

Optimization and Statistical Evaluation of Copper and Nickel Biosorption Capabilities by Dry Biomass of *Penicillium oxalicum* JQ624873

Rania M.A. Abedin

Botany and Microbiology Department, Faculty of Science, Alexandria University, Egypt.

rania.abedin@gmail.com

Abstract: Heavy metal resistant fungus was tested to evaluate its applicability for heavy metal biosorption. A screening experiment revealed that *Penicillium oxalicum* JQ624873 was resistant to CuSO₄, NiCl₂ and PbSO₄. It showed efficient biosorption of Cu(II) and Ni(II) using either alive (59 % and 55 %, respectively) or dead biomass (48 % or 47 % respectively) at 30°C for 72 h at pH (4-4.2). Biosorption was highly depended on pH and maximum biosorption of Cu(II) (65%) and Ni(II) (60%) were obtained at pH 5. Plackett-Burman design was applied to optimize biosorption conditions. The increase in Ni(II) biosorption was mainly affected by time of incubation, washing of cells and temperature. The increase in Cu(II) biosorption was mainly affected by washing of cells, mixing speed and metal concentration. After optimization, biosorption efficiency of Ni(II) and Cu(II) increased up to 80 % and 94 % respectively.

[Rania M.A. Abedin. **Optimization and Statistical Evaluation of Copper and Nickel Biosorption Capabilities by Dry Biomass of *Penicillium oxalicum* JQ624873.** *Life Sci J* 2014;11(2):61-67]. (ISSN:1097-8135). <http://www.lifesciencesite.com>. 8

Key words: *Penicillium oxalicum* JQ624873, Copper biosorption, Nickel biosorption, Dry Biomass, Plackett-Burman.

1. Introduction

Heavy metal release to the environment has been increasing continuously because of industrial activities and technological development and poses a significant threat to the environment, public and soil health. With the rapid development of many industries, wastes containing metals has been directly or indirectly discharged into the environment causing serious environmental pollution and affecting human health (Das et al., 2008).

Contamination of agricultural soil with heavy metals is a major problem on industrial and defense related sites all over the world (Hemambika et al., 2011; Parameswari et al., 2010).

Heavy metals include cadmium, lead, chromium, copper and nickel, which contaminate the soils, ground water, sediments and surface waters are extremely toxic to biological and ecological systems. The response of microorganisms towards toxic heavy metals is of importance in view of the interest in the reclamation of polluted sites (Shankar et al., 2007). Microorganisms uptake metal either actively (bioaccumulation) and/or passively (biosorption) (Hussein et al., 2003). Biological process like biosorption has acquired due attention owing to number of advantages and engaged the scientists from all over the world to identify the potent biomass type (Al-Masri et al., 2010; Xiao et al., 2010). Biosorption refers to the passive metal uptake by different forms of biomass, which may be dead or alive.

Microbial populations in metal polluted environments adapt to toxic concentrations of heavy

metals and become metal resistant. Different species of *Aspergillus*, *Pseudomonas*, *Sporophycticus*, *Bacillus*, *Phanerochaete*, etc., have been reported as efficient chromium and nickel reducers (Yan and Viraraghavan, 2003). Fungus belongs to groups of organisms with very well known heavy metal sorption capacity. Fungi are known to tolerate and detoxify metals by several mechanisms. Fungal biosorption indicated that the cell surface functional groups of the fungus might act as ligands for metal sequestration resulting in removal of the metals from the aqueous culture media (Pal et al., 2010).

The objective of this study is to optimize, evaluate and compare the tolerance and capability of *Penicillium oxalicum* JQ624873 towards biosorption of [Cu(II), Ni(II), Pb(II), Zn(II) and Co(II)] using dead and alive biomass of the fungal cells. Also, to use statistical designs to optimize the conditions for heavy metal biosorption. The influence of pH, contact time, temperature and initial metal ion concentration on metal biosorption was evaluated.

2. Materials and methods

Fungal strain and heavy metals

Penicillium oxalicum JQ624873 was isolated from agricultural Egyptian soil by using potato dextrose agar medium (PDA) and was identified morphologically, microscopically and by molecular methods (Rania et al., 2013). It was maintained on glucose peptone medium containing 20 g/l glucose, 20 g/l peptone, 5 g/l yeast extract, and 15 g/l agar, at pH 7. Heavy metals used in this work were obtained from the

Microbiology Laboratory, Faculty of Science, Alexandria University, Egypt. Stock solutions of analytical grade metals (CuSO₄, NiCl₂, PbSO₄, ZnCl₂, CoSO₄) were prepared in distilled water in different concentrations as (5, 10, 20, 30, 40 and 50 mg/ml) and autoclaved for 15 min at 121°C.

