Myco-agro sorbents: novel heavy metal sequesters

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Abstract

The potential application of biosorption and biosorbents has evoked tremendous interest to prepare cheap and commercial biosorbents through manipulation of indigenous microbial resources along with abundantly available low cost natural plant by-products. In the present study, rice straw was used to cultivate five indigenous fungal species viz., *Aspergillus niger* Van Tieghem, *Aspergillus terreus* Thom, *Aspergillus. flavus* Link ex Gray, *Rhizopus arrhizus* Fischer and *Trichoderma harzianum* Rifai. Substrate colonized by fungi was utilized as adsorbent. Batch adsorption trials were accomplished by taking 0.1 g of powdered biomass of colonized substrate in 100 mL of solution comprised of 10 different concentrations of Ni(II) in the range of 50-500 mg L^{-1}. Results showed that colonized substrate exhibited significantly greater metal removal efficiency (50%) than uncolonized substrate (30%). Substrates colonized with *T. harzianum* and *R. arrhizus* demonstrated a significantly higher biosorption efficiency (20-50%) and capacity (20-250 mg g^{-1}) at metal concentration range of 50-500 mg L^{-1} than the substrate colonized by rest of the fungal species. It was concluded that these two indigenous fungal species can be used as low cost biosorbents by growing their mycelia on rice straw for Ni(II) removal on pilot scale.

Keywords: Lignocellulosic waste, mycoadsorbent, Ni(II), wastewater.

Introduction

Serious kind of heavy metal pollution removal is now felt more than ever because of severe water crises world-wide. However, in practice number of conventional techniques are currently being applied and very little of the set targets has been achieved. As a solution the biosorption technologies have recently emerged as economically and practically viable option to conventional technologies by utilizing any natural source of biomass as a biosorbent for the depollution of industrial heavy metal (Akhtar and Shoaib, 2012).

Easily available and routinely utilized materials comprised of three groups of biosorbent: algae, fungi, and bacteria, the former two perhaps giving broader choices (Wang and Chen, 2009). Of recent, the use of fungi for depollution of heavy metal ions has been gaining advantage due to their filamentous morphology, robust nature, high percentage of cell wall material, required in small amount and efficiently adsorbs metal along with reutilization and desorption capabilities. Among fungi, species of *Aspergillus*, *Rhizopus* and *Trichoderma* have been extensively utilized as potential mycoadsorbents. The hyphal wall was found to be a primary site of metal ions accumulation (Javaid *et al*., 2011; Shoaib *et al*., 2011; Manzoor *et al*., 2012; Shoaib *et al*., 2012).

Furthermore, lignocelluloses waste material has been introduced as a new family of naturally abundant, easily available and cost effective biosorbents derived through byproduct/waste of pre-harvest, post-harvest and fermentation industries. These plant biomass wastes comprised of cellulose, hemicellulose and lignin and are the largest renewable reservoir of potentially fermentable carbohydrates on earth (Mtui and Nakamuvra, 2005). Beside tremendous potential of plant linocellulosic waste in second generation bio-products such as bioethanol, biodiesel, biohydrogen and methane, their prospective in heavy metal ions removal cannot be overlooked. A novel adsorbent can be prepared by culturing fungi on lignocellulosic waste in a very simple and unsophisticated way that may provide better results in metal ions sequestration from industrial effluents in eco-friendly and economic way (Javaid and Bajwa, 2008). The current study was designed to assess the Ni(II) removal potential of rice straw colonized with five fungal species viz. *A. niger*, *A. terreus*, *A. flavus*, *R. arrhizus* and *T. harzianum*.

