BIOSORPTION OF LEAD (II) AND CADMIUM (II) FROM AQUEOUS SOLUTION USINGRAW AND PRETREATED ASPERGILLUS NIGER.

Joycyl P. Albesa II, Alexander Allen S. Alfaras, Aira May G. De Rosas, Shaira B. Sta. Catalina & Engr. Patlawrenze Joseph G. Angeles

Department of Chemical Engineering, College of Engineering, Adamson University, Ermita, Manila, Philippines

Abstract

Heavy metals present in wastewater are found to be detrimental not only to the environment but also to the health of human beings. The biosorption of lead (II) and cadmium (II) from aqueous solution by raw and NaOH--pretreated fungal biomass of Aspergillus niger was investigated. Parameters affecting biosorption such as pH, agitation rate, and metal ions were examined. It was found that the initial pH of the solution strongly affected the degree of biosorption. Metal solutions with pH of 5 and 4 yield the highest percentage biosorption of Aspergillus niger for mono--metal solutions and bi--metal solutions, respectively. The agitation rate of 400 rpm and 150 rpm yield the highest percentage removal on single metal ions and combined metal ions, respectively. Moreover, the lead and cadmium adsorption by pretreated Aspergillus niger was higher than that of raw Aspergillus niger in all cases. The kinetics of heavy metal biosorption matched second order reaction model. The biosorption isotherms indicated that the biosorption of Cd (II) and Pb (II) can be fitted with the Langmuir equation indicating that the biosorption mechanism of Cd (II) and Pb (II) by Aspergillus niger was chemisorption.

Keywords: Aspergillus niger;; Biosorption;; Pretreatment

1.0 INTRODUCTION

Due to the increasing demand for fresh water, numerous methods were developed to treat wastewater and proven to be effective, but most of these technologies are costly and difficult to perform. Recently, great attention has been given to biosorption in treating wastewater containing heavy metals because it is simple and cost effective.

For heavy metal removal, one of the most promising biosorbent is the fungus Aspergillus niger (A. niger). Most biosorbents, including A. niger, needs modification to enhance its biosorption by activating its binding sites and/or remove unwanted substances in its cell wall. Finding new methods in improving its biosorption capacity must be given attention.

The primary objective of the study is to evaluate the adsorbing capacity of raw and alkali pre--treated A. niger in simulated wastewaters containing metal ions (Pb2+ and Cd2+). The specific objectives include the determination and comparison of the effects of pH of the metal solutions, pretreatment of biomass, agitation rate, type of metals in the solution, and determining the isotherm model and kinetic model most fitted for the adsorption process.

2.0 LITERATURE REVIEW

Toxicity of Heavy metals

Heavy metals including chromium, arsenic, cadmium, zinc, mercury, nickel and etc., are noxious metals that can result to severe health effects as seen in Table 1 (Gakwisiri et al., 2012).

Table 1: The Maximum Contamination Limit (MCL) for the most toxic heavy metal standards

Heavy Metal	Toxicities	MCL (mgL^{-1})
Arsenic	Circulatory system disorder, cancer in liver, lung heart etc., skin diseases	50
Cadmium	nephritis, kidney failure, cancers	10
Chromium	Bowel disorder, vomiting, carcinogenic, nausea	50
Copper	Insomnia, liver impairment	250
Nickel	Eczema, cough, chronic asthma	200
Zinc	Neurological disorders, weariness	800
Lead	Kidney damage, fetal brain impairments, disorder in nervous system, and circulatory system	6
Mercury	Kidney failure, disorder in nervous system and circulatory system, rheumatism	0.03

Biosorption

The term "biosorption" focuses mainly to the subject substances to be sorbed. Usually, the term referred specifically to micro material as biosorbents and metals as biosorbates. Most researches about biosorption still give attention on metals and other related elements (Gadd, 2009). On the other hand, biosorption have been also applied to removal of organics such as dyes, to enrichment with biological fertilizers, to the steroid's recovery, proteins that are high--valued and drugs and pharmaceuticals (Kaushik & Malik, 2009;; Michalak, Chojnacka, & Witek--Krowiak, 2013;; Bohumil Volesky, 2007). Consequently, biosorption can evidently describe any system where the biological material interacts with the substance to be sorbed, in which results to sorbate concentration reduction in the solution (Gadd, 2009).

Biosorption binds metal ions (mostly cations) by negatively charge compounds present in the cell membranes. Mechanisms involve in biosorption is necessary to understand prior to achieve optimal process in heavy metal removal. However, due to the complexity of biosorbents, the occurrence of various mechanisms at different rates is possible. The mechanisms involve ion exchange, physical adsorption and complexation. (Babák, Šupinová, Zichová, Burdychová, & Vítová, 2012;; Kanamadi, 2003).

- Ion exchange – is the exchanging ions for the same charge ions occurring on solids containing significant functional groups.
- Complexation – heavy metal ions are limited to the functional groups existing in cell membranes
- Physical adsorption – intermolecular interaction (Van der Waals forces) triggers physisorption and chemical binding does not occur.

