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Potential of *Humicola phialophoroides* biomass for zinc (II) removal from aqueous solution

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Abstract

The purpose of this research was to estimate the Zn^{2+} biosorption by biomass of *Humicola phialophoroides* fungus isolated from creek sediments in a zinc mine area in Thailand. The Langmuir isotherm model gave a better fit for the result data more than Freundlich, Temkin and Dubinin-Radushkevich isotherm models. Zn^{2+} removal ability in all *H. phialophoroides* biomass types as increasing Zn^{2+} concentration had the same pattern, but biomass pretreated by NaOH showed better Zn^{2+} removal ability than viable biomass, non-viable biomass, and HNO_3 pretreated biomass. Maximum Zn^{2+} biosorption of biomass pretreated with NaOH took place at initial solution at pH 8 after 150 min. In addition, Zn^{2+} was well adsorped by NaOH pretreated biomass at temperature higher than 70 °C, while desorption experiments indicated that the desorption efficiency with 0.1 M HNO_3 solution reached 92.31%.

Key words: biosorption, *Humicola phialophoroides*, pretreated biomass, Zn^{2+}.
Abbreviations: ITS, internal transcribed spacer; PDA, potato dextrose agar; PDB, potato dextrose broth.

Introduction

Zinc is a widespread heavy metal ion in natural systems including water, air, sediment and soil. More than 13 million tons of zinc was produced in 2013 worldwide (U.S. Geological Survey 2014). Approximately 70% of zinc comes from mined ores and 30% comes from recycled sources (Hosford 2013). Approximately 80% of zinc is used in galvanizing to protect steel from corrosion (The National Mining Association 2014). At background levels, it is an essential nutrient for human, animal and plant life. However, high concentrations of zinc can be harmful and can cause toxicity.

The commonly used procedures for removing metal ions from aqueous solutions include ion exchange, chemical precipitation, lime coagulation, reduction process, membrane technologies, and activated coal applied to remove these solutions of heavy metals, but these can be expensive and are often limited at high concentration (Zabochnicka-Świątek, Krzywonos 2014). At present, the bioremoval treatment process of metals has received increasing attention in term of biosorption, because of its many advantages such as the ability to treat large volumes of wastewater, rapid kinetics and high selectivity in the removal and recovery of specific heavy metals. Manystudies have reported that fungi biomass possesses high metal removal, intracellular uptake of the metal ions occur by the cells, the process involves metabolism using living cells, and the cell surface sorption allows interaction between toxic metal ions and functional groups such as carboxylate, hydroxyl, sulfate, phosphate and amino groups present on the cell surface (Abbas et al. 2014; Dhankhar, Hooda 2011).

Several fungal biosorbents such as *Phanerochaete chrysosporium* (Marandi et al. 2010), *Penicillium chrysogenum* (Tan, Cheng 2003), *Aspergillus niger* (Vale et al. 2016) and *Rhizopus arrhizus* (Preetha, Viruthagiri 2005) have been used in Zn^{2+} removal from wastewater. *Humicola* sp. fungus occurs in the Mae Toa creek sediments from zinc mine area, Tak province, Thailand (Netpae et al. 2015). Study showed that *Humicola* sp. is an excellent adsorbent for metal ions in aqueous solutions (Netpae et al. 2014). There is no information on the use of *Humicola phialophoroides* for the biosorption of zinc. In this study, adsorption ability by viable, non-viable and two types of pretreated *H. phialophoroides* biomass were investigated for removal of Zn^{2+} from aqueous solution under experimental conditions.

Materials and methods

Microorganisms

The *Humicola* sp. was isolated from Mae Tao creek sediments from the zinc ore area in Mae Sot District, Tak Province, Thailand. The fungus was identified at the species level as follows. Fungal DNA was extracted with a NucleoSpin®Extract Kit (Macherey-Nagel, Germany). The complete internal transcribed spacer (ITS) region of rDNA was amplified with the primers ITS 1 to 4 and sequenced. The resulting 873-bp sequence was deposited in GenBank (GU988752.1). The sequence showed 99% similarity

