

Cd²⁺ Biosorption by Pretreatment Biomass of Highly Cadmium Resistant Fungus *Humicola* sp.

Tinnapan Netpae*

Faculty of Science and Technology, Nakhon Sawan Rajabhat University, Thailand.

* Corresponding author. Tel: +66 (0)5621 9100; Fax: +66 (0)5622 1237; E-mail: tinnapan_net@yahoo.com

Abstract

This research was carried out to estimate the removal of Cd²⁺ from an aqueous solution by pretreatment of *Humicola* sp. biomass with acid (H₂SO₄) and base (NaOH). The influence of different treatments of dried *Humicola* sp. biomass on its Cd²⁺ biosorption activity, indicated that 10% NaOH was increased biosorption of Cd²⁺ in comparison with the untreated and acid treated biomasses. Maximum Cd²⁺ biosorption of pretreated biomass by base took place at initial solution at pH 6 and 7 after 150 minutes, while the maximum adsorption of Cd²⁺ in pretreated biomass by acid was obtained highest at pH 6 after 60 minutes. Cadmium ions removal increase within the temperature has raised more than 40 °C. Desorption experiments indicate that the desorption efficiency with 0.1 M HNO₃ solution reaches 96.35% and 83.32% in acid and base treatment, respectively.

Keywords: *Humicola* sp.; pretreatment; biosorption; cadmium.

1. Introduction

Cadmium (Cd) is extremely toxic, the problem of Cd contamination occurs when aqueous effluents from many industrial processes that contain dissolved heavy metals without treatment are disposed. They may have an adverse impact on the environment. Various types of microorganism including bacteria, algae, yeast and fungi are capable of adsorbing Cd from solution due to metal attracting compositions or natively charged functional groups on their cell wall which is mainly binding properties of such microorganism. *Humicola* sp. fungus is able to grow in the sediments of highly contaminate with Cd [1]. Recent studies have identified the potential for Cd²⁺ biosorption by *Humicola* sp. of viable and non-viable biomass pretreated by heating while Cd concentration were increased in the same pattern, but viable biomass showed better Cd²⁺ removal ability than non-viable biomass [2]. Although living cell showed better Cd²⁺ removal ability than dead cell, but the use of living cells have many disadvantages as they are more sensitive to Cd concentration, toxicity effect and adverse conditions

such as pH and temperature. Moreover, they require nutrient supply and culture maintenance, which cause of increasing of operating cost. According to many workers, the chemical pretreatment methods may provide better results in the list of number of pretreatment protocols owing to change in cell wall chemistry of the biosorbent [3-5].

In this research, adsorption ability non-viable biomass of *Humicola* sp. pretreated by acid (HNO₃) and base (NaOH) were investigated for removal of Cd from aqueous solution under experimental conditions.

2. Materials and Methods

Microorganism

Humicola sp. was isolated from sediment in Mae Tao creek in Mae Sot District, Tak Province, Thailand. Fungal spores were obtained from a 5 day old culture grown on Potato Dextrose Agar (PDA) at 30±2°C. The spores were collected in 0.01 % tween-80 solution.

Biomass preparation

Humicola sp. biomass was cultivated in Potato Dextrose Broth (PDB), using the shake flask method. Spore suspensions (1x10⁸ spores) were cultivated in 250 ml erlenmeyer flask with 50 ml PDB. Once inoculated, flasks were shaken on a rotary shaker at a speed of 150 rpm for 3 days at 30±2 °C. Harvested biomasses (20 g wet wt.) were pretreated with NaOH and HNO₃ by suspending the biomass in 50 ml of 10% NaOH and HNO₃ for 30 minutes at 30±2 °C. The biomass were collected and washed many times with distilled water until the pH of supernatant became 7. Finally, pretreated biomass was dead in an autoclaved at 121°C for 20 minutes and then harvested by filtering through a membrane filter and dried at 80 °C in an oven for 12 hours. This was then ground, using a blender to break cell aggregates into smaller fragments. The biomass was then passed through 100 μm mesh sieve of to obtain particle sizes of less than 0.5 - 1.0 mm diameter. The pretreatment biomass was preserved in airtight jar to be used in the Cd²⁺ uptake studies.

