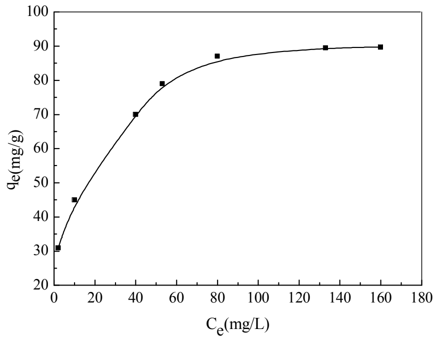

Figure 5. The relationship of C_e and adsorption capacitie for the PF-9

The Langmuir and Freundlich constants and their correlation coefficients (R^2) evaluated from these isotherms for Pb(II) were given in Fig. 6 and Fig. 7. From Fig. 6 and Fig. 7, the value of the correlation coefficient (R^2) for the Langmuir equation (0.9985) was much higher than that for the Freundlich equation (0.8189). Thus, the adsorption data fitted well with the Langmuir isotherm. The adsorption data of Pb(II) according with Langmuir isotherm illustrated that the binding energy on the whole surface of PF-9 was uniform. In other words, the whole surface had identical adsorption activity. The adsorption data of Pb(II) according with Langmuir isotherm also showed that the adsorbed metal ions do not interact or compete with each other, and they were adsorbed by forming a monolayer. This phenomenon, at the same time, indicated that chemosorption was the principal removal mechanism in adsorption process.

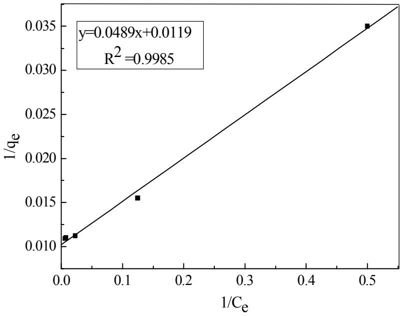

Figure 6. Langmuir biosorption isotherm

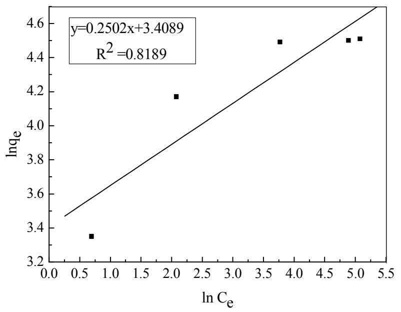

Figure 7. Freundlich biosorption isotherm

IV. CONCLUSION

The present study demonstrated that the biosorbent produced by Pseudomonas fluorescence C-9, an extracellular biopolymer PF-9, can be a potential adsorbent for removing Pb(II) ions from environmental and industrial wastewater samples. Sorption of metal ions was dependent on such experimental conditions as pH, sorbate and sorbent concentrations and sorbate–sorbent contact time. FTIR spectra showed that the principal functional sites taking part in the sorption process included carboxyl , hydroxyl groups, $-SO_3$ stretching and amine groups. Scanning electron microscopic analysis demonstrated the microporous structure of the material. The pseudo-second order kinetics and Langmuir adsorption isotherm models were noted be fit to the experimental data. The maximum binding capacity of Pb(II) according to Langmuir isotherm were 92.66 mg/g at pH 6.0 , shaker speed 150 rpm, at 25°C and for 60 min. The present study offered a scope for significant technological development towards the removal of Pb(II) from environmental and industrial wastewater samples.

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