Table 1. Linear isotherm models forms used in this study. C, the equilibrium concentration (L g^{-1}); B, a constant related heat of sorption (J mol^{-1}) by B = RT / bT; q, the amount of metal ions adsorbed (mg g^{-1}); bT, the Temkin isotherm constant; qm, the maximum monolayer coverage (mg g^{-1}); R, the universal gas constant (8.314 J mol^{-1} K^{-1}); KL, the Langmuir constant (L mg^{-1}); T, the temperature at 298 K; KF, the Freundlich constant related to the adsorption capacity; $\mathcal{E}$, RTln [1+ (1 / C)]; A, the Temkin isotherm equilibrium binding constant (L g^{-1})

Isotherm model	x-axis	y-axis	Linear form
Langmuir	1 / q	1 / C	$1/q = (1 / q_m K_L) (1 / C) + 1 / qm$
Freundlich	log q	log C	log q = 1 / n log C + log KF
Temkin	Q	lnC	q = BlnA + BlnC
Dubinin-Radushkevich	Lnq	$\mathcal{E}^2$	$lnq = ln\ q_m - k\ \mathcal{E}^2$

with sequences of *H. phialophoroides*. Fungal spores were obtained from a 5 days old culture grown on Potato Dextrose Agar (PDA) at 30 ± 2 °C. Colonies grown on PDA were white with abundant aerial hyphae. The colony colour was light yellow initially and later became light brown with irradiation. The spores were collected in 0.01% Tween-80 solution.

Reagents

Zinc nitrate hexahydrate [$Zn(NO_3)_2$ $6H_2O$] was used to prepare 1000 mg L^{-1} standard stock solution of Zn^{2+} ion solution. All chemicals used in this research were of analytical grade, and solutions were prepared using deionised water.

Zinc tolerance test experiment

H. phialophoroides dry biomass was weighed after 3 days incubation on a rotary shake flask at 30 ± 2 °C and 150 rpm in the PDB broth with zinc concentration 10, 25, 50 and 100 mg L^{-1}, as compared to that without zinc.

Biomass preparation

Biomass of *H. phialophoroides* was cultivated in Potato Dextrose Broth (PDB), using the shake flask method. Spore suspension (10^8 spores) were cultivated in a 250 mL Erlenmeyer flask with 50 ml PDB at 30 ± 2 °C with a shaker at a speed of 150 rpm for 3 days. The viable biomass was harvested by filtration and subjected to successive washings with deionised water. Then non-viable biomass was prepared by autoclaving the viable biomass at 121 °C for 20 min and then harvested by filtering through a membrane filter, it was then dried at 80 °C in an oven for 12 h. The pretreated biomass was suspended in 10% HNO_3 and 10% NaOH solutions for 30 min at 30 ± 2 °C. Subsequently, the biomasses were collected and washed with deionized water until the pH of the wash solution was in near neutral range (pH 7). The pretreated biomass was killed in an autoclave and then harvested by filtering through a membrane filter and then dried at 80 °C in an oven for 12 h. Finally, non-viable biomass and pretreated biomass were then ground, using a blender to break cell aggregates into smaller fragments. The biomass was then passed through 100 µm mesh sieves of to obtain particle sizes of less than 0.5 to 1.0 mm diameter.

Batch isotherm experiments

The equilibrium sorption of the Zn^{2+} ions onto biomass was carried out by contacting 0.1 g of the substrate with 50 mL of different concentrations from 0 to 150 mg L^{-1} for 120 min on the shaker at a speed of 150 rpm. The amount of metal bound by the biosorbent was calculated as:

$$q = Ci - Cf \times V \times W,$$

where q is the metal uptake (mg Zn g^{-1} dry mass), and Ci and Cf are the initial and final Zn^{2+} concentrations in the supernatant, respectively (mg L^{-1}), V is the volume of the zinc concentration (mL), and W is the dry mass of the biomass added (g). Linear forms of the isotherms models have been widely adopted to determine the isotherm parameters or the most fitted model for the adsorption system due to the mathematical simplicity. The sorption isotherms of Zn^{2+} were studied by fitting the obtained data to linear forms of the Langmuir (Langmuir 1916), Freundlich (Freundlich 1906), Temkin (Temkin, Pyzhev 1940) and Dubinin-Radushkevich (Dubinin, Radushkevich 1947) isotherm models (Table 1). The best fit model was selected based on the determination coefficient (R^2).