Materials and Methods

The stock solutions of Ni metal ions (1000 mg L^{-1}) were prepared from its nitrate salts of analytical grade by dissolving the exact quantity of salt in double distilled deionized water. Further concentrations were prepared by diluting stock solution with double distilled deionized water. The pure cultures of test fungal species viz. *A. niger*

(FCBP 0074), *A. terreus* (FCBP 0058), *A. flavus* (FCBP 0064), *R. arrhizus* (FCBP 800) and *T. harzianum* (FCBP 0139) were procured from First Fungal Culture Bank of Pakistan, Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan. The pure cultures of the fungi were maintained on 2% malt extract agar in 9-cm diameter Petri plates. Each of the fungal species was cultivated by inoculating 0.5–1 mm disc on pre-autoclaved rice straw filled in plastic bags. Bags were kept in incubator at 25 ± 1 ^{o}C until the mycelium of each fungus had fully penetrated to the bottom of the substrate and ramified the whole substrate. Colonized rice straw was dried in oven at 60 ^{o}C and homogenised to obtain particle size of 150 µm mesh size considering that smaller particle size has greater surface area and more binding sites.

Biosorption experiments were performed by taking 0.1 g of colonized substrate biomass in 100 mL of metal solution in 250 mL Erlenmeyer flask. Metal concentrations were varied over range of 50–500 mg L^{-1}. Each treatment was replicated three times. The mixture (biomass + metal solution) was agitated at 150 rpm at pH 4.5 for 2 hours. After desired contact time, the mixture was filtered through Whatmann filter paper No.1 and residual metal ion concentrations were determined through atomic absorption spectroscopically. Uncolonized rice straw served as control treatment. The amount of Ni(II) accumulated by biomass (q in mg g^{-1}) and efficiency (%) of biosorbents were calculated by formulas described by Javaid and Bajwa (2008).

$$E = \left(\frac{C_i - C_f}{C_i}\right) * 100$$

$$q = \left(\frac{C_i - C_f}{m}\right) V$$

$\mathbf{C_i}$ is the initial concentration of the metallic ion (mg L^{-1}); $\mathbf{C_f}$ is the final concentration of metallic ion (mg L^{-1}); m = dried mass of the biosorbent in the reaction mixture (g); **V** = volume of reaction mixture (mL).

Data regarding biosorption capacity and efficiency were analyzed through Tukey's HSD test at 5% level of significance (Richard, 2008).

Results and Discussion

Generally, rice straw either colonized or uncolonized found to be good adsorbent of Ni(II) ions (Table 1 & 2). In a number of previous findings, rice straw was regarded as good biosorbents for removal of different metal ions (Dhir and Kumar, 2010; Singha and Das, 2013). Probably, active binding site on cell wall of straw contains configuration of cellulose molecules surrounded by hemicellulosic, lignin and pectin along with small amounts of protein (Mtui, 2009). Metallic ions in the aqueous solution would likely to attract with large surface area of cell wall component preferably with amorphous structure of lignocellulosics material than highly crystalline structured cellulose Colonized substrate exhibited significantly greater adsorption capacity/efficiency for Ni(II) ions than uncolonized substrate. Yakout and Elsherif (2010) stated that native agriculture by-products possessed generally low sorption capacity therefore needs to be modified. In current study, the modification of rice straw by fungal growth may cause delignification and solubilization of the polymer core of straw that could make further provision of supplementary binding sites in straw. Whereas, the colonized has additional functional groups in form of chitin of fungal cell wall that is regarded as excellent adsorbent of metal ions.

Maximum adsorption efficiency of 30% and 50% of either uncolonized and colonized rice straw adsorbents, respectively, was comparatively low than previously reported efficiency of the same substrate (El-Said, 2010; Dhir and Kumar, 2010; Naiya *et al*., 2011). It is most probably due to utilization of a very low amount of biomass i.e. 0.1 g for the adsorption even at very high concentration of Ni(II) ions. While different native characteristic of the adsorbents along with varied experimental conditions may cause more differences in the adsorption behaviour of the adsorbent.