Biosorption major advantage is that the metabolic--independent cells bound the heavy metals. This permits the dead organisms to remove the contaminants and makes the process cost--effective and simpler because living biomass demands additional energy and nutrients supply (Babák et al., 2012;; Kanamadi, 2003).

Cell membranes take a significant role in biosorption process. All metal ions pass through a cell wall before going to the cytoplasm and cell membrane. The structure of cell wall includes different proteins and polysaccharides, and thus many active spots are present for binding of metal ions. Also, the diverse compositions and surface of microbial cell have an important effect on different number of adsorbed metal ions. The microbial cell surface is composed of very large molecules with highly charged functional groups including phosphate, carboxylic, hydroxyl, and amino (Taricska et al., 2006).

For most cell surface, its charge is negative due to lack of phosphate and carboxylic residues which results to a passive binding of cations on the surface of the cell (Chojnacka, 2010). In the solution, metal ions with positively charge are attached to the anionic surface of the cell. The whole process takes place with no cell metabolism engage and said to be passive. The passive process of biosorption makes the metal rejection rates faster than those processes that are metabolic--dependent. This biosorption property would cause it the most efficient in treating large volume of water carrying metal ions at low concentrations, including the final treatment process for aqueous streams in order to meet the regulated standards of metal concentrations prior to its discharge to the environment (Southichak, Nakano, Nomura, Chiba, &

Nishimura, 2006). Biosorption treatment can be performed not only in small experiments, but also in larger scales application. It was observed that the most effective configuration for biosorption process is a packed bed column reactor (B. Volesky, 2001).

3.0 MATERIALS AND METHODOLOGY

Materials

Aspergillus niger were procured at and cultured by Adamson University Technology Research and Development Center (AUTRDC). This fungus was used as the adsorbent in the experiment. Most of the glass wares, specifically Erlenmeyer flasks, petri dishes, and vials, were bought at Patagonian Enterprises. Other apparatus used were borrowed from Adamson University Chemical Engineering Laboratory. Equipment used such as analytical balance, LAQUA pH meter, Gyrotory® water bath shaker model G 76, and convection oven were also from the same laboratory. The Fourier transform infrared spectroscopy, FTIR (PerkinElmer), and autoclave used were from Adamson University Chemistry Laboratory. Atomic absorption spectroscopy, AAS (Shimadzu), from De La Salle University, Manila was used.

Reagents

The reagents, NaOH, HCl, PbCl2, and CdSO4, in pellets were bought at Patagonian Enterprises located at 1612 Remigio St, Sta. Cruz 121, Manila, 1003 Metro Manila. In this study, all reagents used were analytical grade.