Effect of temperature, pH and contact time on Zn removal by fungus

In order to evaluate the effect of temperature, pH and contact time on the Zn^{2+} uptake, the experiment was conducted in the same manner, except the temperature of zinc solution was changed to 30, 40, 50, 60 and 70 °C. The pH of the solution was prepared to be in the range between 3.0 and 8.0 before mixing biomass. The pH was adjusted to

Table 2. Dry weight of mycelium of *H. phialophoroides* in PDB with and without Zn^{2+} after 3 days of incubation. Means followed by the same letter are not significantly different ($p < 0.05$)

Zinc concentration (mg L^{-1})	Mycelium dry mass of *Humicola phialophoroides*	
	(g)	(%)
0	0.85 ± 0.01 a	100.00
10	0.85 ± 0.02 a	99.73
25	0.87 ± 0.03 a	102.45
50	0.86± 0.02 a	100.84
100	0.71 ± 0.02 b	83.50

Table 3. Zn^{2+} uptake in biiomass of *H. phialophoroides*. Means followed by the same letter are not significantly different ($p < 0.05$)

Zinc concentration (mg L^{-1})	Zn^{2+} uptake (mg Zn g^{-1} DM)			
	Viable biomass	Non-viable biomass	HNO_3 pretreated biomass	NaOH pretreated biomass
0	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
1	0.25 ± 0.01b	0.23 ± 0.01b	0.27 ± 0.01b	0.31 ± 0.05b
5	1.21 ± 0.012c	1.17 ± 0.012c	1.30 ± 0.032c	1.51 ± 0.65d
10	1.69 ± 0.01d	1.68 ± 0.01d	1.90 ± 0.01e	2.12 ± 0.02f
25	2.17 ± 0.01f	2.14 ± 0.03f	2.42 ± 0.02g	2.74 ± 0.01h
50	5.12 ± 0.12j	5.13 ± 0.10j	4.81 ± 0.16i	6.42 ± 0.08no
100	5.77 ± 0.07m	5.34 ± 0.18k	5.49 ± 0.13kl	6.35 ± 0.33n
150	5.78 ± 0.12m	5.59 ± 0.18l	5.51 ± 0.11kl	6.53 ± 0.27p

the required value with 0.1 M NaOH or 0.1 M HNO_3. The period of contact time was studied up to 180 min by using the procedure described earlier, and samples were collected every 30 min.

Zn desorption experiments

The 0.1 M HNO_3 solution was used to elute Zn^{2+} from the biomass. Following the Zn^{2+} sorption experiments, the Zn-loaded biomass was prepared by centrifugation, washed and returned to 25 mL of the effluent 0.1 M HNO_3 for 30 min on a rotary shaker (125 rpm). Zinc concentration was determined after separating the biomass from eluting agent by filtration.

Atomic absorption analysis

The concentration of Zn^{2+} was measured using an atomic absorption spectrophotometer (Perkin Elmer model PinAAcle 900T) by the flameless method of graphite system.

Statistical analysis

All the experiments were run in triplicate. Mean values were used in analysis of data by using the analysis of variance (one-way ANOVA) and Post Hoc Duncan test ($p < 0.05$).

Results and discussion

Minimum inhibitory concentrations

The dry biomass was not significantly different between the control and the treatment at Zn^{2+} concentrations from 0 to 50 mg L^{-1} (Table 2). In the 100 mg Zn^{2+} treatment, mycelial dry weight of *H. phialophoroides* was significantly lover. This indicates that the more tolerant species have mechanisms and physiological adaptation to resist higher Zn^{2+} concentrations and to avoid its toxic effect response to the concentrations of the Zn^{2+} ions, which makes it an attractive potential candidate for further investigation regarding its ability to remove Zn^{2+} from contaminated water.

Table 4. Isotherm parameter models for Zn^{2+} adsorption onto different types of *H. phialophoroides* biomass

Isotherms	Viable biomass	Non-viable biomass	HNO_3 pretreated biomass	NaOH pretreated biomass
Langmuir				
q_m (mg Zn g^{-1} DM)	5.495	5.942	5.695	6.835
K_L (l mg^{-1})	0.529	0.548	0.475	0.351
R^2	0.9961	0.9962	0.9972	0.9964
Freundlich				
K_F (mg g^{-1}) (l $mg^{-1})^{1/n}$	0.401	0.379	0.450	0.508
N	1.691	1.672	1.785	1.742
R^2	0.9464	0.9388	0.9392	0.932
Temkin				
A (l g^{-1})	1.162	1.152	1.007	1.039
B	1.180	1.135	1.103	1.336
R^2	0.8836	0.8853	0.9207	0.8824
Dubinin-Radushkevich				
q_m (mg Zn g^{-1} DM)	3.187	3.106	3.257	3.843
k (mol² kJ^{-2})	0.0006	0.0006	0.0006	0.0006
R^2	0.7385	0.7538	0.7771	0.7618