Amongst the colonized adsorbents, rice straw colonized either with *R. arrhizus* and *T. harzianum* exhibited the highest and parallel metal removal efficiency (40-50%) in contrast to three species of *Aspergillus* (20-40%) at different concentration of Ni(II) (50-500 mg L^{-1}). Difference in the composition of cell walls, texture and porosity in different fungi could be responsible for this difference e.g. *R. arrhizus* cell wall has high chitin and chitosan content of the cell walls than chitin and glucan in cell walls of *Aspergillus* spp. (Manzoor *et al*., 2012). Besides, compact mycelial mat of *Aspergillus* species might have blocked some of micro-pores in rice straw, making it difficult for the metal ions to reach the adsorption sites, as the adsorption takes place in these micro-pores.

It was noticed that concentration range of 100-500 mg L^{-1} apparently exhibited significant elevation in uptake capacity of the biosorbents. The increase in adsorption capacity with increase in metal concentration may be due to the higher adsorption rate and utilization of all active sites available for the adsorption even at higher concentration. While adsorption efficiency of the adsorbents increased up to certain level then levelling off. Occurrence of more unoccupied surface binding sites on the adsorbent at low concentration of metal ions could possibly be responsible for higher adsorption efficiency. Whereas, on increasing metal concentration equilibrium between metal ion and adsorbent's active site establish very soon thus efficiency decreased due to competitive effect of metal ions for adsorption site. Likewise, at higher concentrations, metal needed to diffuse to the biomass surface by intraparticle diffusion and greatly hydrolyzed ions will diffuse at a slower rate (Horsfall and Spiff, 2006).

Conclusion

It is evident from the contemporary research that colonized rice straw either with *T. harzianum* or *R. arrhizus* hold good Ni(II) uptake potential and could be utilized as economic agro-myco-sorbents in removal of Ni(II) ions from aqueous solution within concentration range of 50-500 mg L^{-1}.

Table 1. Biosorption capacity of uncolonized and colonized substrate for Ni(II) ions from aqueous solution.

Metal Concentration (mg L^{-1})	Biosorption capacity (mg g^{-1})					
	Control (UCS)	**CS with *R. arrhizus***	**CS with *T. harzianum***	**CS with *A. niger***	**CS with *A. terreus***	**CS with *A. flavus***
50	9±1.7 B	18±0.6 A	18±1.2 A	11±1.3 B	10±1.6 B	9±1.8 B
100	16±3.5 B	33±0.9 A	36±2.1 A	29±2.2 A	31±3.0 A	28±1.5 A
150	30±3.0 B	57±1.5 A	59±1.1 A	54±3.8 A	56±1.9 A	48±4.4 A
200	49±2.2 C	82±0.7 AB	87±1.8 A	76±4.2 AB	72±1.2 B	71±4.6 B
250	65±4.8 D	110±2 AB	113±2.1 A	97±1.45 C	94±1.0 C	101±1.5 BC
300	80±1.7C	141±1.8 A	143±2.5 A	123±1.53 B	117±1.2 B	119±3.0 B
350	100±1.7 D	166±1.9 A	165±1.8 A	150±1.6 B	150±2.3 B	139±3.4 C
400	120±4.1 C	196±2.2 A	195±0.6 A	184±2.1 A	184±2.2 A	156±4.4B
450	133±3.4 D	220±2.6 A	227±2.5 A	200±3.5 B	198±4.0 B	170±1.7 C
500	153±3.3 C	243±1.9 A	250±1.7 A	200±3.6 B	197±1.8 B	190±3.8 B

Table 2: Biosorption efficiency of uncolonized and colonized substrate for Ni(II) ions from aqueous solution.