3.1 METHODOLOGY

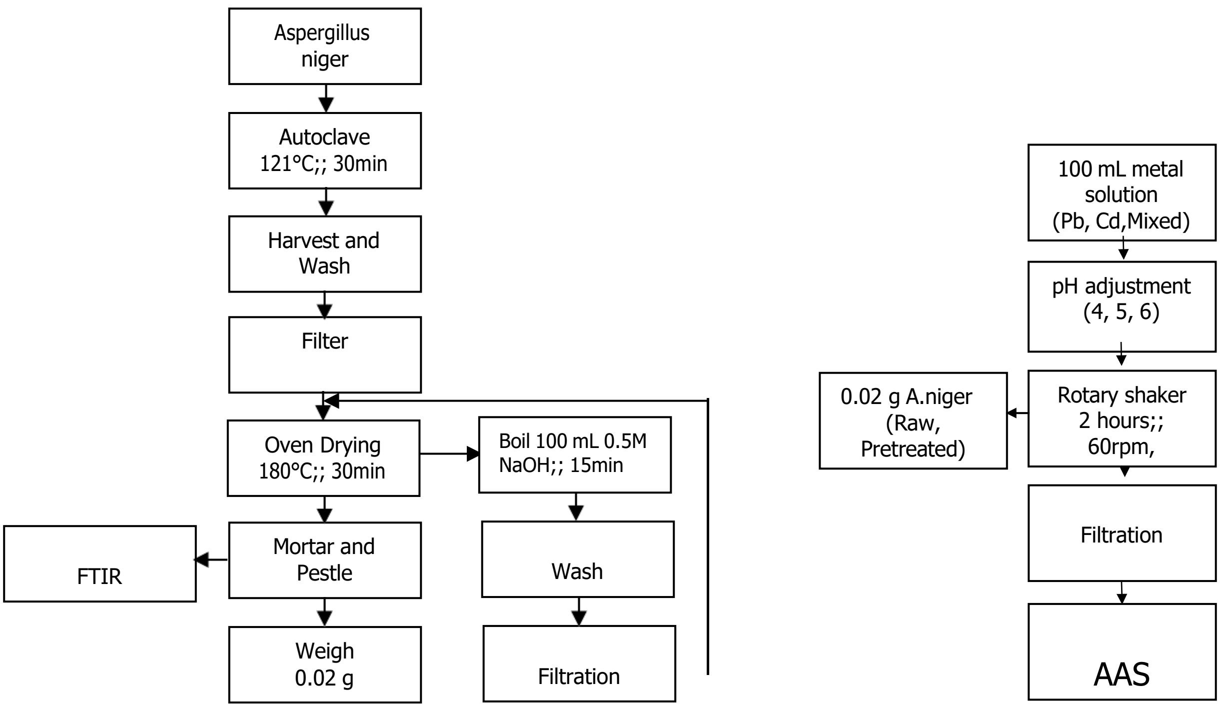

Figure 1: Methodology

Preparation of Biomass

Batches of procured A.niger were subjected to sterilization by autoclave at 121°C for 30 minutes. Resulting A. niger were harvested, washed several times, filtered using

Whatman 42, and oven dried at 181°C for 30 minutes. The A. niger was separated into two parts. One was directly grounded using mortar and pestle, the other was first boiled in 100mL 0.5M NaOH for 15 minutes before being grounded.

Biosorption Experiment
Metal solutions of $PbCl_2$, $CdSO_4$, and its mixture were diluted to 20 mg/L from the stock solutions. Each metal solution sample of 100 mL was placed in a 250--mL Erlenmeyer flask. The metal solutions were adjusted to pH 4, 5, and 6 by putting infinitesimal amounts of 0.1M HCl and NaOH. The Erlenmeyer flasks were placed in the rotary shaker.

Biomass weighing 0.02 g each was used. Biosorption started when each of the biomass was placed in the Erlenmeyer flask. The biosorption experiments were done in a rotary shaker at different speeds, 2 (60 rpm), 5 (150 rpm), 8 (400 rpm) and at constant temperature. The biosorption lasted for 2 hours. At the end of the biosorption, each sample was filtered using Whatman42 filter paper and the filtrates were placed in the vials for testing.

Kinetic Experiments
Metal solutions with concentrations 20 mg/L, 40 mg/L, and 60 mg/L were used. The pH and agitation rate that yielded highest metal uptakes was chosen and biomass weighing 0.02 g each were used in this part of experiment. The samples with the biomass were placed in a rotary shaker for 5 hours. Equal amounts of aliquots were withdrawn in the sample throughout the duration of the biosorption.

4.0 ANALYSIS

Samples of raw and pretreated fungus were analyzed by Fourier Transform Infrared Spectroscopy, FTIR (PerkinElmer), in order to determine the alteration in functional groups due to pretreatment. Atomic absorption spectroscopy, AAS (Shimadzu), was used to analyze the metal concentration of the solution after the biosorption process.

Statistical Treatment
Each set of samples were analyzed in triplicates. In order to determine the effects of the different parameters (e.g. pH, agitation rates, metal solutions) to the adsorption capacity of A. niger, one--way ANOVA was done. On the other hand, Independent T--test was used to compare the adsorption capacity of raw and alkali pretreated biomass.

5.0 FINDINGS

Effect of pH, Pretreament of Biomass, Agitation Rate, and Metal Solution on the Adsorption of Pb^{+2} and Cd^{+2}

Effect of pH
As stated by several studies, the efficiency of the adsorbent to remove the heavy metal in the solution is heavily dependent on the pH which can interact with another variable such as the agitation magnitude (Bai & Abraham, 2003;; Li et al., 2015;; Rostami &

Joodaki, 2002;; Sepehr et al., 2012). The figures below show the percentage removal at pH 4, 5 and 6 for Pb^{2+} and Cd^{2+} at 400 rpm and combined metal solution (Pb^{2+} and Cd^{2+}) at 150 rpm with 20mg/L as initial concentration.