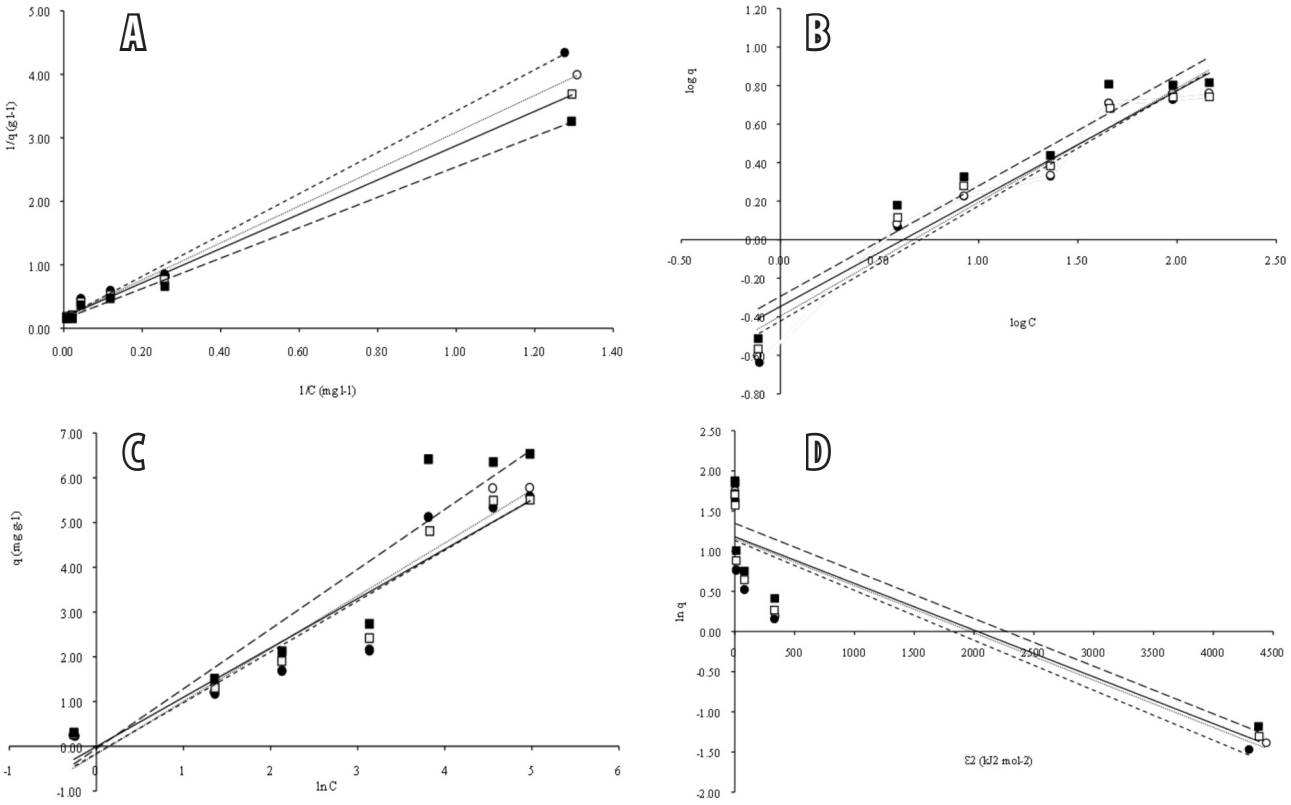

Fig. 1. Langmuir (A), Freundlich (B), Temkin (C) and Dubinin-Radushkevich (D) isotherms of Zn^{2+} removal by different types of *H. phialophoroides* biomass: viable biomass (❍), non-viable biomass (●), HNO3 pretreated biomass (❑) and NaOH pretreated biomass (■).

Zn^{2+} uptake by H. phialophoroides *biomass*

The results related to Zn^{2+} biosorption by viable, non-viable and two types of pretreated biomasses are presented in Table 3. The NaOH pretreated *H. phialophoroides* had the highest biosorbent activity for Zn^{2+} among the biomasses studied. An increase in biosorption of Zn^{2+} as a result of pretreatment by NaOH could be due to exposure of active metal binding sites embedded in the cell wall or chemical modifications of the cell wall components (Javaid et al. 2011).