Batch isotherm experiments

The isotherm experiments were studied using 0.1 g of cell dried. Biosorbent was suspended in 50 ml of metal solution and initial Cd concentrations were varied over the range 0 to 150 mg l⁻¹. The working solutions adjusted to pH 5 were shaken at 150 rpm for 2 hours at 30±1 °C. Samples were taken at time intervals, centrifuged and then analyzed for residual metal concentration.

The adsorption capacity, q , is calculated from the difference between initial and equilibrium concentrations as shown in equation:

$$q = \frac{(C_i - C_f)V}{W}$$

where q is the Cd uptake (mg g⁻¹ dry wt.), C_i and C_f are the initial and equilibrium concentration of metal solution (mg l⁻¹), W is the adsorbent dosage (g), and V is the volume of the Cd-bearing solution contacted with sorbent (ml). Several adsorption isotherms originally used for gas phase adsorption are available and rapidly adopted to correlated adsorption equilibrium in heavy metal adsorption. The most widely use among them is Langmuir equation. The resulting values of C_f/q were plotted against C_i to obtain a Langmuir plot typical of the sorption behavior [6].

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

In order to evaluate the effect of temperature, pH and contact time on the Cd²⁺ uptake, the experiment was conducted in the same manner, except the temperature of Cadmium 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 9.0 before mixing biomass. The pH was adjusted to the required value with 0.1M NaOH or 0.1M HNO₃. The period of contact time was studied up to 180 min by using the procedure described earlier, samples were collected every 30 min (30, 60, 90, 120, and 180 min, respectively).

Cd desorption experiments

The 0.1M HNO₃ solution was used to elute Cd²⁺ from both biomass. Following the Cd sorption experiments, the Cd-loaded biomass was prepared by centrifugation, washed and returned to 25 ml of the effluent 0.1 M HNO₃ for 30 min on a rotary shaker (125 rpm). Metal concentrations were determined after separating the biomass from eluting agent by filtration.

Atomic absorption analysis

Cd remaining in solutions was analyzed by using an atomic absorption spectrophotometer (Variance spectra model AA-220 FS) with and oxidizing flame of an air acetylene mixture at a ratio of air flow at 13.50 l min⁻¹ and acetylene flow 2 l min⁻¹.

Statistical analysis

All the experiments were triplicate. Mean values were used in the analysis of data by using the

analysis of variance (one - way ANOVA) and Post Hoc. Duncan test ($p < 0.05$).

3. Results and Discussion

Uptake Mechanism of Cd by biomass pretreated with acid and base

Pretreatment of *Humicola* sp. biomass with H₂SO₄ and NaOH were proved to increase or maintain adsorption efficiency and capacity in comparison to untreated biomass. At Cd concentration of 100 mg l⁻¹, acid and base pretreatment biomass removed Cd of 62.53±1.54 mg Cd g⁻¹ dry wt. and 71.33±2.18 mg Cd g⁻¹ dry wt., respectively. The Cd²⁺ uptake by pretreated biomass was showed in the Table 1. This value is better than untreated of biomass because the maximum removal extent of viable biomass of *Humicola* sp. is 61.77±3.25 mg Cd g⁻¹ dry wt., under identical conditions [2].