Alive and dead Biomass production of test fungal strain

P. oxalicum JQ624873 was cultured in a medium containing 20 g/l glucose, 1 g/l KH₂PO₄, 0.5 g/l (NH₄)₂SO₄, 0.5 g/l MgSO₄·7H₂O, 0.01 g/l yeast extract, 0.01 g/l CaCl₂·2H₂O, 0.001 g/l CuSO₄·5H₂O, 0.001 g/l Fe₂(SO₄) and 0.001 g/l MnSO₄·H₂O at pH 4-4.2. One liter of the previous medium was distributed into 250 ml conical flasks and was inoculated by taking active inoculums from preserved stock culture. Cultures were incubated for 7 days at (25±1) °C and 150 rpm. After the incubation period, biomass was separated from culture broth by filtration and was subjected to successive washings with double distilled deionized water. The biomass was dried at 50 °C for 24 h. These washed and dried cells were thereafter called alive biomass. In case of dead biomass, the filtrated mycelial mass was killed by autoclaving at 121°C for 20 min, washed several times with double distilled deionized water and then dried at 50 °C for 24 h (Park et al., 2005). The alive and dead biomass was kept in a desiccator for the biosorption experiments.

Determination of heavy metal-resistance of the fungal isolate by plate diffusion method

Heavy metal resistance of the fungus was determined by plate diffusion method (Hassen et al., 1998). Heavy metal salt solutions were prepared in different concentrations as (5, 10, 20, 30, 40 and 50 mg/ml). Each plate containing 15 ml of solidified glucose peptone medium was spread and inoculated with 1 ml of 72 h old *P. oxalicum* culture. To each plate, 100 µl of appropriate metal salt solutions were added in each well of 10 mm in diameter and 4 mm in depth. *P. oxalicum* plates were incubated at 30°C for

48 h. After incubation, the zone of inhibition was measured. An inhibition zone size less than 1 mm was scored as resistance strain for that heavy metal.

Measurement of metal biosorption

Aliquots (0.02 g) of alive or dead biomass were suspended in 4 ml solution containing 10 mg/ml heavy metal (pH 4-4.2) and were incubated for 72 h at 30°C and 150 rpm. After centrifugation (6000 rpm for 15 min) the concentration of the remaining heavy metal in the supernatant was measured by Atomic Absorption Spectrophotometry (Perkin Elmer-2380) in Central Laboratory in Mohrem bek, Faculty of Science, Alexandria. Biosorption efficiency was characterized by the following formula:

Percentage of metal biosorption = $100 \frac{(I - F)}{I}$, here, I is the initial metal concentration of the solution and F is the remaining metal concentration after biosorption. Each measurement was carried out in triplicates and mean values were calculated using the data of at least 2 independent experiments.

Optimization of pH on heavy metal biosorption

In separate experiments, the pH of the heavy metal solution was varied from 3 to 11 and the pH with the highest percentage of metal biosorption was regarded as optimal pH for biosorption.

Statistical analysis: Plackett-Burman design

The effect of 11 variables on metal biosorption was studied by applying the Plackett-Burman experimental design (Plackett and Burman, 1946; Rajendran et al., 2007) (Table 1 and 2). In this experiment, 11 factors (metal concentration, washing cells, incubation time, temperature, dry weight, glucose concentration, addition of (NH₄)₂SO₄, MgSO₄·7H₂O and KH₂PO₄, sterilization and mixing speed) were screened in 12 combinations organized according to the Plackett-Burman matrix. A 50% increase of the original component level of metal concentration is represented by the "+" sign, while 50% decrease of the original component level is represented by the "-" sign.

Table 1. Different levels of the eleven independent variables used in the Plackett-Burman design.