The graphs of all adsorption isotherm models are shown in Fig. 1; the correlation coefficients and the intercept values are shown in Table 4, the highest R^2 was obtained by fitting experimental data into the Langmuir isotherm model, as compared with the Freundlich, Temkin and Dubinin-Radushkevic isotherm models. The Langmuir isotherm model assumes monolayer adsorption in which adsorbates are adsorbed to a finite number of definite localised sites that are identical and equivalent with no lateral interaction. This isotherm model reflects the homogeneous adsorption mechanism where each molecule owns its enthalpy and activation energy at the same time. This model does not permit transmigration of the adsorbate in the plane of the surface (Perez Marín et al. 2007). The values of maximum monolayer adsorption capacity of *H. phialophoroides* biomass were 5.495, 5.942, 5.695 and 6.835 mg Zn g^{-1} dry wt. for viable biomass, non-viable biomass, HNO_3 treated biomass and NaOH treated biomass, respectively. These values are higher than for many fungal biomasses such as, *Aspergillus niger* (Vale et al. 2016), *Penicillium chrysogenum* (Tan, Cheng 2003), but lower than for *Rhizopus arrhizus* (Kapoor, Viraraghavan 1995) and *Streptoverticillium cinnamoneum* (Puranik, Paknikar 1997).

Table 5. Desorption of Zn^{2+} from different types of biomass of *H. phialophoroides*

Biomass	Zinc uptake (mg Zn g^{-1} DM		Removal efficiency (%)
	Before desorption	After desorption	
Viable biomass	5.29 ± 0.08	4.68 ± 0.63	88.47
Non-viable biomass	5.15 ± 0.59	4.84 ± 0.34	93.98
HNO_3 pretreated biomass	4.86 ± 0.07	4.49 ± 0.66	92.39
NaOH pretreated biomass	6.63 ± 0.65	6.12 ± 0.34	92.31

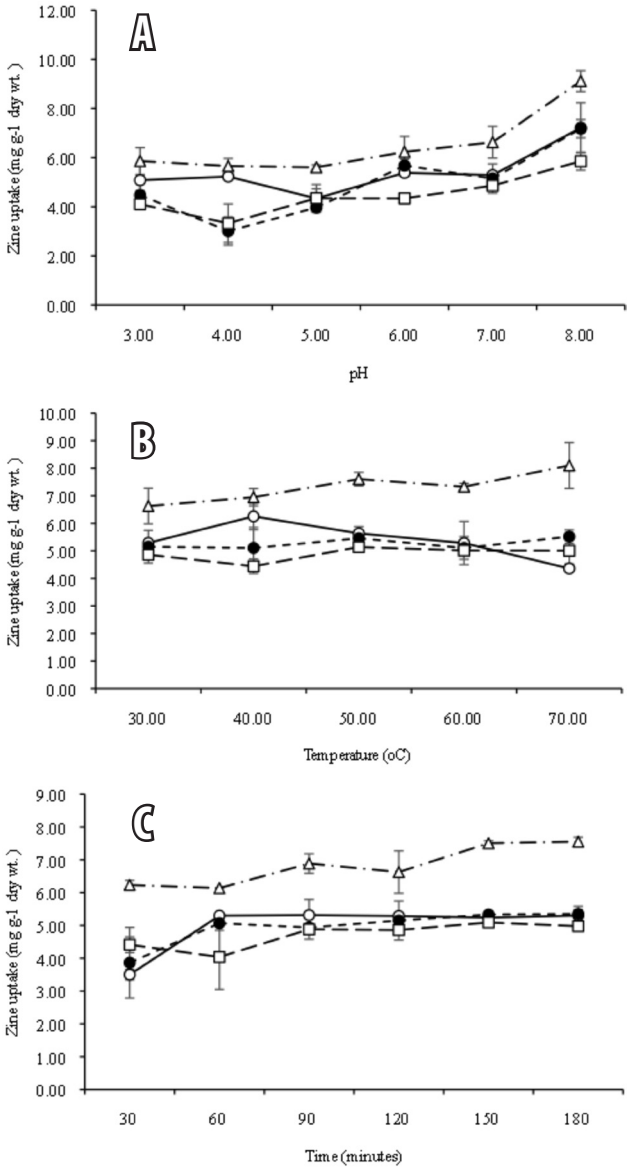

Fig. 2. Effect of pH (A), temperature (B) and contact time (C) on Zn^{2+} removal by *H. phialophoroides* biomass: viable biomass (❍), non-viable biomass (●), HNO_3 pretreated biomass (❑) and NaOH pretreated biomass (Δ).