Metal Concentration (mg L^{-1})	Biosorption efficiency (%)					
	Control (UCS)	**CS with *R. arrhizus***	**CS with *T. harzianum***	**CS with *A. niger***	**CS with *A. terreus***	**CS with *A. flavus***
50	9±1.33 B	37±0.9 A	35±2.3 A	21±2.6 B	19±3.3 B	19±3.5 B
100	15±3.5 B	35±0.9 A	36±2.1 A	29±2.2 A	31±2.9 A	28±1.5 A
150	20±2.0 B	38±0.9 A	40±1.2 A	36±2.5 A	38±1.2 A	32±2.9 A
200	24±1.0 C	41±0.3AB	44±1.0 A	38±2.2 AB	36±0.6 B	36±2.3 B
250	26±1.2 D	44±0.8 AB	45±0.6 A	40±0.7 C	38±0.4 C	40±0.6 BC
300	27±1.6 C	47±0.6 A	48±1.2 A	41±0.5 B	39±0.6 B	40±1.0 B
350	28±0.5 D	48±0.5 A	47±0.2 A	43±0.4 B	43±0.4 B	40±1.0 C
400	29±0.4 C	49±0.5 A	49±0.8 A	46±0.5 A	46±0.4 A	39±1.1 B
450	30±0.9 D	49±0.5 A	50±0.4 A	45±0.9 B	44±0.9 B	37±0.4C
500	31±0.7 C	48±0.4 A	50±0.3 A	40±0.8 B	38±0.6 B	37±0.8 B

UCS: Uncolonized substrate; CS: Colonized substrate
Note: ± indicates standard errors of means of three replicates. Values with different letter in each row shows significant ($p \leq 0.05$) difference among the treatments as determined Tukey's HSD Test.

References

Akhtar S, Shoaib A, 2012. Biosorption, Solution to Arsenic Pollution. *J. Anim. Plant Sci*., **22**: 659-664.

Dhir B, Kumar R, 2010. Adsorption of heavy metals by *Salvinia* Biomass and agricultural residues. *Int. J. Environ. Res.,* **4**: 427-432.

El-Said AG, 2010. Biosorption of Pb(II) Ions from Aqueous Solutions Onto Rice Husk and its Ash. *J. Am. Sci*., **6**: 143-150.

Mtui GYS, 2009. Recent advances in pretreatment of lignocellulosic wastes and production of value added products. *Afr. J. Biotechnol*., **8:** 1398-1415.

Horsfall JM, Spiff AI, 2004. Effect of metal ion concentration on the biosorption of Pb^{2+} and Cd^{2+} by *Caladium bicolor* (wild cocoyam), *Afr. J. Biotechnol*., **4:** 191-196.

Javaid A, Bajwa R, 2008. A new approach of utilizing plant by-products colonized by fungal mycelia for sorption of industrial heavy metal ions. *Pak. J. Phytopathol.,* **20**: 101-107.

Javaid A, Badar T, Aslam N, 2011. Removal of Pb(II), Cu(II) & Cd(II) from aqueous solution by some fungi and natural adsorbents in single- & multiple metal systems. *Pak. J. Bot*., **43**: 2997-3000.

Javaid A, Badar T, Aslam N, 2011. Removal of Pb(II), Cu(II) & Cd(II) from aqueous solution by some fungi and natural adsorbents in single- & multiple metal systems. *Pak. J. Bot*., **43**: 2997-3000.

Manzoor T, Shoaib A, Bajwa R, 2012. Mycoremmediation of Cu(II) and Ni(II). *Afr. J. Microbiol. Res*., **6:** 236-244.

Naiya TK, Singha B, Das SK, 2011. FTIR Study for the Cr(VI) Removal from Aqueous Solution Using Rice Waste. *IPCBEE.,* **10**: 114-119.

Shoaib A, Naureen A, Tanveer F, Aslam N, 2012. Removal of Ni(II) ions through Filamentous Fungi. *Int. J. Agric. Biol.,* **14**: 831-834.

Singha B, Das SK, 2013. Adsorptive removal of Cu(II) from aqueous solution and industrial effluent using natural/agricultural wastes. *Colloids Surf.,* **107**: 97-106.

Wang J, Chen C, 2009. Biosorbents for heavy metals removal and their future. *Biotechnol. Adv*., **27**: 195-226.

Yakout SM, Elsherif E, 2009. Batch inetics, isotherm and thermodynamic studies of adsorption of strontium from aqueous solutions onto low cost rice-straw based carbons**.** *Carbon Sci. Technol*., **1**: 144-153.

Richard L. 2008. One Way ANOVA – Independent Samples. Vassar.edu. Retrieved on December 4th, http://www. faculty. vassar.edu/.