Table 1. Cadmium uptake on acid and base pretreated biomass of *Humicola* sp.

| Cd concentration (mg l ⁻¹) | Cadmium uptake (mg Cd g ⁻¹ dry wt.) | |
|--|--|-------------------------|
| | HNO ₃ treated biomass | NaOH treated biomass |
| 0 | 0.00±0.00 ^a | 0.00±0.00 ^a |
| 1 | 0.92±0.01 ^a | 0.92±0.10 ^a |
| 5 | 5.52±0.20 ^b | 6.57±0.30 ^b |
| 10 | 11.61±0.18 ^{de} | 13.33±0.81 ^e |
| 25 | 26.83±0.85 ^g | 33.55±2.03 ^h |
| 50 | 43.63±1.34 ^j | 56.90±2.72 ^l |
| 100 | 62.53±1.54 ^{no} | 71.33±2.18 ^p |
| 150 | 63.87±3.40 ^o | 69.50±1.67 ^p |

Note: For a Cd uptake, mean concentrations followed by the same letter are not significantly different ($p < 0.05$).

The adsorption performance of the Cd²⁺ biosorbent can be described by mathematical model in term of Langmuir isotherm. These isotherms follow the typical Langmuir adsorption pattern as shown by the linear transformation (Figure 1). The linearised form of Langmuir equation is represented by the following expression:

$$\frac{C_{eq}}{q} = \frac{C_{eq}}{q_{max}} + \frac{1}{q_{max} b}$$

where C_{eq} is the the equilibrium solution concentration (mg l⁻¹), q_{max} is the amount adsorbed at equilibrium (mg g⁻¹), the Langmuir constants q_{max} and b are related to adsorption capacity and energy of adsorption, respectively [7]. The linear plot between C_{eq}/q with C_{eq} shows that investigated metal ions adsorption by pretreated biomass. As compared in Table 2, the base pretreated biomass has a greater capacity (q_{max}) than acid pretreated biomass and untreated biomass for Cd adsorption. Conversely, the untreated *Humicola* sp. biomass has higher binding constant than the pretreated biomass.

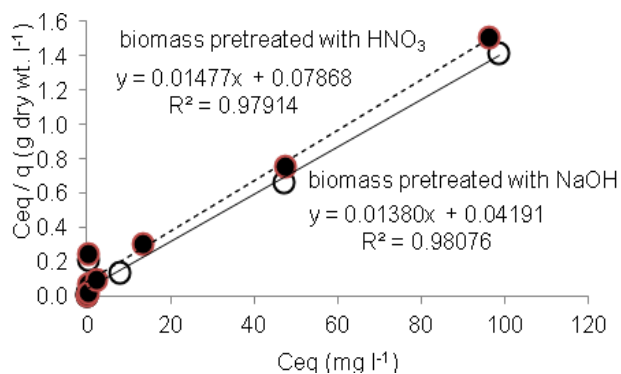


Figure 1. Langmuir adsorption isotherm of Cd removal by *Humicola sp.*, HNO₃ treated biomass (●, ---) and NaOH treated biomass (○, —).

Table 2. Comparison of the Langmuir constants for Cd adsorption by *Humicola sp.* biomass.

| | qmax (mg Cd g ⁻¹ dry wt.) | b (mg l ⁻¹) |
|----------------------------------|---|----------------------------|
| HNO ₃ treated biomass | 67.70 | 5.33 |
| NaOH treated biomass | 72.46 | 3.04 |
| Untreated biomass* | 67.61 | 9.78 |

* Reference: Netpae, Suckley and Phalaraksh, 2014.

Effect of temperature, pH and contact time on Cd removal

Temperature and pH were the most important factor influencing the biosorption process. Under the same condition, the base pretreatment biomass was high accumulation than biomass pretreated by acid. The binding of Cd²⁺ to pretreated biomasses were enhanced when temperature increases. From the results, Cd²⁺ removal increase rapidly within the temperature has raised more than 40 °C in base pretreated biomass while the biosorption by acid pretreated biomass increased slowly during the temperature increase (Figure 2A). Higher temperatures usually enhance sorption as a result of the increased surface activity and kinetic energy of the solute [11]. However, physical damage to the biosorbent can be expected at higher temperatures [12].