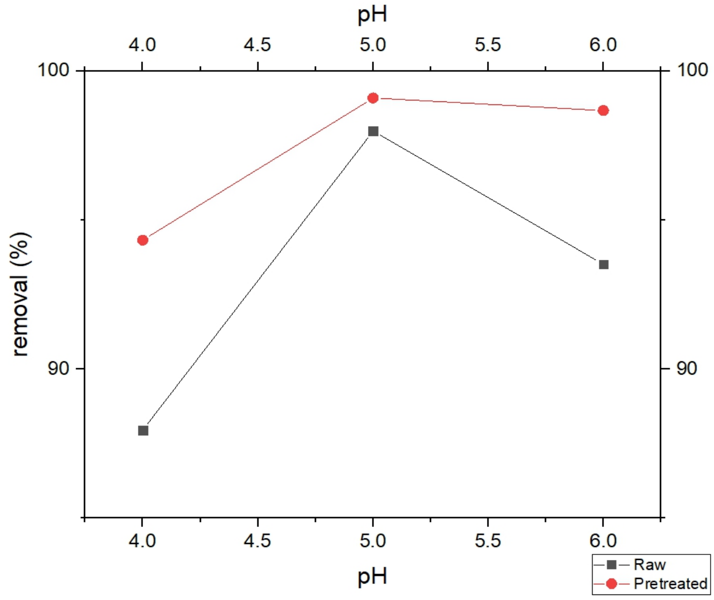

Figure 2: Effect of pH to percentage removal of Pb^{2+} at 400 rpm

It can be observed in Figure 2 that the highest percentage removal (97.99%) for both raw and (99.10%) pre--treated A.niger was at pH 5 and the lowest percentage removal (87.96%) for raw and (94.33%) pre--treated A.niger was at pH 4. It was expected that biosorption should increase as the solution pH was also increased. The effect of solution pH can be attributed that pH at low values, hydronium ions form and occupy the binding sites where less lead ions are sorbed because of the repulsion. As pH increases, the biosorbent surface becomes less positively charged so therefore increasing its biosorption capacity (Amirnia, 2015). However, there was a slight decrease in the percentage removal at pH 6. This was because at solution pH greater than 5, soluble hydroxylated complexes of the ions formed competing with the ions themselves for the biosorbent binding site surfaces.

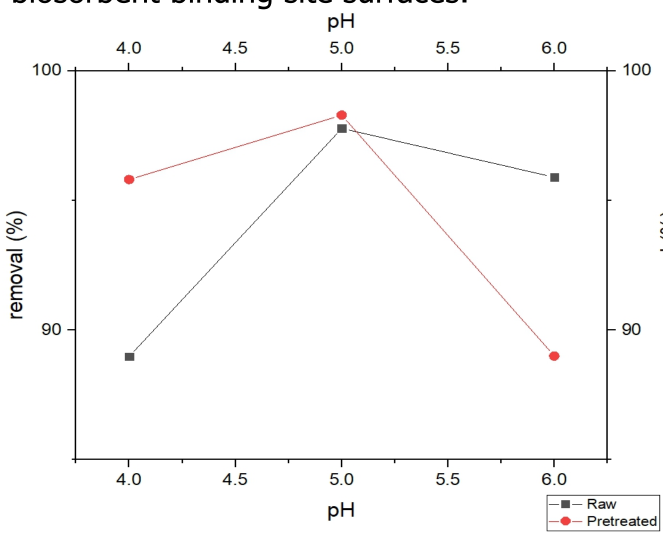

Figure 3: Effect of pH to percentage removal of at 400 rpm.

Looking at Figure 3, data showed highest percentage removal at pH 5, 97.89% and 98.30% removal for raw and pretreated A.niger, respectively. The lowest percentage removal was at pH 4 with 88.98% for raw A.niger and 89.00% pretreated A.niger at pH 6. Same can be said with the results of the Pb^{+2}. The highest percentage removal for for raw A.niger was at pH 5 while for the pretreared A.niger was at pH 4. For , both the raw and pretreated A.niger yields to a highest percentage removal at pH 5. Study shows that at low pH values, the strong affinity of protons onto metal binding sites on cell walls of biomass results in a competitive inhibition for metal biosorption (Chen, 2004). From these results, the best biosorbent at the best pH condition and best agitation rate may already be chosen. Pretreated biosorbent at pH 5 and 400 rpm will be used for the Kinetic Modelling of single metal ions. On the other hand, pretreated biosorbent at pH 4 and 150 rpm will be used for the Kinetic Modelling of combined metal ions.

It was found that the initial pH of the solution affected to the degree of biosorption. For this case, the ANOVA confirms that the pH has significant difference in the adsorption of heavy metals since the p--value is 0.044. Although the multiple comparison shows that there are no significant differences among the pH 4, 5, and 6 since the computed p--value is greater than 0.05. This means that if the environment is acidic, it will yield a better adsorption.

Effect of Pretreatment of Biomass

Looking Figure 2 and Figure 3, it can be observed that the alkali pretreated biosorbent at different pH levels and agitation magnitudes gave a higher percentage removal than the raw biosorbent. Pretreatment of the biosorbent increased the percentage removal from the aqueous solution. In order to determine the characteristics functional groups in charge for biosorption of metal ions, FTIR spectroscopy was applied. The results of the spectra and the comparisons of raw and pretreated fungi before biosorption were depicted in the Figure 4.

