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Kinetics and Equilibrium Biosorption of Nano-ZnO Particles on Periphytic Biofilm under Different Environmental Conditions

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ABSTRACT. As engineered nanoparticles (ENPs) were widely used in industry and commerce, increasing amounts of ENPs are expected to enter aquatic environment where their fate and potential impacts are unknown. Understanding the interaction between ENPs and periphytic biofilm, ubiquitous in the natural water environment, will help to better predict the behavior and fate of ENPs in aquatic media. This study focuses on ZnO NPs biosorption mechanism by periphytic biofilm dominated by bacteria and diatoms. Batch experiments were performed, in which the effect of pH, natural organic matter (NOM), extracellular polymeric substances (EPS) and temperature on the biosorption were investigated. The biosorption of ZnO NPs were found to be pH dependent, and more biosorption was observed under neutral and acidic conditions. Increasing concentration of NOM negatively affected on the biosorption of ZnO NPs onto periphytic biofilm due to the adsorption of NOM molecules on the surface of nanoparticles and the resulting steric stabilization of the particles suspension. The removal of loosely bound EPS enhanced the biosorption capacity of periphytic biofilm. The biosorption of nano-ZnO on the biofilm was related to electrostatic force of attraction, confirmed from the zeta potential study. The biosorption of ZnO NPs on biofilm was correlated better with Langmuir isotherms as compared to Freundlich isotherms under the concentration range studied. The biosorption process was thermodynamically feasible and spontaneous. The present study shows that periphytic biofilm can be employed for an environmentally benign and effective biosorbent for removal of ZnO NPs.

Keywords: engineered nanoparticles, biosorption, natural biofilm, NOM, EPS

1. Introduction

Engineered nanoparticles (ENPs) are widely applied in many commercial industrial and consumer products such as semiconductors, cosmetics, textiles, and pigments (Gottschalk et al., 2009). Given these materials broad use, it is inevitable for the increasing quantities of ENPs released directly or through discharges of municipal wastewater into the aquatic environment (Nowack and Bucheli, 2007).At the same time, there is an increasing concern regarding the risks of ENPs to human and ecosystem health (Holden et al., 2014; Sharifi et al., 2012).

The potential environmental and health risks of nanoparticles are greatly dependent on their fate and behavior in the na-tural environment in terms of exposure scenarios (Klaine et al., 2008). The discarded ENPs, whether intentional or not, will follow a variety of pathways, whose ultimate destiny will be into the natural water environment (Weinberg et al., 2011). Studies have demonstrated that the release of ENPs negatively affected the microbial communities in the aqueous environment (Fabrega et al., 2011; Zhang et al., 2012). AgNPs exposure negatively impacted natural bacterioplankton activity and made changes in bacterial community composition in natural waters (Das et al., 2012a, b). Extracellular polymeric substances (EPS) mitigated the toxicity of TiO_2 nanoparticles on planktonic bacteria pseudomonas aeruginosa (Hessler et al., 2012). Nevertheless, we are still far from understanding the behavior and transport of ENPs in the natural water environment. According to the literature (Nowack and Bucheli, 2007), the environmental fate of nanoparticles is strongly influenced by sorption processes on microbial communities in the aqueous environment. Periphytic biofilm, ubiquitous in the natural environment (Flemming and Wingender, 2010; Sheng et al., 2010), are highly sensitive to levels of ENPs concentration, and play a significant role on the behavior of ENPs in aquatic environment (Battin et al., 2009). However, little research has been carried out to evaluate the adsorption of periphytic biofilm for nanoparticles.

Recently, the environmental fate of ZnO nanoparticles is becoming of great interest. It has been shown that ZnO nanoparticles have acute toxic effect to bacteria (Li et al., 2011), daphnia magna (Adam et al., 2014), and microalgae (Aruoja et al., 2009). In order to quantitatively evaluate the potential ecological risk of nano-ZnO, it is extremely crucial to understand the influence of biofilms on the fate of ZnO NPs in natural environment. In addition, natural organic matter (NOM) is ubi-

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quitous in aqueous environment. It could alter the aggregation/-suspension behavior and transport of ENPs, which has been addressed in earlier studies (Mohd et al., 2014; Zhao et al., 2013). It is known from colloid sciences that NOM is likely to change the properties of NPs concerning their stability and their transport behavior by adsorbed on ENPs (Pelley and Tufenkji, 2008). As NOM were composed primarily of humic substances in the environment (Kiser et al., 2010), humic substances were also important components of EPS (Sheng et al., 2010). EPS, secreted by microbial community, provide the mechanical stability of biofilm and affect the properties of microbial aggregates, such as surface characteristics, adsorption ability etc (Flemming and Wingender, 2010).Therefore, it is important to investigate what roles NOM and EPS play in ZnO NPs biosorption to periphytic biofilm in natural water environment. In this paper, biosorption is used to refer to the net effect of all biomass-particle sorption mechanisms that remove NPs from water, including adsorption to cell surfaces, adsorption to EPS, and uptake into cells (absorption) through active or passive transport across the cytoplasmic membrane or through membrane disruption (Kiser et al., 2010).

For the above-mentioned reasons, the overall aim of this study was to investigate biosorption of ZnO nanoparticles to periphytic biofilm. More specifically, our main objectives were to (1) quantify the effects of pH, NOM and EPS on the biosorption of ZnO NPs to periphytic biofilm; (2) study the influence of surface charge on biosorption; and (3) investigate the adsorption kinetics, isotherms and the thermodynamics.

2. Materials and Methods

2.1. Periphytic Biofilm Preparation

Glass slides (25 × 75 mm) sterilized by 0.1 M HCl solution for 30 min were submerged and fixed in reactor (Supplementary materials, Figure S1). The experimental reactor (Wang et al., 2013) was built with Plexiglas sides (10 mm thich) and a PVC base (20 mm thick). It was 4 m long, 0.3 m wide, 0.3 m deep. The reactor was fed with contaminated water collected from a eutrophic river. After two weeks of biofilm seeding, the fed water was filtered (through 0.45 um) to allow the free growth of periphytic biofilm (Wu et al., 2010). The characteristics of the filtered river water are detailed in Table S1 (Supplementary materials). During the whole experimental period, physicochemical and biological measurements were performed weekly. All analyses were performed in accordance with standard methods (APHA, 1998). All the determined values were based on results from triple duplicates. The oxygen level was monitored regularly using a HACH Dissolved Oxygen Monitor. The DO remained stable at 7 ± 1 mg/L, and the temperature maintained at 288 ± 2 K. After 41 days, according to our earlier study (Wang et al., 2013), the dense biofilm samples (dry biomass reached steady state) were harvested from the reactor and used for further experiments.

2.2. Removal of Loosely Bound EPS from Biofilms

Among the biofilm EPS components, those that can be readily removed are defined as 'loosely bound EPS', while those that need vigorous removal processes are defined as 'tightly bound EPS'. Extraction reagents such as ethanol, which can damage bacterial cells, are often applied to remove tightly bound EPS. To eliminate loss of microbial activity and viability, only loosely bound EPS (LB-EPS) were removed in present study. The periphyton samples were scraped from the glass slides with a sterile toothbrush. Periphyton samples were rinsed three times with phosphate-buffered solution (5 mM PBS, pH = 7) and then centrifuged (2,000 g) for 10 min; the centrifuged supernatant was discarded (Li and Yang, 2007; Ras et al., 2013). The rinsed samples (referred to as original biofilm) were divided into two portions. One portion (original biofilm) was stored at 277 K without further processing. The other portion was processed to extract LB-EPS from the biofilms following the procedure described by Sheng and Liu (2011) as briefly summarized here.

Biofilms were resuspended in phosphate-buffered solution (5 mM PBS, pH = 7). A 30-s vortex was performed to mix biofilm fragments with PBS. The biofilm suspension was vortexed at the maximum speed for 1 min, then centrifuged at 277 K, 4,000 g, for 20 min. Pellet resuspension, vortexing, and centrifugation were repeated three times to improve the efficiency of the extraction of EPS. The loosely bound EPS-extracted biofilms were stored at 277 K until used in experiments.5 mM PBS was used to provide a pH (7.0) and was prepared by dissolving 0.44 g/L KH_2PO_4, 0.27 g/L Na_2HPO_4 in ultra-pure water (Millipore).

Inorder to estimatebiomass, the wet original biofilms were weighted and then let for drying for 24 h at 105 °C and weighted again. The moisture content of the periphyton was determined to be 84 ± 2%.

2.3. Characterization of ZnO NPs

ZnO NPs were purchased from Shanghai Aladdin Ltd., China. The specific surface area of ZnO NPs was measured via a Micromeritics Tristar 3000 analyzer by nitrogen adsorption at 77 K using the Brunauer-Emmett-Teller (BET) method. Before use the NPs stock suspension of 100 mg/L were prepared by dispersion 0.1 g of ZnO NPs in 1 L of phosphate-buffered solution (5 mM PBS, pH = 7), followed by 1 h of ultrasonication (298 K，250 W, 40 kHz) (VGT-1048, Weigute Ultrasonic Machine Company, China) according to the literature (Zheng et al., 2011). The morphology of ZnO NPs in the stock suspensions was imaged using a SEM (Hitachi S-4800), by placing a drop of aqueous sample on a carbon-coated copper sheet andthe sheet was dried at room temperature (298 K) for one day. During the whole experimental period, the particle-size distribution and zeta potential of the ZnO NPs were measured using a Mal−vern Zetasizer Nano ZS90 (Malvern Instruments, UK) and the release of Zn^{2+} from nano-ZnOdissolutionin the phosphate-buffered solution was monitored by inductively coupled plas−ma mass spectrometry (ICP-MS, Agilent 7700X) (Zheng et al., 2011).