Biosorption of pretreatment of *Humicola sp.* biomass with H₂SO₄ and NaOH have the similar trend that the pH of the metal solution increase from 3 to 9. The accumulation level was found the optimum pH 6 to 7. Accumulations at extreme pH 2-4 were rare (Figure 2B). Other studies showed that increasing of heavy metal biosorption capacity of pretreated fungi occurred as pH increased [13] [14]. Optimum contact time for Cd adsorption by Alkaline biomass treatment by NaOH was 150 minutes, while the rate of biosorption by biomass pretreated with acid was faster and contributed significantly (p < 0.05) to equilibrium uptake 99.14 % recovery being achieved within 60 minutes (Figure 2C).

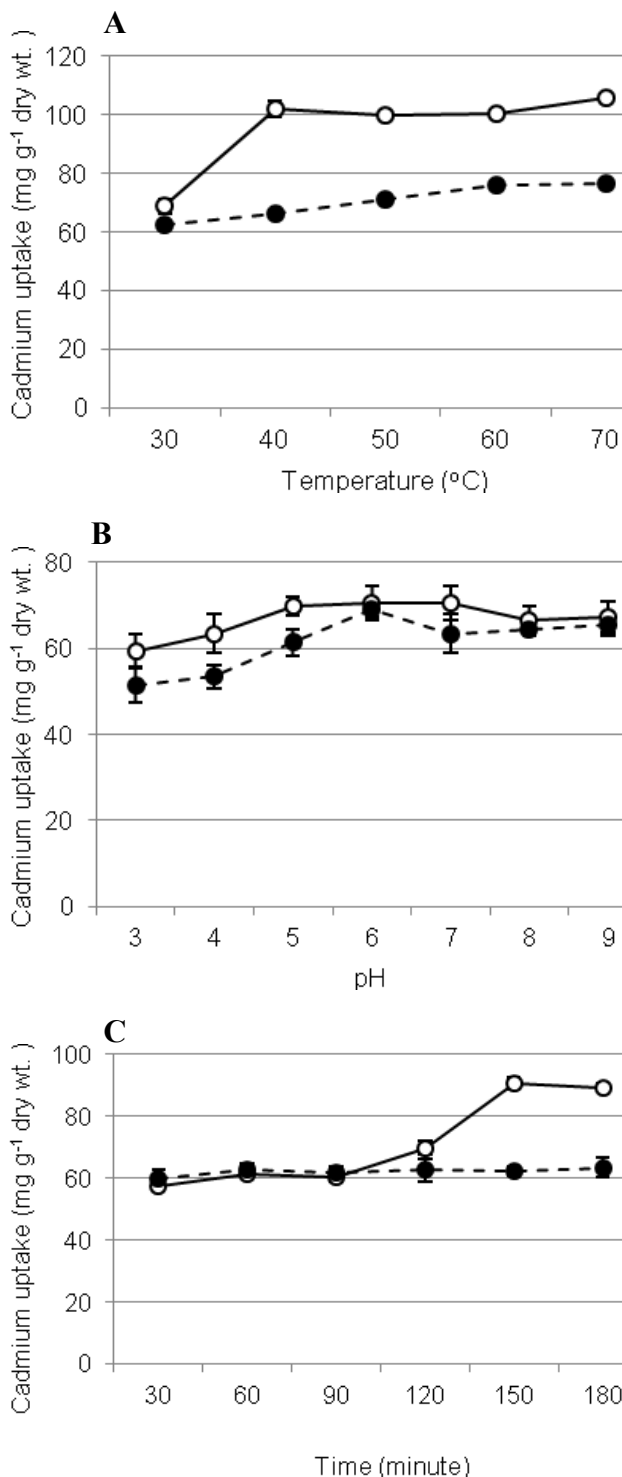


Figure 2. Effect of temperature (a), pH (b) and contact time (c) on Cd²⁺ removal by *Humicola sp.* by -●- is HNO₃ treated biomass and —○— is NaOH treated biomass.

Cadmium desorption

0.1M nitric acid solution was able to effectively elute the biosorbed Cd²⁺ (Table 3). The Removal efficiency of Cd²⁺ was decreased by about 96.35% and 83.32% in acid and base treatment respectively. The decrease in Cd²⁺ uptake by acid desorbent might be due to the increase of the concentrations of competing hydronium ions. It is also possible that

the physical structure of the biomass becomes damaged by this acid [15].