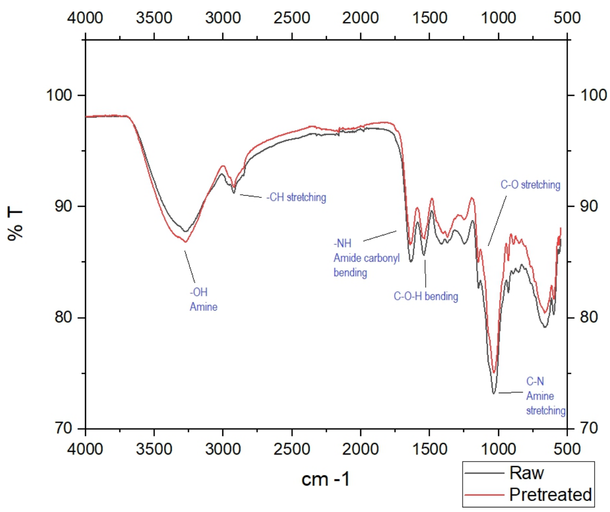

Figure 4: FTIR Spectra for raw and pretreated A.niger before biosorption

FTIR spectrum presents distinct peaks at 4000 to 550 cm^{-1}. The broad and strong bands at 3500 to 3000 cm^{-1} can be attributed to overlapping of –OH and amine group. The overlap makes it hard to distinguish between primary and secondary amine. The band at 2900 cm^{-1} the –CH stretching, 1631 cm^{-1} –NH or Amide Carbonyl bending, 1500 cm^{-1} C--O--H bending, 1100 cm^{-1} C--O stretching, and 1000 cm^{-1} to C--N or Amine stretching. Thus, Aspergillus niger biomass contain hydroxyl and amine groups on its surface. The functional groups for raw and pretreated Aspergillus niger before biosorption have no visible change. However, there is a change in the %Transmittance. From the study of Alavi et.al., since there is a pH change, it can be assumed that deprotonation took place on the binding sites of A.niger upon the pretreatment process. Thus, giving higher yield of metal removal than the raw biosorbent.

The data gathered for this study were treated using Independent T--test. The results showed that the alkali pretreated A.niger yields a higher percentage of metal removal than the raw A.niger. it was found that raw or pretreated A.niger has significant difference in the adsorption capacity of the biosorbent since the computed p--value is 0.001. This concludes that the pretreated biomass is a better biosorbent than the raw biomass.

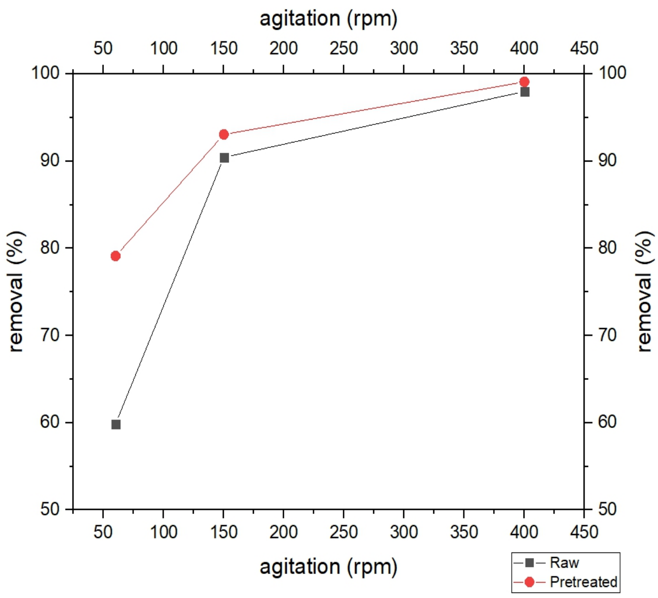

Figure 5: Effect of agitation rate to percentage removal of at pH 5

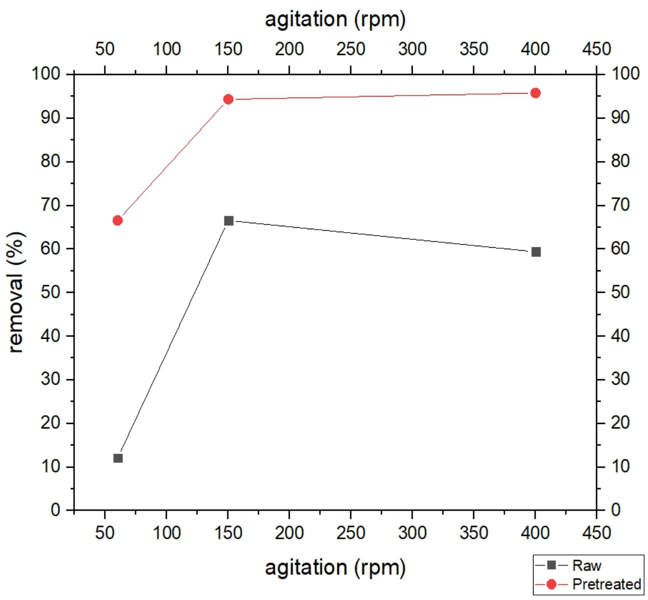

Figure 6: Effect of agitation rate to percentage removal of at pH 4

.

Maximum percentage adsorbance for (97.99% for raw, 99.10% for pretreated A.niger) was observed at 400rpm. It can be observed that the adsorption rate was highly influenced by the agitation rate. The adsorption rate increases with the agitation speed because shaking encourages the availability of the cell wall binding spots for the uptake of metal ions (Azizian, 2004;; Liu et al., 2006).

Same can be said with the results of the . 97.79% removal was observed for raw and 98.30% for pre--treated A.niger. Agitation speed enhanced the biosorptive removal rate of adsorptive pollutant by minimizing the transfer resistance (Abdi & Kazemi, 2015).