Factor	Abbreviation	High Level (+)	Low Level (-)
Metal concentration	M	10 mg/ml	5 mg/ml
Washing cells	W	Washed cells	Unwashed
Incubation time	I	2 days	5 days
Temperature	Tm	30 °C	25 °C
Dry weight	D	5 mg/ml	2.5 mg/ml
Glucose	G	2 %	1 %
(NH ₄) ₂ SO ₄	Am	0.05 %	0 %
MgSO ₄	Mg	0.05 %	0 %
KH ₂ PO ₄	Po	0.1 %	0 %
Viability	V	Alive biomass	Dead biomass
Mixing speed	S	150 rpm	Static

Table 2. Response to Plackett Burman design of 11 variables and 12 trials

Trial no	Variables											Response (% of biosorption of Ni)	Response (% of biosorption of Cu)
	M	W	I	Tm	D	G	Am	Mg	Po	V	S		
1	+	+	-	+	+	+	-	-	-	+	-	55	62
2	+	-	+	+	+	-	-	-	+	-	+	47	73
3	-	+	+	+	-	-	-	+	-	+	+	65	68
4	+	+	+	-	-	-	+	-	+	+	-	65	66
5	+	+	-	-	-	+	-	+	+	-	+	15	63
6	+	-	-	-	+	-	+	+	-	+	+	2	71
7	-	-	-	+	-	+	+	-	+	+	+	22	96
8	-	-	+	-	+	+	-	+	+	+	-	48	68
9	-	+	-	+	+	-	+	+	+	-	-	55	66
10	+	-	+	+	-	+	+	+	-	-	-	62	65
11	-	+	+	-	+	+	+	-	-	-	+	56	71
12	-	-	-	-	-	-	-	-	-	-	-	15	70

The main effect of each variable was determined using the following equation:

$$Exi = (\sum mi+ - \sum mi-) / N$$

Where, Exi is the variable main effect, Mi+ and Mi- are the sum of the recorded results of biosorption percentage in trials which contain + and - levels of independent variables (xi), respectively, (Table 1) and N is the number of trials divided by two. A main effect figure with a positive sign indicates that the + level of this variable is nearer to optimum metal biosorption percentage, while a negative sign indicates that the - level of the variable is closer to the optimal value. Using the Microsoft Excel program, statistical t-values for equal unpaired samples were calculated for the determination of variable significance. Each treatment was carried out in triplicates and the results obtained throughout the work were the arithmetic mean of at least 2 experiments.

Factors affecting Biosorption

Effect of temperature on Ni Biosorption

The influence of incubation temperature with increasing of the incubation time on the biosorption of nickel by *P. oxalicum* JQ624873 was studied by varying the temperature of the metal solution in the biosorption experiments in the range (30-45°C) during the incubation period from (3-8 days) at pH 5, Ni concentration (10 mg/ml) and biomass concentration (2.5 mg/ml) under static conditions. Based upon the heavy metal removal, the optimal temperature was determined.

The Influence of concentration of synthetic copper solution on Cu(II) biosorption

To explore the tolerance of the isolate to the heavy metal, optimal culture conditions were used with varying initial copper concentration in the range (2.5-10 mg/ml) during the incubation period from (1-5

days). Experiment was carried out at pH 5, temperature of (30°C) and biomass concentration (2.5 mg/ml) with the purpose of observing the effect of copper concentration on the rate of metallic biosorption. All the experiments were carried out in triplicates.

3. Results and Discussion

Heavy metal resistance efficiency and screening experiment

P. oxalicum JQ624873 was resistant to CuSO₄ and NiCl₂ followed by PbSO₄ but not to ZnCl₂ or CoSO₄ (data not shown). The highest biosorption efficiency was observed with Cu(II) and Ni(II) using either alive (59 % and 55 %, respectively) or dead biomass (48 % or 47 % respectively) (Figure 1). The observed differences in data obtained in biosorption efficiency between alive and dead biomass may be explained with the heat modification of the cell wall due to sterilization process.

Fungi offer a wide range of chemical groups that can attract and sequester the metals in biomass. Cell walls are composed of structural polysaccharides, proteins and lipids that offer metal-binding functional groups (Veglio and Beolchini, 1997). Cu(II) and Ni(II) biosorption were selected for widely used due to the toxicity of them to living organisms, their presence in the environment cause serious toxicological concerns (Yilmaz et al., 2010). According to Bayramoglu et al. (2005) and Wang and Chen (2006) fungal biosorbents were characterized with high affinity for Cu(II) and Ni(II) as compared to other metal ions.