Table 3. Desorption of Cd on treated biomasses of *Humicola* sp. used with 0.1 M HNO₃.

| pretreated biomasses | Cadmium uptake (mg Cd g ⁻¹ dry wt.) | | removal efficiency (%) |
|----------------------|--|------------------|------------------------|
| | before desorption | after desorption | |
| HNO ₃ | 62.15±1.47 | 59.88±6.01 | 96.35 |
| NaOH | 90.55±2.02 | 75.45±7.22 | 83.32 |
| Untreated biomass* | 61.77±2.57 | 48.77±8.35 | 78.95 |

* Netpae, Suckley and Phalaraksh, 2014

4. Conclusions

According to the results of this research, it is obvious that the pretreatment biomass of *Humicola* sp. by acid (HNO₃) and base (NaOH) are able to remove Cd²⁺ from aqueous solution. The base treated biomass of *Humicola* sp. can give rise the higher Cd²⁺ removal efficiency in solution than untreated and acid treated biomasses. Maximum Cd²⁺ biosorption of pretreated biomass by base took place at initial solution at pH 6 and 7 after 150 minutes. The use of nitric acid as eluting agent is effective for desorbing of Cd²⁺ loaded biomass.

Acknowledgments

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References

[1] Netpae T., Suckley S., Phalaraksh C. (2014) Biosorption of Cd²⁺ from Aqueous Solutions by Tolerant Fungus *Humicola* sp. *Advances in Environmental Biology*, **8**(21): 308-312.

[2] Netpae T., Suckley S., Phalaraksh C. (2015) Cadmium Tolerance Fungi Isolated from Polluted Sites in the

Mae Tao creek, Thailand. *Advanced Studies in Biology*, **7**(1): 29-37.

[3] Das N., Charumathi D., Vimala R. (2007) Effect of pretreatment on Cd²⁺ biosorption by mycelia biomass of *Pleurotus florida*. *African Journal of Biotechnology*, **6**(22): 2555-2558.

[4] Wang J., Chen C. (2009) Biosorbents for heavy metals removal and their future. *Biotechnology Advances*, **27**: 195-226.

[5] Javaid A., Bajwa R., Manzoor T. (2011) Biosorption of heavy metals by pretreated biomass of *Aspergillus niger*. *Pakistan Journal of Botany*, **43**(1): 419-425.

[6] Langmuir I. (1916) The constitution and fundamental properties of solids and liquids, Part. I: Solids. *Journal of the American Chemical Society*, **38**: 2221.

[7] Ahalya N., Kanamadi R.D., Ramachandra, T.V. (2006) Biosorption of Iron (III) from aqueous solution using the husk of *Cicer arietinum*. *Indian Journal of Chemistry Technology*, **13**: 122 -127.

[11] Mameri N., Boudries N., Addour L., Belhocine D., Lounici H., Grib H., Pauss A. (1999) Batch zinc biosorption by a bacterial nonliving *Streptomyces rimosus* biomass. *Water Research*, **33**: 1347-1354.

[12] Dhankhar R., Hooda A. (2011) Fungal biosorption – an alternative to meet the challenges of heavy metal pollution in aqueous solutions. *Environmental Technology*, **32**(5): 467-491.

[13] Rao P.R., Bhargavi C. (2013) Studies on biosorption of heavy metals using pretreated biomass of fungal species. *International Journal of Chemistry and Chemical Engineering*, **3**(3): 171-180.

[14] Joshi J., Sahu O. (2014). Adsorption of Heavy Metals by Biomass. *Journal of Applied & Environmental Microbiology*, **2** (1): 23-27.

[15] Pimpa W., Netpae T., (2004) Use of pelleted biomass of *Aspergillus oryzae* for lead removal. *Thai Environmental Engineering Journal*, **18**(1): 21-28.