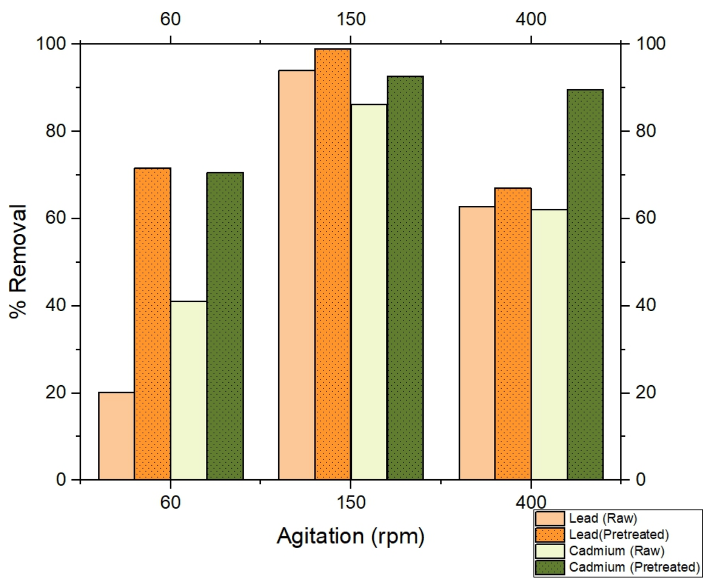

Figure 7: Effect of agitation rate to percentage removal of the mixture of at pH 4

From Figure 7, mixture of maximum percentage removal was observed at 150rpm with For the mixture of with agitation rate higher than 150rpm, adsorption capacity decreased. Studies showed that at very high agitation rates, the adsorbate particles and adsorbate molecules increased their kinetic energy. The gained increase sufficiently aided the hasty collision of molecules with each other causing in the detachment of the loosely bound adsorbate molecules (Mondal, Samanta, Dutta, & Chattoraj, 2017).

The results showed that the agitation rate has contributed greatly on the adsorption of lead and cadmium. It was also found from ANOVA that changing the agitation has significant difference in adsorption of the heavy metals since the p--value is 0.000. However, the multiple comparisons showed that the 150 rpm is not significantly different from the 400 rpm since the p--value is 0.885. Better adsorption will yield if the agitation rate is within 150 -- 400 rpm.

Effect of Metal Solution

As shown in the Figure 8, biosorption capacities for both single metal ions, , were higher than when it is combined. Studies showed that competitive biosorption happens when two or more metals are in contact with each other (Chen, 2004). The decrease in percentage removal is due to decrease in affinity of the metal ions because of the competition between metals for binding sites present in the biomass wall. Studies also showed that biosorption capacity for each metal decreases as the number of metals in the solution is increased (Sulaymon, Mohammed, & Al--Musawi, 2013).

The single metal ions and combination of the two metal ions have no statistical significance in the adsorption of lead and cadmium since the computed p--value from ANOVA is 0.127. The multiple comparison also showed that there are no significant differences among the metal ions. This means that the A. niger can be used as a good biosorbent for removing both single metal ions and combined metal ions.

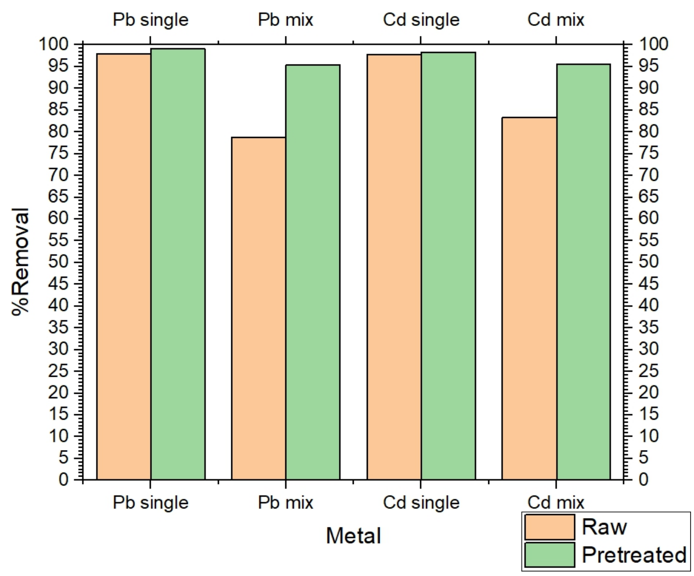

Figure 8: Effect of Metal solution

Kinetic Modeling

Studies on the kinetics of the process of metal ion removal by A. niger were carried out with the purpose of observing the development of the process until the system reached equilibrium. The most common approach in describing the biosorption kinetics is the Pseudo--First Order and Pseudo--Second Order Lagergren Model. These are functions of the amount of metal adsorbed at equilibrium, and amount of metal adsorbed at time t,.