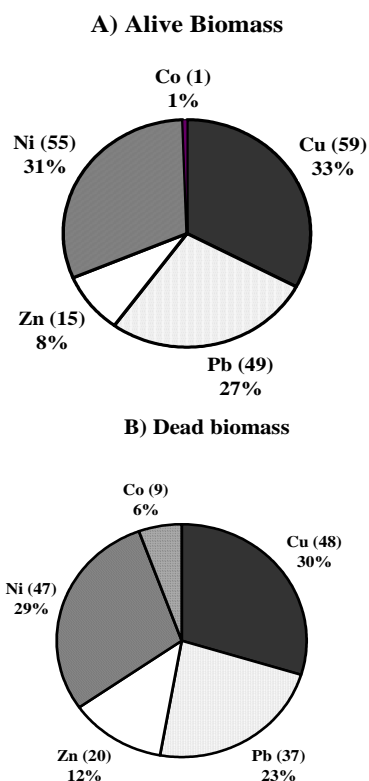


Figure 1. Biosorption percentage of different heavy metals by a) Alive biomass and b) Dead biomass of *P. oxalicum* JQ624873. Temperature: 30 °C, incubation time: 72 h, concentration: 10 mg/ml and pH (4-4.2).

Optimal pH for heavy metal biosorption by *Penicillium oxalicum* JQ624873

Biosorption efficiency was highly depended on the pH. Best results were obtained at pH 5 with both Cu(II) and Ni(II) (Figure 2); therefore we used this pH value in all the further experiments. Above pH 5, the percent biosorption for both the metals decreased gradually. At higher alkaline pH values (8 and above), a rapid reduction in biosorption capacity of both metals was observed.

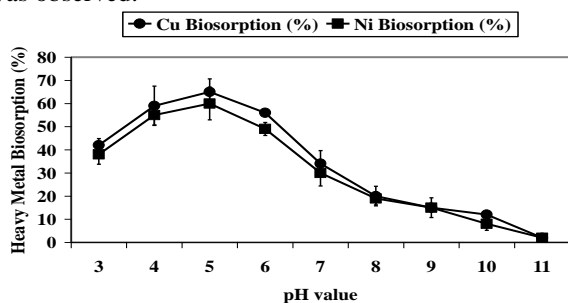


Figure 2. Biosorption of Cu(II) and Ni(II) by *P. oxalicum* JQ 624873 at various pH values. Mean±S.D. values calculated from 3 independent experiments are presented.

It was proven previously by Yilmaz et al. (2010), that maximum heavy metal biosorption capacities were attained at low pH values, 5.0 and 6.0. Low removal of nickel at low pH by *P. chrysogenum* has also been reported (Tian et al., 2004). Fungal surfaces have a negative charge on pH range from 2 to 6, which can be beneficial for biosorption (Yan and Viraraghavan, 2003). The increase in percent removal of metal with increase in pH up to 5 is due to the strong relations of bioaccumulation to the number of surface negative charges, which depends on the dissociation of functional group (Yakup et al., 2004).

Elucidation of different factors affecting metal biosorption by *Penicillium oxalicum* JQ624873 Plackett-Burman design for Ni and Cu Biosorption

The Plackett-Burman design was used to identify factors affecting significantly the biosorption. 11 variables were tested (Table 1). All experiments were performed in duplicate and the average results as percentage of copper and nickel biosorption were presented in Table 2. The results of main effect were presented in Table 3, 4 and Figure 3. According to the data, Ni(II) biosorption highly depended on the incubation time and temperature (Table 3, Figure 3). Washing of the cells and shaking of the cultures were also important (Table 3, Figure 3). In case of Cu(II), the tested variables had less effect on biosorption (Table 4, Figure 3). Concentration of the metal, $(\text{NH}_4)_2\text{SO}_4$ and MgSO_4 as well as washing of the cells and shaking of the cultures had the biggest effect (Table 4, Figure 3).

The Plackett-Burman statistical design examined 11 factors affecting Nickel and copper biosorption (Andreazza et al., 2010). These factors affect general microbial metabolism and specifically promote or decrease biosorption (Ozer et al., 2009; Tunali et al., 2006).

Time course of Ni biosorption by *Penicillium oxalicum* JQ624873 at various temperatures

Metal biosorption by *P. oxalicum* JQ624873 increased with the increase in incubation time and incubation temperature but to a certain limit. Maximum nickel biosorption efficiency (80%) was obtained at 35°C after 6 days of incubation (at pH 5 using 10 mg/ml Ni(II) and 2.5 mg/ml biomass concentrations under static conditions) (Figure 4). Bioaccumulation of nickel by fungal species appears to be temperature dependent.

Asku et al. (1992) reported that temperature does not influence the biosorption processes in the range of (20-35°C). The range of optimal temperature values (30-35 °C) were comparable to the range of room temperature that was used when isolating the microorganisms (Shankar et al., 2007). The temperature of the adsorption medium could be

important for energy dependent mechanisms in metal removal by microorganisms. Temperature is known to affect the stability of the cell wall, its configuration and can also cause ionization of chemical moieties. These

factors may simultaneously affect the binding sites on isolated fungal species causing reduction in heavy metal biosorption (Gulay et al., 2003).