2.4. Adsorption Kinetics

To investigate the adsorption kinetics of biofilm for ZnO

NPs, 0.2 g wet biofilm samples (corresponding dry weight of 0.032 g), including original biofilm and loosely bound EPS-extracted biofilm were added into a series of glass vials each with working volume of 200 mL. The freshly prepared ZnO NPs stock suspension was diluted into the glass vials with a concentration (50 mg/L) at pH = 7. Then, the mixtures were equilibrated on a shaker at 150 rpm for 24 h (298 K). After that, the concentration of ZnO NPs remained in the supernatant and adsorbed on the biofilm were determined at various time intervals by ICP-MS (Agilent 7700X). The suspension was centrifuged for 10 min at 2000 g to separate out the biomass and adsorbed nanoparticles. Centrifugation force and time were chosen to sediment all the biomass, as well as maintain the ZnO NPs fraction remains in the supernatant (Khan et al., 2012). The relative sorption to biofilms at each time point was calculated from the relative loss of NPs from the aqueous phase correcting for controls (e.g. vessel sorption). The amount of adsorption at time 't', 'q_t' (mg/g) was calculated by:

$$q_t = \frac{(c_0 - c_t)}{W} \quad (1)$$

where 'C_0' and 'C_t' (mg/L) are the concentration of ZnO NPs at initial and the time 't', respectively. 'V' is the volume of the solution (L) and 'W' is the mass of biofilm sample used (g). All the tests were carried out in triplicate, and mean values of the results were reported. The experimental error limit was strictly kept within ± 5%.

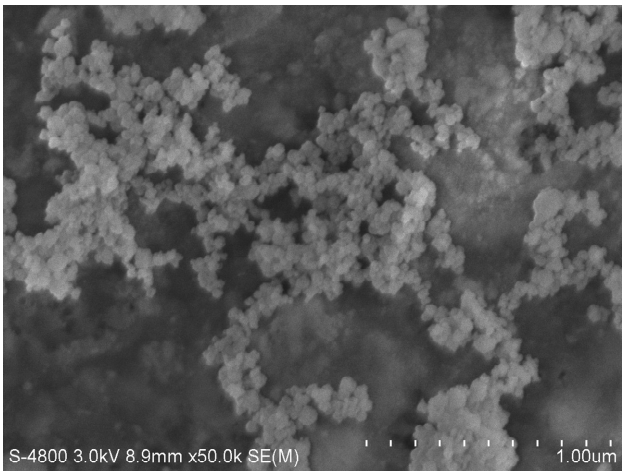

Figure 1. SEM image of ZnO NPs.

2.5. Adsorption Isotherm

The isotherm of adsorption of ZnO NPs on biofilm was carried out with a series concentration of 0 ~ 50 mg/L at different temperatures (288, 298 and 313 K). Basically, the isotherm experiments were identical to those of adsorption kinetic experiments. The effect of pH on biosorption was investigated at 298 K with initial nano-ZnO concentration of 50 mg/L. The desired pH of the suspensions was maintained by adding 0.01 M HCl or NaOH solution. For the NOM, Suwannee River NOM (SRNOM, International Humic Substances Society, pH = 7) was employed as a surrogate NOM sample. The effect of natural organic matter (NOM) on adsorption was evaluated at different NOM concentration (0 ~ 10 mg/L). The amount of ZnO NPs adsorbed at equilibrium 'q_e' (mg/g) on biofilm was calculated from the following equation:

$$q_e = \frac{(c_0 - c_e)V}{W} \quad (2)$$

where 'C_0' and 'C_e' (mg/L) are the concentration of ZnO NPs at initial and equilibrium, respectively. 'V' is the volume of the solution (L) and 'W' is the mass of biofilm sample used (g). All the tests were carried out in triplicate, and mean values of the results were reported. The experimental error limit was strictly kept within ± 5%.

2.6. Measurement of the Zeta Potential

The zeta potential of ZnO NPs and biofilm suspension were measured using a Malvern ZetaSizer Nano ZS90. The NPs and biofilmsampleswere suspended in phosphate-buffered solution (5 mM PBS) and adjusted pH by 0.01 M HCl or NaOH solution. The zeta potential at different NOM concentrations at pH = 7 was also measured.

3. Results and Discussions

3.1. Characterization of ZnO Nanoparticles

Size and morphology of ZnO NPs were characterized by SEM. A typical SEM micrograph was shown in Figure 1. ZnO nanoparticles used in this study were nearly spherical in shape and polydispersed. The specific surface area of the ZnO NPs was determined to be 52.2 ± 3.5 m^2/g. The DLS measurements indicated that the ZnO NPs were poorly dispersed and readily aggregated from 37 ± 5 to 735 ± 12 nm within 180 min in solution (Figure S2, Supplementary materials). During the whole experimental period, only a small fraction of ZnO NPs dissolution (< 5%) in the PBS was observed from the ZnO NPs control experiments, which was consistent with the experimental results reported by Li et al. (2011).

3.2. Adsorption Kinetics

The biosorption of ZnO NPs on periphytic biofilms as a function of contact time (pH = 7) were shown in Figure 2. The biosorption of ZnO NPs on biofilms occurred quickly and 90 min was enough to achieve the biosorption equilibrium. Thus, the optimal equilibrium time in this study was targeted for 120 min, to ensure all the experimental equilibrium reached. The adsorption kinetics is important for adsorption studies, which can provide valuable data for understanding the mechanism of adsorption processes (Yan et al., 2012). To investigate the biosorption kinetics of nano-ZnO onto the periphytic biofilms, the pseudo-first-order model was used in this study. The pseudo-first-order kinetic rate equation can be expressed as:

$$q_t = q_e(1 - e^{-k_1 t}) \quad (3)$$

where k_1 (min^{-1}) is the rate constant of the pseudo-first-order adsorption, q_e (mg/g) and q_t (mg/g) are the amount of ZnO NPs adsorbed onto biofilms at equilibrium and at time t (min), respectively.

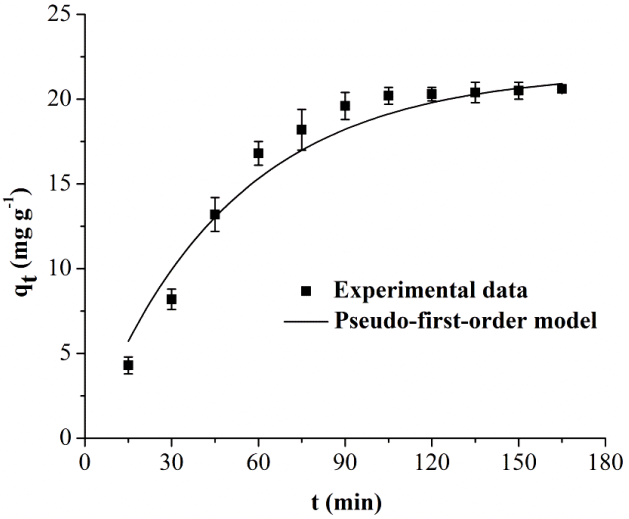

Figure 2. Adsorption kinetics of nano-ZnO on periphytic biofilm at 298K (pH = 7), with an initial concentration of 50 mg/L. Values are the mean of n = 3 (mean ± standard deviation).

The experimental results expressed a very good compliance with the pseudo-first-order equation for its high correlation coefficient (0.9626). The rate constant (k_1) and qe of the plot value were 0.02 (min^{-1}) and 22.15 (mg/g), respectively. The theoretical 'q_e' value agreed well with the experimental 'q_e'. The results suggested that the pseudo-first-order adsorption model can be used to satisfactorily describe the biosorption kinetics of ZnO NPs onto periphytic biofilm.

3.3. Effect of pH on Biosorption

Sorption potential is defined in this context as the potential degree of interaction between sorbent and sorbate surface, which is determined by the surface properties of the two materials. The effect of pH on the biosorption of ZnO NPs onto periphytic biofilm was evaluated in a pH range of 6 ~ 11 and the results were shown in Figure 3(a). The figure showed that pH had a significant effect on the biosorption of ZnO NPs onto biofilms. Generally, the biosorption capacity for biofilms was much greater at pH < 8 than pH > 10 and kept almost invariable from pH = 5 to 8. These phenomena can be explained by considering the influence of pH on the surface charge of nanoparticles and biofilm suspension. The pH had a direct effect on zeta potential, and it was reported that the zeta potential of particles decreased with increase in pH (Peng et al., 2004; Jiang et al., 2009).

Figure 3(b) showed the zeta potentials of biofilm suspension and ZnO NPs at different pH values. Periphytic biofilm

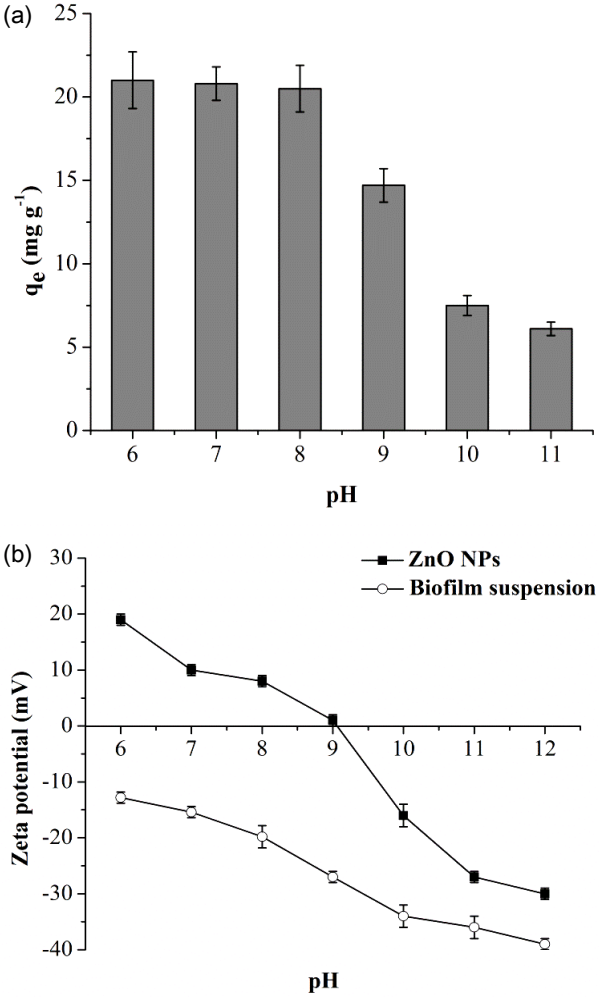

Figure 3. (a) Effect of pH on biosorption of nano-ZnO on biofilm at 298K, with an initial concentration of 50 mg/L; (b) Zeta potential of the nanoparticles and biofilm suspension at different pH values. Values are the mean of n = 3 (mean ± standard deviation).

showed negative surface charge in tested pH range. Due to the carboxyl groups, phosphate groups and amino groups on the cellular membrane of the bacteria, the bacterial species generally exhibit negative surface charge (van der Wal et al., 1997). The ZnO NPs exhibited positive zeta potential up to pH = 9 and turned to negative when pH < 9. The zeta potential value agreed well with the biosorption mechanism. With increase in pH, the zeta potential of ZnO NPs decreased and the amount of adsorbed ZnO nanoparticles on biofilms also decreased. When solution pH is above 9, the surface charge of both ZnO NPs and biofilms were negative, leading to large electrostatic force. The electrostatic repulsion forces predominate, thus, reducing the biosorption. When at pH < 9, however, electrostatic interaction became one of the driving forces for the biosorption. In this region, ZnO NPs expressed positive charge while microbial biofilms had negative charge, the electrostatic force of attraction promoted the bio-

sorption of ZnO NPs onto biofilms. Khan et al. (2012) investigated the effect of pH on the adsorption of silver nanoparticles onto *A. punctate*, and similar results were found.

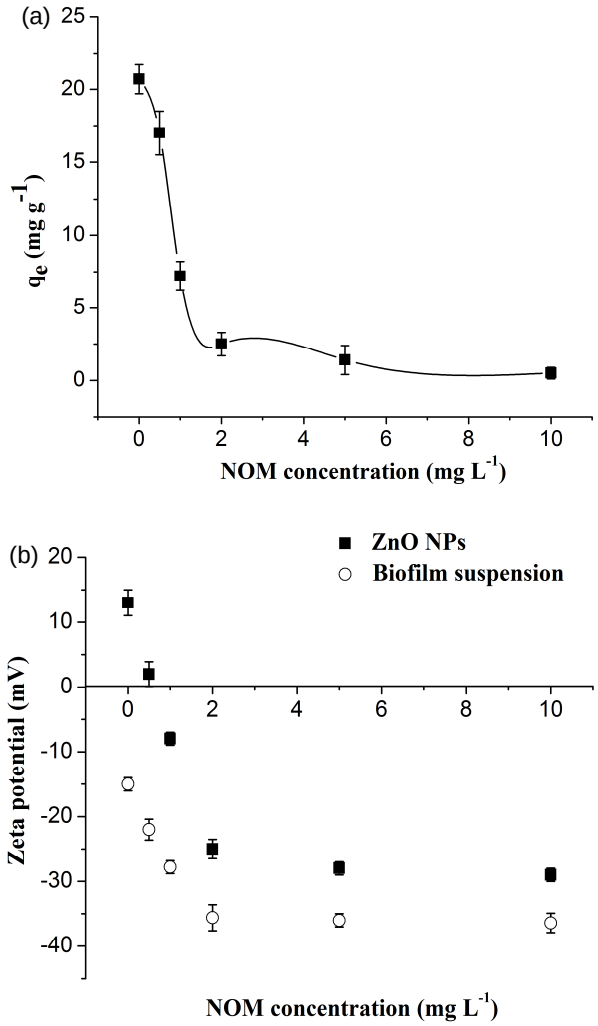

Figure 4. (a) Effect of NOM on biosorption of nano-ZnO on biofilm at 298K (pH = 7), with initial concentration 50 mg/L; (b) Zeta potential of the nanoparticles and biofilm suspension at different NOM concentrations. Values are the mean of n = 3 (mean ± standard deviation).

3.4. Effect of NOM and EPS on Biosorption

The effect of NOM on the biosorption of ZnO NPs on biofilm was studied with initial concentration of 50 mg/L ZnO NPs. Results showed that there was no much difference in adsorption when NOM concentration increased from 0 to 0.5 mg/L. However, when NOM concentration increased above 2 mg/L, a significant decrease in biosorption was observed (Figure 4(a)). High concentration of NOM could significantly affectthe particle dispersion of nanoparticles in aqueous suspension (Gao et al., 2012; Akaighe et al., 2013). NOM adsorption can impart negative charge to the surface of nanoparticles and then stabilize or at least reduce aggregation of nanoparticles (Zhang et al., 2009). Thus, the interaction between nanoparticles and bacterial cells were influenced. Fabrega et al. (2009) found that natural organic macromolecules mitigated the short-term nanosilver toxicity to bacteria in natural water environment.

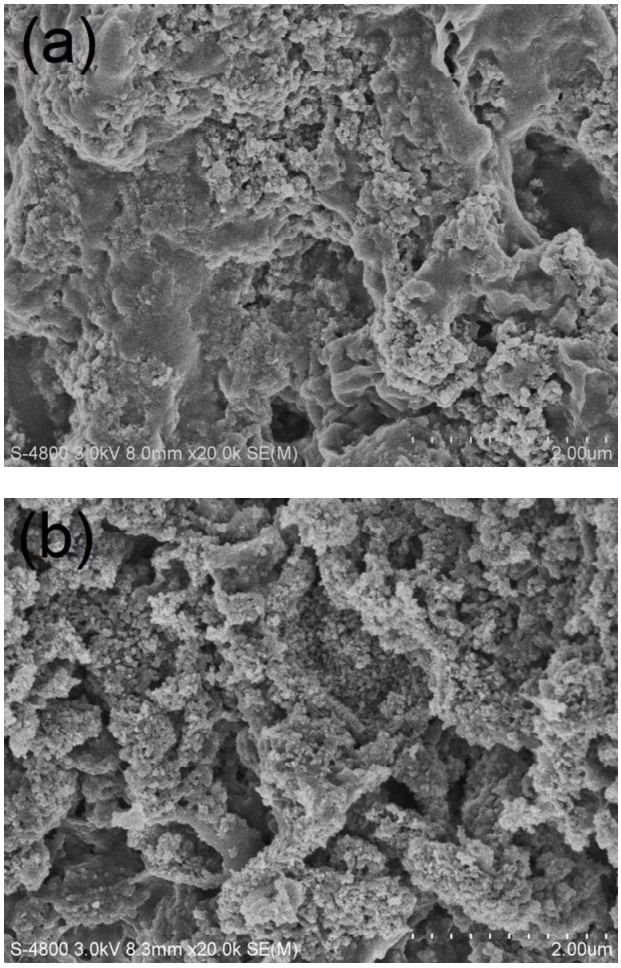

Figure 5. (a) SEM image of original periphytic biofilms; (b) SEM image of periphytic biofilms with loosely bound EPS removed. Bar size: 2 um.

Figure 4(b) showed the zeta potentials of ZnO NPs suspension and periphytic biofilm at different concentrations of NOM. Results showed that when NOM concentration increased from 0 to 0.5 mg/L, ZnO NPs exhibited positive charge and above that the zeta potential turned to negative. Biofilm suspension remained negative charge within the increase concentration of NOM. When the concentration of NOM was below 0.5 mg/L, the electrostatic force of attraction favored the biosorption of positively charged ZnO NPs on negatively charged biofilm suspension. While, with the NOM concentration increasing, there was a high degree of repulsion between the negatively charged ZnO NPs and the biofilm suspension, which formed an electrostatic barrier that limited the biofilm-particle interactions, thus hindered the biosorption of nanoparticles onto natural biofilm. It is proposed that this behavior is due to the adsorption of NOM

molecules onto the surface of nanoparticles and the resulting steric stabilization of the particles suspension (Keller et al., 2010; Delay et al., 2011).

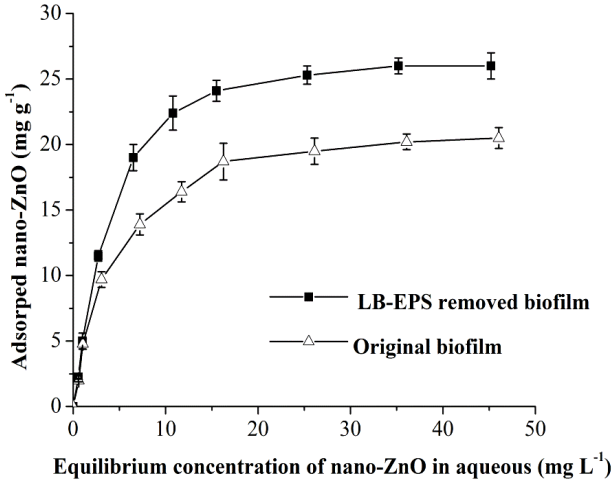

Figure 6. Effect of LB-EPS on the biosorption of nano-ZnO on periphytic biofilm at 298 K (pH = 7). Values are the mean of n = 3 (mean ± standard deviation).

The fate and behavior of contaminant such as nanoparticles (Joshi et al., 2012) and heavy metals (Sheng et al., 2013), may be greatly influenced by the presence of biofilm EPS. To some extent, EPS perform as a physical barrier keeping ZnO NPs from reaching the biofilm cells. Figures 5(a) and 5(b) showed SEM images of natural biofilm with and without LB-EPS, respectively. As observed in Figure 5a, diatoms and bacteria in the original periphytic biofilm were immersed in compact extracellular polymers. While biofilm cells are much more exposed after the removal of LB-EPS (Figure 5(b)), although tightly bound EPS (TB-EPS) were still present. Comparison was made for the biosorption capacity between original biofilm and the LB-EPS-extracted biofilm (Figure 6). For the concentration of ZnO NPs below 10 mg/L, the biosorption capacity of the two different biofilms were nearly equivalent. However, when the concentration of ZnO nanoparticles increased, the LB-EPS-extracted biofilm exhibited higher biosorption capacity than the original biofilm. EPS have the potential to interact with hydrophobic or positivelycharged components, and protect the cells from contacting with the toxic chemicals (Henriques and Love, 2007). In present study, the positively charged ZnO NPs in aqueous solution (pH = 7) bounded to the EPS, which impeded the interactions between ZnO NPs and biofilm cells. The mechanisms of this phenomenon are likely to be the same as those of NOM: increasing electrical repulsion and decreasing van der Waals forces of attraction (Kiser et al., 2010).

3.5. Biosorption Isotherms and Thermodynamic Data

The equilibrium adsorption isotherm model is fundamental indescribing the interactive behavior between adsorbate and adsorbent. In this study, the ZnO NPs are the adsorbate and periphytic biofilms are theadsorbent. Isotherm studies were carried out at three different temperatures (T = 288, 298 and 313 K) at pH = 7 (Figure 7). The data generated were evaluated by Langmuir and Freundlich adsorption isotherm equations to interpret the nature of nanoparticle biosorption on the biofilm.

Langmuir isotherm model assumes monolayer adsorption, and ispresented by the Equation (4):

$$q_e = \frac{q_{\max} K_L C_e}{1 + K_L C_e} \tag{4}$$

where q_e is the nanoparticle amount adsorbed per unit mass of adsorbent (mg/g), C_e is the equilibrium concentration of ZnO in the solution (mg/L), $q_{\max}$ is the maximum adsorption capacity (mg/g) and K_L is the constant related to the free energy of adsorption.

The Freundlich model is expressed as Equation (5):

$$q_e = K_F C_e^{\frac{1}{n}} \tag{5}$$

where K_F ((mg/g) (mg/L)$^{-1/n}$) and n are empirical constants. K_F is related to the adsorption capacity of the adsorbent and n represents the intensity of adsorption.

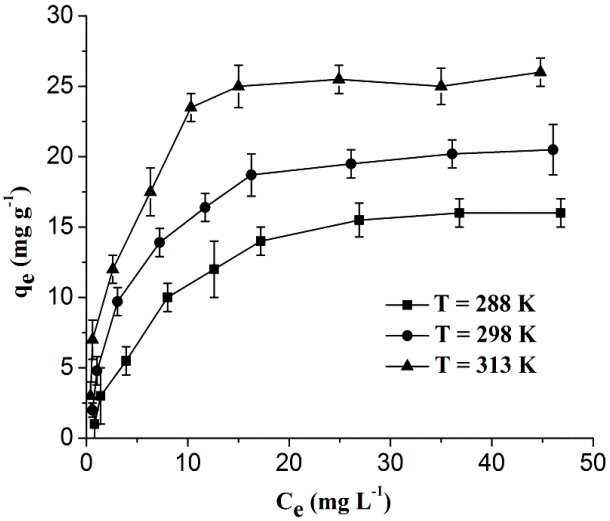

Figure 7. Adsorption isotherm of nano-ZnO on periphytic biofilmat three different temperatures (pH = 7). Values are the mean of n = 3 (mean ± standard deviation).

Figure 7 shows the biosorption isotherms at three different temperatures. The biosorption of ZnO NPs increased with the increase of experimental temperature. The adsorption isotherm is the highest at T = 313 K and is the lowest at T = 288 K, illustrating that the biosorption of nano-ZnO onto periphytic biofilm was promoted at higher temperature. The measured data were regressively analyzed with the Langmuir and Freundlich models and their calculated parameters were shown in Table 1.

Table 1. Adsorption Isotherm Parameters of ZnO-NPs onto Periphytic Biofilm at Three Different Temperatures, at PH = 7

T (K)	Langmuir			Freundlich		
	q_{max} (mg/g)	K_L	R^2	K_F((mg/g)(mg/L)$^{-1/n}$)	1/n	R^2
288	19.68	0.12	0.987	3.77	0.40	0.904
298	23.05	0.21	0.994	5.02	0.41	0.914
313	28.32	0.33	0.980	9.54	0.29	0.867

Table 2. Thermodynamic Parameters of ZnO NPs Adsorbed on Periphypic Biofilm

C_0 (mg L^{-1})	ΔH^0(**kJ** mol^{-1})	ΔS^0(**kJ** mol^{-1} K^{-1})	ΔG^0(**kJ** mol^{-1})		
			288 K	298 K	313 K
5	15.69	0.103	-13.98	-15.01	-16.55
10	16.86	0.111	-15.11	-16.22	-17.88
30	24.93	0.146	-17.12	-18.58	-20.77
50	35.40	0.184	-17.59	-19.43	-22.19

Comparing the correlation coefficients (R^2) deduced that the biosorption process is better defined by Langmuir model (R^2 > 0.980) than the Freundlich model, which indicated that almost complete monolayer coverage of the adsorbent particles (Sahle-Demessie and Tadesse, 2011). In addition, biofilm has a limited biosorption capacity, thus the biosorption could be better described by the Langmuir model rather than Freundlich model, as an exponentially increasing adsorption is assumed in the Freundlich model (Kiser et al. 2010).

The thermodynamic parameters (ΔH^0, ΔS^0, and ΔG^0) for nano-ZnO adsorption on biofilm can be calculated from the temperature dependent adsorption. The values of enthalpy (ΔH^0) and entropy (ΔS^0) can be calculated from the slope and y-intercept of the plot of ln K_d vs. 1/T (Figure 8) via applying the following equations:

$$K_d = \frac{C_0 - C_e}{C_e}\frac{V}{m} \tag{6}$$

$$\ln K_d = \frac{\Delta S^0}{R} - \frac{\Delta H^0}{RT} \tag{7}$$

where C_0 is the initial concentration (mg/L), C_e is the equilibration concentration (mg/L), V is the volume (mL) and m is the mass of biofilm (g), R (8.314 J mol^{-1} K^{-1}) is the ideal gas constant, and T (K) is the temperature in Kelvin.

Free energy changes (ΔG^0) of specific biosorption are calculated from:

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \tag{8}$$

The relevant thermodynamic parameters were summarized in Table 2. The Gibbs free energy change (ΔG^0) was negative as expected for a spontaneous process under the conditions applied. The value of ΔG^0 became more negative with the increase of temperature, which indicated that more efficient biosorption occurred at higher temperature. The positive value of enthalpy (ΔH^0) demonstrated that the biosorption process was endothermic in nature. Positive values of ΔS^0 suggested increased randomness at the biofilm-suspension interface, when ZnO NPs were fixed on the active sites of the biosorbents.

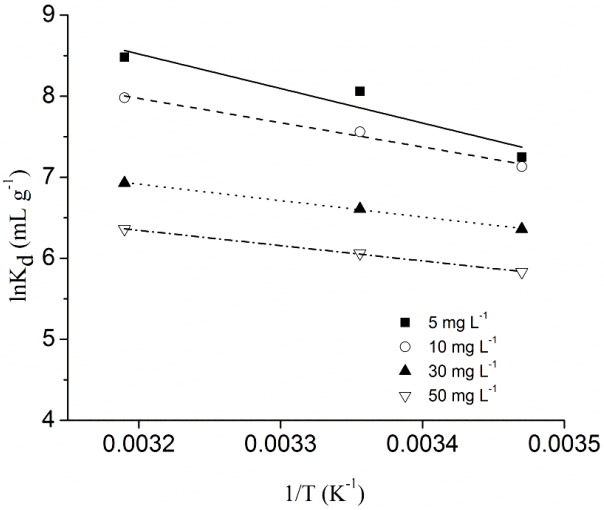

Figure 8. Variation of lnK_d with reciprocal of temperature.

4. Conclusions

This study aimed to understand the biosorption mechanisms of periphytic biofilm for ZnO nanoparticles. The effect of environmental factors such as pH, NOM, EPS and temperature on the adsorption and removal of ZnO NPs by periphytic biofilm were investigated. Results showed that pH and NOM concentration can influence the zeta potential of biofilm suspension and ZnO nanoparticles. The adsorption of ZnO NPs was directly related to zeta potential and significantly different biosorption were observed due to the changes of zeta potentials of nanoparticles and biofilm suspension. EPS hindered the interaction between ZnO NPs and biofilm cells and decreased the biosorption capacity of biofilm for ZnO NPs in the conditions tested. The experimental data were well fit to the pseudo-first-order rate

equation. Langmuir isotherm fit the experimental data better than Freundlich isotherm. Meanwhile, the negative values of ΔG^0 and positive value of ΔS^0 showed that the ZnO NPs biosorption onto periphytic biofilm was feasible and spontaneous. This study indicated that the fate and transport of ZnO nanoparticles in the natural environment were strongly dependent on the presence of periphytic biofilm and influenced by the environmental factors. More work need to be done to conduct studies on the interaction between other kinds of nanoparticles and natural biofilm.

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Supporting Material. The supporting materials containing Figures S1 and S2, and Table S1 are available at http://www.iseis.org/jei/download/Supplement_201400267.docx

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