Table 2, 3 and 4 show the summary of the rate constants and equilibrium concentration and the correlation coefficients for the Pseudo--First Order and Pseudo--Second Order Largergen Model. The correlation coefficients, R^2, were calculated from the linear plots to compute the validity of each model. The model with the higher R^2 values was considered. The data suggest that the adsorption process follows the linearized Lagergren pseudo second order. The model was computed using the equation:

This means that the rate of biosorption using A. niger follows the assumption that it is proportional to the square of the number of unoccupied binding sites. This also indicates that the rate limiting step in the adsorption was chemisorption.

Table 2: Rate Kinetics for Adsorption using pretreated Aspergillus niger

	Lagergren Pseudo--First Order		Lagergren Pseudo--Second Order	
20 mg/L	0.000802	0.5087	0.009993	0.9996
40mg/L	--0.000147	0.0600	0.005384	0.9984
60mg/L	--0.000290	0.0167	0.005482	0.9963

Table 3: Rate Kinetics for Adsorption using pretreated Aspergillus niger

	Lagergren Pseudo--First Order		Lagergren Pseudo--Second Order	
20 mg/L	0.00062	0.288	0.009972	0.9989
40mg/L	0.00196	0.385	0.005572	0.9924
60mg/L	0.00016	0.053	0.003401	0.9992

Table 4: Rate Kinetics for mixture of Adsorption using pretreated Aspergillus niger

	Lagergren Pseudo--First Order		Lagergren Pseudo--Second Order	
20 mg/L	0.000788	0.326	0.010604	0.9965
40mg/L	--0.000200	0.015	0.00601	0.9894
60mg/L	--0.000587	0.256	0.004396	0.9957

Adsorption Isotherm

Modelling of the heavy metal biosorption by A. niger were carried out using adsorption isotherms, Freundlich and Langmuir models. The following are the equations used for the purpose of this study.

For Langmuir model: For Freundlich model:

Correlation coefficient, R^2, was used to quantify the validity of each model. Linear plots C_e/Q_e vs. C_e describes the Langmuir isotherm model for the adsorption of Pb^{+2}, Cd^{2+} and combination of Pb^{+2} and Cd^{2+} while linear plots lnQe vs. lnCe describes the Freundlich isotherm.

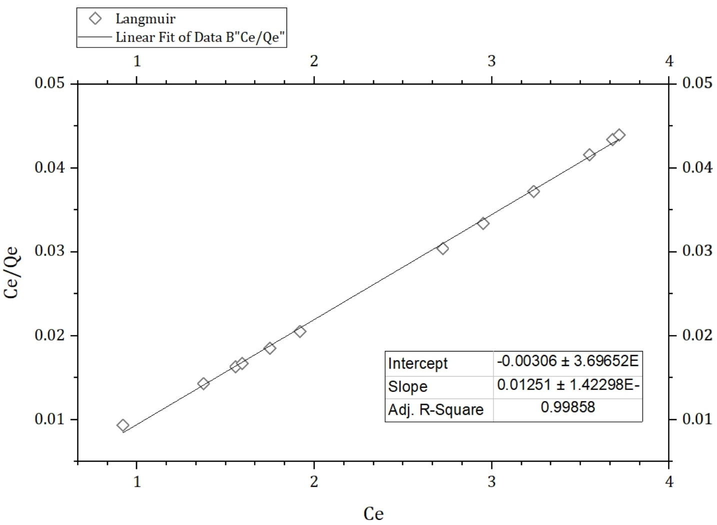

Figure 9: Linearized Langmuir Isotherm for Pb^{+2} and Cd^{+2}

From the data, it is evident that the isotherm model more fitted for adsorption of Pb^{+2}, Cd^{+2} and mixture of Pb^{+2} and Cd^{+2} is the Langmuir model where R^2 = 0.9987 for Pb^{+2}, R^2 = 0.9989 for Cd^{+2} and R^2 = 0.9987 for the mixture of Pb^{+2} and Cd^{+2}.

Since Langmuir isotherm model was followed, this means that the metals were homogeneously distributed over the adsorbent surface. The binding sites of A.niger for adsorption of monomolecular layer have the similar affinity. It was also found that there is no interaction in between the adsorbed molecules.

6.0 CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

In conclusion, biosorption of heavy metals, Cd^{+2} and Pb^{+2}, was successfully accomplished using biomass Aspergillus niger. It was later found that A. niger may be utilized more effectively when pretreated. Pretreatment instigated better metal uptake because it revealed more binding sites for biosorption. Parameters such as pH and agitation rate directly affect the biosorption capacity of the biomass. The biosorption process was observed to be best when the pH is 5 and at the agitation rate of 400 rpm. Any alterations may decrease the biosorption capacity. For pH lower or higher than 5, hydronium ions or soluble hydroxyl complexes compete with the metal ions, thus, lowering the biosorption capacity. On the other hand, higher agitation rate increases the transmission of particles around the biosorbent. The results also showed that A. niger may be used for the removal of Cd^{+2} and Pb^{+2} from aqueous solution whether present individually or in combination. The biosorption data fitted well in the Lagergren Pseudo--Second Order Model. This means that the rate of biosorption using

A. niger follows the assumption that it is proportional to the square of the number of unoccupied binding sites. This also shows that the rate limiting step in the adsorption was chemisorption. The biosorption of metals from single metal solutions and combined metal solutions follows the Langmuir isotherm. This indicates that the biosorption mechanism of Cd^{+2} and Pb^{+2} was monolayer.