Table 3. Main effect values for each variable for Ni(II) biosorption based on the Plackett Burman experiment

Variables	M		W		I		Tm		D		G		Am		Mg		Po		V		S	
	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
	55	65	55	47	47	55	55	65	55	65	55	47	65	55	65	55	47	55	55	47	47	55
	47	22	65	2	65	15	47	15	47	65	15	65	2	47	15	47	65	65	65	15	65	65
	65	48	65	22	65	2	65	2	2	15	22	65	22	65	2	65	15	2	65	55	15	48
	15	55	15	48	48	22	22	48	48	22	48	2	55	15	48	22	22	62	2	62	2	55
	2	56	55	62	62	55	55	56	55	62	55	62	48	55	56	48	56	22	56	22	62	62
	62	15	56	15	56	15	62	15	56	15	56	15	56	15	62	15	55	15	48	15	56	15
Mean	41	43	52	33	57	27	51	33	44	41	43	41	44	41	41	43	42	42	43	42	34	50
Main Effect	-2.5		19.1		29.9		17.5		3.1		1.5		2.9		-2.1		-0.5		1.1		-15.5	
t-statistic	-0.184		1.581		3.066		1.414		0.234		0.11		0.209		-0.16		-0.036		0.086		-1.226	
P-value	0.428		0.072		0.006		0.094		0.410		0.457		0.419		0.438		0.486		0.466		0.124	

Table 4. Main effect values for each variable for Cu(II) biosorption based on the Plackett Burman experiment

Variables	M		W		I		Tm		D		G		Am		Mg		Po		V		S	
	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
	62	68	62	73	73	62	62	66	62	68	62	73	66	62	68	62	73	62	62	73	73	62
	73	96	68	71	68	63	73	63	73	66	63	68	71	73	63	73	66	68	68	63	68	66
	66	68	66	96	66	71	68	71	71	63	96	66	96	68	71	66	63	71	66	66	63	68
	63	66	63	68	68	96	96	68	68	96	68	71	66	63	68	96	96	65	71	65	71	66
	71	71	66	65	65	66	66	71	66	65	65	66	65	68	66	71	68	71	96	71	96	65
	65	70	71	70	71	70	65	70	71	70	71	70	71	70	65	70	66	70	68	70	71	70
Mean	67	73	66	74	68	71	72	68	68	71	71	69	72	67	67	73	72	68	72	68	74	66
Main Effect	-6.5		-7.8		-2.8		3.5		-2.8		1.8		5.2		-6.2		4.2		3.8		7.5	
t-statistic	-1.380		-1.7		-0.535		0.666		-0.535		0.343		1.01		-1.232		0.8		0.732		1.556	
P-value	0.110		0.065		0.302		0.260		0.302		0.369		0.168		0.123		0.221		0.240		0.075	

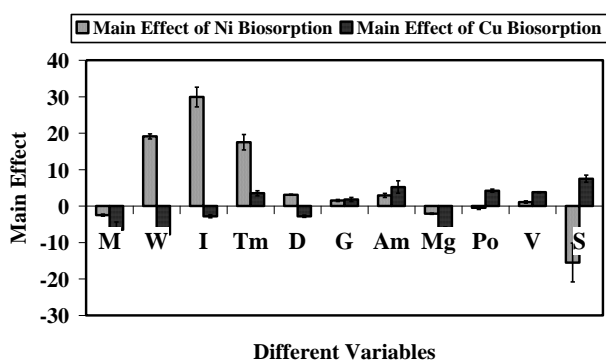


Figure 3. Main effects of Ni and Cu biosorption obtained by *P. oxalicum* JQ624873 at pH 5 according to Plackett-Burman design. Mean±S.D. values calculated from 3 independent experiments are presented.

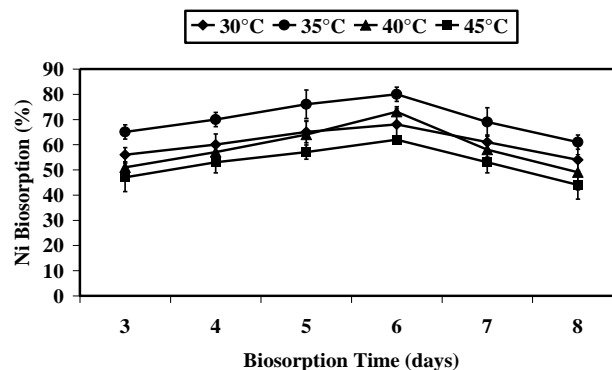


Figure 4. Time course of Ni Biosorption obtained by *P. oxalicum* JQ624873 at various incubation temperatures at 10 mg/ml of Ni, dry live biomass (2.5 mg/ml), pH 5 and static incubation. Trial 3,4. Mean±S.D. values calculated from 3 independent experiments are presented.

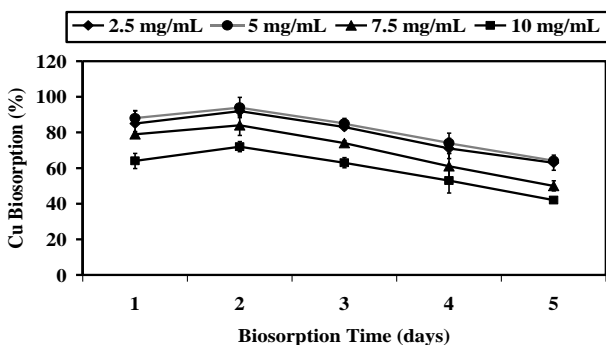


Figure 5. Time course of Cu Biosorption (%) obtained by *P. oxalicum* JQ624873 at various concentrations of copper at dry live biomass (2.5 mg/ml), 30 °C, pH 5 and in shaking conditions (150 rpm) Trial 7. Mean±S.D. values calculated from 3 independent experiments are presented.

Time course of Cu biosorption by *Penicillium oxalicum* JQ624873 at various concentrations of copper

Experiments on the influence of concentration of synthetic copper solution with an increasing of the incubation time on the biosorption of copper by *Penicillium oxalicum* JQ624873 at pH (5), temperature of (30°C) and biomass concentration (2.5 mg/ml) were carried out with the purpose of observing the effect of copper concentration in the range (2.5-10 mg/ml) during the incubation period from (1-5 days) on the rate of metallic biosorption. Maximum copper biosorption efficiency (94%) was obtained at (5 mg/ml) of $\text{CuSO}_4 \cdot 7 \text{H}_2\text{O}$ solution after two days of incubation (at pH 5 and 30°C under static conditions using 2.5 mg/ml biomass) (Figure 5).

The initial metal ion concentration seems to have an impact on biosorption, with a higher concentration resulting in a high solute uptake (Binupriya et al., 2007). An increase in copper concentration had a negative impact on biosorption efficiency (Das et al., 2008). According to Shankar et al. (2007), as the heavy metal concentration increases the fungal culture growth of all the isolates were inhibited. *Penicillium* sp. has the ability to withstand only at 20 mg/L concentrations of Cd. It was explained that at low metal concentrations (as encountered in effluent samples) the biosorption capacity of the biosorbents is not fully utilized (Rani and Harapriya, 2003).

Corresponding Author:

Assoc. Prof. Rania Mohamed Ahmed Abedin
Botany and Microbiology Department
Faculty of Science, Alexandria, Egypt
Email: rania.abedin@gmail.com

References

1. Al-Masri MS, Amin Y, Al-Akel B, Al-Naama T. Biosorption of Cadmium, Lead, and Uranium by Powder of Poplar Leaves and Branches. *Appl. Biochem. Biotechnol.* 2010; 160: 976-987.
2. Andrezza R, Pieniz S, Wolf L, Lee MK, Camargo FAO, Okeke BC. Characterization of copper bio-reduction and biosorption by a highly copper resistant bacterium isolated from copper-contaminated vineyard soil. *Sci. Total Environ.* 2010;408: 1501-1507.
3. Asku Z, Sag Y, Kutsal T. The biosorption of copper by *C. vulgaris* and *Z. ramgera*. *Environ. Technol.* 1992; 13: 579-586.
4. Bayramoglu G, Celik G, Yalcin E, Yilmaz M, Arica MY. Modification of surface properties of *Lentinus sajor-caju* mycelia by physical and chemical methods: evaluation of their Cr^{6+} removal efficiencies from aqueous medium. *J. Hazard. Materials* 2005; 119: 219-229.
5. Binupriya AR, Sathishkumar M, Kavitha D, Swaminathan K, Yun SE, Mun SP. Experimental and isothermal studies on sorption of Congo red by modified mycelial biomass of wood rotting fungus. *Clean* 2007; 35: 143-150.
6. