Jr. of Industrial Pollution Control 35(1)(2019) pp 2251-2257
www.icontrolpollution.com
Research Article

TOLERANCE AND REMOVAL OF ZINC (II) AND MERCURY (II) BY DEAD BIOMASS OF *ASPERGILLUS TUBINGENSIS* MERV4

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(Received 28 February, 2019; accepted 04 July, 2019)

Key words: Zinc, Mercury, Removal, Phylogenetic, A. tubingensis, Tolerance.

ABSTRACT

Zinc and mercury are causing a problem worldwide owing to highly toxicity and this problem will intensify if obligatory actions not taken. In present study searching about bioactive fungal strain can. We screened ten endophytic fungal species derived from different plants grow in polluted sites for tolerating and removing both zinc and mercury efficiently from aqueous solutions. Among these isolates *Aspergillus. tubingensis* Merv4 showed the highest resistant toward mercury (Hg^{2+}) with the highest tolerance against zinc (Zn^{2+}). Moreover, simultaneously it was tolerant to different other toxic metals like pb^{2+}, Cr^{6+}, Cd^{2+} and Ni^{2+} with varied degree of resistant. Dead biomass of *A. tubingensis* Merv4 strain showed capable of totally removing (100%) of both zinc and mercury, respectively from aqueous solution under optimized conditions. In real industrial disposal water the maximum removal values of zinc and mercury were (96% and 91%, respectively) by dead biomass of *A. tubingensis* Merv4 under optimized conditions. In near future these promising fungi can be used in bioremediation of mercury, zinc and other metal contaminates from industrial wastewaters onto microbial dead biomass.

INTRODUCTION

Heavy metal contamination is widespread globally due to manufacturing which seriously threatens the ecosystem significantly, principally the health of individuals because of their severe toxicity. With the purpose of reduce the effects of metal pollutions; wastewater must be treated before disposal into water bodies. Recently, zinc and mercury toxicity have been increased extensively (Tahir, *et al.*, 2017) as a result of abundant industrial purposes for example, pharmaceuticals, cosmetics, electrical appliances, pulp and paper industries (El-Gendy and El-Bondkly, 2016). Biological treatment is an important technique to reduce metal contamination because of its low cost and high efficiency. The United States environmental protection agency (EPA) reported mercury as a polluted priority because it can pass simply into the blood brain barrier and Hg^{2+} at high concentrations causes dysfunction in the lung, kidney, chest pain and shortness of breath.

Many previous reports recommended the isolation of resistant fungi to heavy metals and the usage of their biomass for removing metal pollutions from industrial effluents like the like *Rhizopus stolonifer* (lead, cadmium, copper, and zinc), *Pleurotus ostreatus* HAAS (lead, cadmium, and chromium), *Rhodotorula mucilaginosa* (mercury, copper, and lead), *Aspergillus niger (*removal of lead, cadmium, copper, and nickel*)*, *A. niger* (aluminum, iron, lead, and zinc), immobilized cells of *A. niger* (copper, manganese, zinc, nickel, iron, lead, and cadmium), *endophytic Drechslera hawaiiensis* (cadmium, copper and lead), *endophytic penicillium lilacinum* (cadmium, copper and lead) (El-Gendy, *et al.*, 2011 and 2017; Acosta-Rodríguez, *et al.*, 2018; Martínez-Juàrez, *et al.*, 2012). Furthermore, the World Health Organization recommended that the maximum acceptable concentration of zinc in drinking water is 0.05 mg/L. higher concentrations in humans can cause anemia, nausea, vomiting, nephritis, corrosive effect on skin, metal emission

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fever, damage pancreas, lungs and nerve membrane (Mali, *et al.*, 2014).

The aim of this work is to examine some mineral-resistant fungal strains that can be used to treat zinc and mercury contamination efficiently with optimizing the remediation process parameters including process time, absorbed dose, temperature and pH to improve the biological absorption of zinc and mercury under batch mode to potent safe removal of hazardous metal in the near future.

MATERIALS AND METHODS

Microorganisms and culture conditions

Ten endophytic fungal strains belong to different 8 genera namely, *Rhizopus oryzea* (Merv2), *Aspergillus luchuensis* (Merv3), *Aspergillus tubingensis* (Merv4), *Monacrosporium elegans* (Merv5), *Penicillium duclauxi* (Merv6), *Curvularia lunata* (Merv7), *Penicillium lilacinum* (Merv8), *Drechslera hawaiiensis* (Merv9), *Verticillium Fungicola* (Merv10) and *Pestalotiopsis clavispora* (Merv11) were isolated and identified according to morphological, physiological and biochemical characterizations in a previous study (El-Gendy, *et al.*, 2011). Fungal inoculums were obtained after culturing in potato dextrose broth in dark for 10 days at 28ºC and 180 rpm. Endophytic fungi were maintained on potato dextrose agar and reserved at 4°C.

Experimental reagents and apparatus

Metals under study, sodium hydroxide and nitric acid were of analytical grade (Merck), stock solutions of mercury and zinc as well as the standard calibration were performed, pH was amended using HNO_3.

Preparation of adsorbent for uptake experiments

Inoculum of 10^6 spore/mL ten days old culture of each fungal strain were inoculated into 250 mL Erlenmeyer flasks containing 100 mL of YMG medium (yeast extract, 5; malt extract, 10; glucose, 10, g/L) under static conditions in dark for ten days at 28ºC and then fungal biomasses were harvested by passing 100 mL of each fungal culture through a previously weighted filter papers (Whatman No. 1). The harvested mycelia were washed, autoclaved at 121°C, dried in oven at 60°C until reach constant weights and then stored at - 20°C (El-Gendy and El-Bondkly, 2016; El-Gendy, *et al.*, 2011 and 2017).

Adsorbate solution preparations

Various concentrations of metal solutions 100 mg/L, 200 mg/L, 300 mg/L, 400 mg/L and 500 mg/L of mercury chloride and zinc chloride were prepared.

Phylogenetic analysis of the selected biosorbent fungal strain

Extraction and purification of genomic DNA of the most active biosorbent endophytic fungal strain (Merv4), amplification of ribosomal DNA and the PCR program were done according to a previous study (El-Bondkly, 2006 and 2012; El-Bondkly and El-Gendy, 2012; White, *et al.*, 1990). Sequence relationships were recognized by the BLAST tool from the National Center for Biotechnology Information (NCBI). Phylogenetic analyses were conducted with the neighbor-joining method through MEGA6 software.

Batch mode adsorption experiment

In this study, biosorption capacity of fungus was determined in batch conditions. Adsorption was performed using died fungal biomass wherein weighed amount of fungal biomasses were added to constant concentration of zinc and mercury metal solution, individually. Mixtures were then kept at room temperature, taken sample were withdrawn at different contact time intervals. The adsorbent and adsorbate were separated through centrifugation at 3000 rpm for 5 min. The residual metal ion concentration was determined by A Perkin-2380 atomic absorption spectrophotometry.

Removal of zinc and mercury under different adsorption mathematical models

Adsorption data achieved in this study were defined using Langmuir and Freundlich isotherms. Langmuir isotherm equation stated as RL= 1/1+bC◦, where RL and C are equilibrium parameter and initial heavy metal concentration. The RL refer to the profile of isotherm if it unfavorable (RL > 1), linear (RL= 1), favorable (0 < RL < 1) or irreversible (RL= 0). The Freundlich equation is prvided as: q_{exp}= n Ce Kf, where q_{exp} refer to quantity of absorbed metal at time (mg/g), Ce is the equilibrium concentration (mg/L) but Kf (mg/g) and n (g/L) are the equilibrium constants (Muneer, *et al.*, 2013; El-Morsy, *et al.*, 2013).

Effect of initial metal concentration

Zn^{2+} and Hg^{2+} solutions of 100, 200, 300, 400 and 500 mg/L at pH 5 were added individually a fixed biomass of 200 mg/L in 250 mL Erlenmeyer flasks in triplicate at room temperature for different times (1, 2, 4, 6, 8, 12 and 24 h) with shaking at 180 rpm for mixing the samples well. Experimental and control (contain no biomass) were analyzed using A Perkin-2380 atomic absorption spectrophotometry (Mali, *et al.*, 2014; Muneer, *et al.*, 2013; El-Morsy, *et al.*, 2013).

Effects of pH and temperature at different treatment times

We evaluate the impact of pH values equal to 4.0, 4.5, 5.0, 5.5, 6.0 and 6.5 at various time periods (1, 2, 4, 6, 8, 12 and 24 h) on Zn^{2+} and Hg^{2+} adsorption by Merv4 dead biomass to assess optimum pH and the equilibrium time for removing Zn^{2+} and Hg^{2+}. On the other hand, the impact of different temperature values (30°C, 35°C, 40°C and 45°C) on reduction potentially of Zn^{2+} and Hg^{2+} was evaluated. 200 mg/L of biomass was added to 50 mL of Zn^{2+} or Hg^{2+} solution at an optimum concentration for each metal in triplicate and then analyzed at different treatment times.

Factory effluents

The heavy metals of wastewater resulted from electrical instruments electroplating industries in Egypt removal by the selected strain were evaluated under optimized conditions.

Determination of zinc and mercury tolerance of endophytic fungi

Metal tolerance was estimated in terms of minimum inhibitory concentration (MIC) in agar plates supplemented with different concentration ranged from 50 to 1000 µg/mL of Cd (II), pb (II), Cu (II), Hg (II), Ag (I), Cr (VI), Ni (II), Zn (II), Fe (III) or Al (III) and inoculated with 10^6 spore/ml incubated at 30 °C for 10 days as previously described (El-Gendy, *et al.*, 2011; Bedioui, *et al.*, 2015; Akhtar, *et al.*, 2013).

RESULTS AND DISCUSSION

Evaluation the bioremoval potentiality of isolated endophytic fungi as biosorbents

Studies in Table 1 showed the quantity of zinc and mercury metals removed (%) from aqueous solutions by the biomasses of different endophytic fungi. Among them, *Aspergillus tubingensis* Merv4 showed the highest removal of zinc (80.13%) followed by *Penicillium lilacinum* MERV8 (71.69%) and *Monacrosporium elegans* MERV5 (70.09%), respectively but the highest biosorption of mercury from aqueous solutions was obtained by *A. tubingensis* Merv4 (70.52%) followed by *M. elegans* Merv5 (61.25%) and *Pestalotiopsis clavispora* Merv11 (51.74%), respectively. So the hyperactive strain *A. tubingensis* Merv4 was selected for the further bioremoval studies. The comparison of the most active biosorbent strain Merv4 with the previous sequences data of fungi within genomic database banks supported 100% similarity with *A. tubingensis* (Fig. 1). Accordingly the effective strain Merv4 was identified and designated as *A. tubingensis* Merv4.

In the mercury detoxification process, work is still necessary to explain the occurrence and biodiversity of microbiome under pressure of metal ions for use in the biological treatment of these toxic contaminants, individually or collectively to achieve greater efficiency. Also, some mercury biosorbent fungi can detoxify mercury with ability to remove other toxic metals such as cadmiumand lead (Joo and Hussein, 2012). Martínez-Juàrez, *et al.* (2012) evaluated the adsorption ability of 14 fungal biomasses toward mercury including *Aspergillus flavus*, *A. fumigatus*, *Helminthosporium* sp., *Cladosporium* sp., *Mucor rouxii*, and *Candida albicans* and they supported the biomasses of *M. rouxii* for efficient removing of metal in solution. The efficiency of endophytic fungi as biosorbet materials for toxic heavy metals was reported by many authors such as El-Gendy, *et al.* (2011) and Pietro-Souza, *et al.* (2017).

Adsorption isotherms

To define the affinity among the metal ions and the waste biomass Langmuir and Freundlich adsorption models were used. Obtained results showed that the Langmuir equation describes well equilibrium adsorption data of zinc and mercury by dead biomass of *A. tubingensis* Merv4 (Table 2). The values of Q^o (95.23 and 147.05 mg/g for Zn^{2+} and Hg^{2+}, respectively), R^2 (0.94 and 0.98 for Zn^{2+} and Hg^{2+},

Table 1. Efficiency of different fungal strains in the removal (%) of Zn^{2+} and Hg^{2+} from aqueous solution.

Fungal strain	Removal (%)	
	Zn^{2+}	**Hg^{2+}**
Rhizopus oryzea Merv2	52.16	28.84
Aspergillus luchuensis Merv3	67.37	50,08
Aspergillus tubingensis Merv4	80.13	70.52
Monacrosporium elegans Merv5	70.09	61.25
Penicillium duclauxi Merv6	48.48	37.10
Curvularia lunata Merv7	35.81	49.32
Penicillium lilacinum Merv8	71.69	44.00
Drechslera hawaiiensis Merv9	65.42	49.95
Verticillium fungicola Merv10	35.32	11,97
Pestalotiopsis clavispora Merv11	59.00	51.74

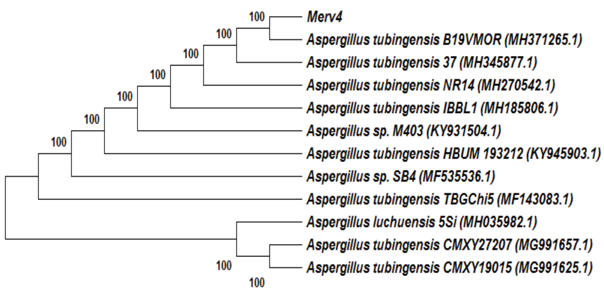

Fig. 1 Phylogenetic analysis of ITS sequence constructed by neighbor-joining method for endophytic fungal strain Merv4 and the highest similar fungi.

respectively) and the small b values (0.085 L/mg for Zn^{2+} and 0.040 L/mg for Hg^{2+}) obtained in this research implicit strong binding of metal ions into *A. tubingensis* Merv4 biomass. El-Morsy, *et al.* (2013) and Muneer, *et al.* (2013) examined the biosorption capacity of fungal strains for Zn (II) and Hg (II) biosorption from aqueous effluents and they also stated that the Langmuir equation describes well the biosorpton procedure with the highest biosorption capability for the dead sorbents.

Effect of various concentrations of Zn^{2+} and Hg^{2+} in aqueous solutions

The biosorption efficiency was evaluated with variable initial metal concentrations from 100 to 500 mg/L, individually. We observed that with the increased concentration of metal ion, its absorption increased and a plateau occurred for both heavy metals. Total removal 100% of 400 and 500 mg/L of zinc was obtained after treatment for 24 and 8 h of incubation periods, respectively while removing of 85% and 98% of 100 and 200 mg/L of mercury occurred after 24 h of treatment (Table 3). These results may be clarified by increasing the number of competing ions on the available binding sites as well as because of the inadequate active sites on biomass at greater concentrations. Similar findings were described for the deletion of zinc from effluents by adsorption into fungal biomass by Mali, *et al.* (2014) and Hg (II) utilization of mercury contaminant by *A. flavus* strain KRP1 (Kurniatia, *et al.*, 2014).

Effect of different pH values in removing Zn^{2+} and Hg^{2+} processes

pH of the solutions plays a major role in the biosorption process because it affects the balance by influencing the differentiation of ions in the solution. Metal ions charge in the solution and the availability of active sites for heavy metals attraction onto the surface of biosobent depend mainly onto pH of the working solution. With a pH increase of 4.0 to 6.5 zinc removal increased (Table 4). Zinc at pH 5.0, 5.5, 6.0 and 6.5 was totally removed after (8, 12, and 24 h), (6, 8, and 12 h), (4, 6, and 8 h) and (4, and 6 h), respectively (Table 4). The highest Hg (II) biosorption was detected at pH 5.5 after 12, and 24 h of treatment (100%, Table 4) and then decreased by (13% and 20%) and (25% and 40%) at pH 6.0 and 6.5 after 12, and 24 h contact times in aqueous solution, respectively. At lower or higher pHs the bioremoval of these heavy metals was decrease due to in acidic pH, protonated cell wall components negatively influenced the adsorption capacity of biomass and with increase in pH the negative charge density on the cell surface increased by the deprotonating of the metal binding sites. Then the efficient use of fungal genera and species in biological treatment of Zn^{2+} and Hg^{2+} is greatly affected by pH (El-Gendy and El-Bondkly, 2016; El-Gendy, *et al.*, 2011and 2017).

Effect of varying temperatures on removal of Zn^{2+} and Hg^{2+}

Maximum zinc absorption capacity was found at 30°C after 12 to 24 h contact time (100%) as well as at 35°C after 8 h of incubation and then the adsorption capacity of died *A. tubingensis* Merv4 biomass reduced with temperature higher than 35°C (62% and 60% at 40°C and 45°C, respectively, Table 5).

Table 2. The Langmuir and Freundlich isotherm models for adsorption constants for Zn^{2+} and Hg^{2+} by autoclaved *A. tubingensis* Merv4.

Metal ions	Freundlich isotherm model				Langmuir isotherm model		
	Q_{exp} (mg/g)	n (g/L)	K_F (mg/g)	R^2	$Q_\circ$ (mg/g)	b (L/mg)	R^2
Zn^{2+}	35.51	5.37	4.75	0.60	95.23	0.085	0.94
Hg^{2+}	31.25	2.56	3.38	0.069	147.05	0.040	0.98

Table 3. The effect of the concentration of zinc and mercury in solution on the removal by *A. tubingensis* Merv4 at different contact times.

Contact time (h)	Metal concentration (mg/L)									
	100		200		300		400		500	
	Removal potentiality (%)									
	Zn^{2+}	Hg^{2+}	Zn^{2+}	Hg^{2+}	Zn^{2+}	Hg^{2+}	Zn^{2+}	Hg^{2+}	Zn^{2+}	Hg^{2+}
1	38	40	41	38	45	30	50	18	52	12
2	50	51	55	49	61	41	64	31	70	29
4	59	59	60	60	65	52	73	33	80	31
6	68	69	73	72	80	61	83	37	90	33
8	74	80	80	80	87	65	92	39	100	39
12	77	82	80	85	90	71	97	40	100	40
24	81	85	90	98	97	75	100	42	100	42

On the other hand, Hg^{2+} was totally adsorbed from aqueous solution (100% removal) after 12 to 24 h of treatment at 30-35°C then adversely affected (80% at 40°C and 70% at 45°C, Table 5). Temperature of the process can be crucial in energy-based mechanisms in heavy metal absorption by fungi as reported by Martínez-Juàrez, *et al.* (2012) for mercury absorption by fungus *M. rouxii* IM-80.

Determination of *A. tubingensis* Merv4 resistance profile against different heavy metals

Aspergillus tubingensis Merv4 showed resistance to heavy metals at variable level as indicated *by* the MIC value of these metals towards them (Table 6). MIC values of cadmium, lead, copper, mercury, silver, chromium (VI), nickel, zinc, ferric and aluminum ions were found to be 650, 510, 700, 615, 430, 500, 1000, 810, 422, and 1000 µgmL^{-1}, individually. These data have confirmed endophytic fungi isolated from manufacturing zone as potential multi-metal resisted fungi. It seems that the constant exposure of fungal community in the manufacturing zone against the heavy metals of effluents has exerted selective pressure on the fungal population, leading to the development of multi resistance to different heavy metals. Our results are consistent with the previous study of El-Gendy, *et al.* (2017); Bedioui, *et al.* (2015); Akhtar, *et al.* (2013) and Alzahrani, *et al.* (2017).

Removal of different metal ions from industrial wastewater using endophytic *A. tubingensis* Merv4

The biosorption activities of different metals including Zn^{2+} and Hg^{2+}, which are the focus of our study from real effluents, were estimated with wastewater of electroplating industry using dead fungal biomass of *A. tubingensis* Merv4 under the optimized conditions (Table 6). It reduce metals ions as follow Cd^{2+} (70%), pb^{2+} (81%), Cu^{2+} (65%), Hg^{2+} (91%), Ag^{+} (93%), Cr^{6+} (92%), Ni^{2+} (90%), Zn^{2+} (96%), Fe^{3+} (70%) and Al^{3+} (91%), respectively from the real industrial wastewater. Our results are in agreement with the previous study of El-Gendy and El-Bondkly, (2016) and El-Gendy, *et al.* (2011 and 2017).

FTIR spectral analysis

Light shift was observed for other distinctive bands, which showed the involvement of functional groups in the adsorption process. The predominant peaks were attributed to OH (3200, 3500, 3442/cm), CH_2 (2930, 2918, 2920/cm), C=O (1651, 1656, 1658/cm), amides (1447, 1440, 1431/cm), CO (1360, 1374, 1382/cm) and C-O-C (1079, 1083, 1071/cm) for unloaded biomass, biomass loaded with zinc and biomass loaded with mercury, respectively (Table 7). Our data were in consistent with El-Gendy, *et al.* (2017) study.

Table 4. Reduction potentially of Zn^{2+} and Hg^{2+} by *A. tubingensis* Merv4 at different pHs with different contact times.

Contact time (h)	Reduction (%) of zn^{2+} and Hg^{2+} $^{+}$ at different pHs											
	4		4.5		5.0		5.5		6.0		6.5	
	Zn^{2+}	Hg^{2+}	Zn^{2+}	Hg^{2+}	Zn^{2+}	Hg^{2+}	Zn^{2+}	Hg^{2+}	Zn^{2+}	Hg^{2+}	Zn^{2+}	Hg^{2+}
1	20	29	23	40	29	45	38	50	38	50	38	50
2	38	33	40	49	45	62	50	64	64	64	54	64
4	39	44	41	52	50	69	60	73	100	93	100	73
6	42	47	46	59	62	77	100	83	100	93	100	63
8	45	50	50	59	100	81	100	92	100	92	60	82
12	48	58	57	64	100	85	100	100	63	87	52	75
24	49	65	60	70	100	85	93	100	50	80	49	60

Table 5. The effect of temperature on reduction potentially of Zn^{2+} and Hg^{2+} by *A. tubingensis* Merv4 at different contact times.

Contact time (h)	Reduction (%) of $Zn^{2+}$and Hg^{2+} at different temperatures							
	30°C		35°C		40°C		45°C	
	Zn^{2+}	Hg^{2+}	Zn^{2+}	Hg^{2+}	Zn^{2+}	Hg^{2+}	Zn^{2+}	Hg^{2+}
1	38	50	39	50	20	41	13	33
2	50	64	60	64	28	52	21	39
4	59	73	76	73	39	56	32	42
6	70	83	89	83	49	61	43	44
8	80	92	100	92	52	70	49	50
12	100	100	100	100	74	75	63	57
24	100	100	100	100	62	80	60	70

Table 6. Minimum inhibition concentration (MIC) of different heavy metals against *A. tubingensis* Merv4 and removal of different heavy metals from industrial wastewater

Metal ions	MIC (μg/mL)	Reduction of metal from industrial waste water (%)
Cd^{2+}	650	70
Pb^{2+}	510	81
Cu^{2+}	700	65
Hg^{2+}	615	91
Ag^{+}	430	93
Cr^{6+}	500	92
Ni^{2+}	1000	90
Zn^{2+}	810	96
Fe^{3+}	422	70
Al^{3+}	1000	91

Table 7. Wave numbers of the dominant peaks obtained by FTIR spectral analysis of unloaded biomasses and loaded biomasses with zinc and mercury individually

Treatment	Functional groups/cm					
	OH	CH_2	C=O	Amides	C-O	C-O-C
Unloaded Biomass	3200	2930	1651	1447	1360	1079
Biomass + Zn^{2+}	3500	2918	1656	1440	1374	1083
Biomass + Hg^{2+}	3442	2920	1658	1431	1382	1071

CONCLUSION

This investigation indicated that mercury and zinc removal by different autoclaved fungal biomasses was estimated and then *A. tubingensis* Merv4 was selected as the hyperactive strain. The performance of the biosorbent was studied as a function of the operating circumstances, especially initial metal concentration, biomass dose, incubation time, different operation pH, temperature and time. It was proved that *A. tubingensis* Merv4 is a very promising biological material to remove the metal ion studied.

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