6.2 RECOMMENDATION

For further study, it is recommended to use Aspergillus niger as biosorbent in actual wastewater in order to further test the adsorption efficiency of biosorbent. Another recommendation would be varying of biomass dosage to determine the effect and the ideal biomass--wastewater ratio that would yield to best biosorption results. Lastly, it is recommended to conduct detailed studies on evaluation of the effects of NaOH pretreatment on sorptive characteristics of the fungal biomass.

7.0 References

Abdi, O., & Kazemi, M. (2015). A review study of biosorption of heavy metals and comparison between different biosorbents. Journal of Materials and Environmental Science, 6(5), 1386–1399.

Amirnia, S. (2015). Biosorption Processes for Removal of Toxic Metals from Wastewaters, (July).

Azizian, S. (2004). Kinetic models of sorption: A theoretical analysis. Journal of Colloid and Interface Science, 276(1), 47–52. https://doi.org/10.1016/j.jcis.2004.03.048

Babák, L., Šupinová, P., Zichová, M., Burdychová, R., & Vítová, E. (2012). Biosorption of Cu, Zn and Pb by thermophilic bacteria -- Effect of biomass concentration on biosorption capacity. Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, 60(5), 9–18.

Bai, R. S., & Abraham, T. E. (2003). Studies on chromium(VI) adsorption--desorption using immobilized fungal biomass. Bioresource Technology, 87(1), 17–26. https://doi.org/10.1016/S0960--8524(02)00222--5 Biosorption, metals. (2010).

Chen, B. (2004). Biosorption of Zn (II) and Cu (II) by Indigenous Thiobacillus thiooxidans Biosorption of Zn (II) and Cu (II) by the indigenous Thiobacillus thiooxidans, 8947(FEBRUARY). https://doi.org/10.1016/S1385--8947(03)00210--9

Chojnacka, K. (2010). Biosorption and bioaccumulation -- the prospects for practical applications. Environment International. https://doi.org/10.1016/j.envint.2009.12.001

Gadd, G. M. (2009). Biosorption: Critical review of scientific rationale, environmental importance and significance for pollution treatment. Journal of Chemical Technology and Biotechnology. https://doi.org/10.1002/jctb.1999

Gakwisiri, C., Raut, N., Al--saadi, A., Al--aisri, S., & Al--ajmi, A. (2012). A Critical Review of Removal of Zinc from. Proceedings of the World Congress on Engineering, I, 6–9.

Kanamadi, R. D. (2003). Biosorption of Heavy Metals, 7(4).

Kaushik, P., & Malik, A. (2009). Fungal dye decolourization: Recent advances and future potential. Environment International. https://doi.org/10.1016/j.envint.2008.05.010

Michalak, I., Chojnacka, K., & Witek--Krowiak, A. (2013). State of the art for the biosorption process----a review. Applied Biochemistry and Biotechnology. https://doi.org/10.1007/s12010--013--0269--0

Mondal, N. K., Samanta, A., Dutta, S., & Chattoraj, S. (2017). Optimization of Cr(VI) biosorption onto Aspergillus niger using 3--level Box--Behnken design: Equilibrium, kinetic, thermodynamic and regeneration studies. Journal of Genetic Engineering and Biotechnology, 15(1), 151–160. https://doi.org/10.1016/j.jgeb.2017.01.006

Southichak, B., Nakano, K., Nomura, M., Chiba, N., & Nishimura, O. (2006). Phragmites australis: A novel biosorbent for the removal of heavy metals from aqueous solution. Water Research, 40(12), 2295–2302. https://doi.org/10.1016/j.watres.2006.04.027

Sulaymon, A. H., Mohammed, A. A., & Al--Musawi, T. J. (2013). Competitive biosorption of lead, cadmium, copper, and arsenic ions using algae. Environmental Science and Pollution Research, 20(5), 3011–3023. https://doi.org/10.1007/s11356--012--1208--2

Taricska, J. R., Wang, L. K., Hung, Y.--T., Li, K. H., Huang, J.--C., Shang, C., … Scovazzo, P. (2006). Advanced Physicochemical Treatment Processes. Advanced Physicochemical Treatment Processes. https://doi.org/10.1007/978--1--59745--029--4

Volesky, B. (2001). Detoxification of metal--bearing effluents: Biosorption for the next century. Hydrometallurgy, 59(2–3), 203–216. https://doi.org/10.1016/S0304--386X(00)00160--2

Volesky, B. (2007). Biosorption and me. Water Research. https://doi.org/10.1016/j.watres.2007.05.062