Effect of pH on biosorption

The effect of pH on Zn uptake was measured in the pH range from 3 to 8 (Fig. 2A). The graph shows that adsorption was low at pH 3 to 6, while the Zn^{2+} was well adsorbed in the high pH range from 7 to 8 ($p < 0.05$). The NaOH treated biomass showed highest detoxification of Zn^{2+} at pH 8, with a value of 9.11 ± 0.04 mg g^{-1} dry mass.

Many studies have shown that pH is an important factor affecting biosorption of Zn^{2+} (Vale et al. 2016; Vaishnav et al. 2012; Javaid et al. 2011). The solution pH influences the adsorbent surface charge. Zinc ion adsorption is affected by the initial solution pH and the change in pH influences the adsorption process. The biosorption capacity of biomass is low at acid conditions, because large quantities of proton compete with the metal cations for the adsorption sites. As the pH of the metal solution increases, the number of protons dissociated from functional groups of carboxylate on the cell wall increases and thus more negative groups for complexation of metal cations are provided (Kapoor et al. 1999).

Effect of temperature on biosorption

The effect of temperature on Zn^{2+} removal from aqueous solution by *H. phialophoroides* biomass was measured in the temperature range between 30 to 70 °C at pH 7. The Zn^{2+} removal in viable biomass was lower at 50 °C (Fig. 2B), while the Zn^{2+} biosorption by non-viable biomass and two pretreated types of biomass were not significantly affected in the temperature range of 30 to 70 °C. The highest value of Zn^{2+} removal (8.10 ± 0.83 mg g^{-1} dry mass) occurred at 80 °C in NaOH treated biomass. Previous studies related to metal sorption by fungi indicated that the effect of temperature on biosorption depended on the metal biosorbent systems (Marandi et al. 2010; Netpae et al. 2014).

Effect of contact time on biosorption

The uptake of Zn^{2+} by the *H. phialophoroides* biomasses was examined at different time intervals and the results are shown in Fig. 2C. Studies have shown that biosorption efficiency of Zn^{2+} increases with increased contact time. Viable biomass, non-viable biomass and HNO_3 treated biomass could also remove Zn^{2+} in solution and reached the equilibrium within 90 min, while the rate of Zn^{2+} adsorption by NaOH treated biomass was increased with contact time up to 150 min and then remained stable until 180 min. The initial faster uptake might be due to the availability of abundant metal species and empty metal binding sites of microbes. Slower phase might be due to saturation of metal binding site (Mathivanan, Rajaram 2014).

Desorption

Desorption of biosorbed Zn^{2+} was achieved by elution with 0.1 M HNO_3 (Table 5). The Zn^{2+} removal was decreased by about 88.47, 93.98, 92.39, and 92.31% for viable biomass, non-viable biomass, HNO_3 treated biomass and NaOH treated biomass, respectively. The decrease in Zn^{2+} uptake by 0.1 M HNO_3 desorbent might be due to the increase of the concentrations of competing H_3O^+. It is also possible that the physical structure of the biomass becomes damaged by HNO_3 (Sun et al. 2010).

Conclusions

This study demonstrated that *H. phialophoroides* possesses good capacity for Zn^{2+} biosorption from aqueous solution and industrial wastewater. The adsorption equilibrium of Zn^{2+} sorption at low concentration can be described by the Langmuir isotherm model. The values of maximum monolayer adsorption capacity of *H. phialophoroides*

biomasses were 5.495, 5.942, 5.695 and 6.835 mg Zn g^{-1} dry mass for viable biomass, non-viable biomass, HNO_3 treated biomass and NaOH treated biomass, respectively. Maximum Zn^{2+} biosorption of biomass pretreated with NaOH occurred at pH more than 8 after 150 min. Moreover, the Zn^{2+} was well adsorped by biomass at high temperature (70 °C). Desorption experiments indicated that the desorption efficiency with 0.1 M HNO_3 solution reached 92.31%.

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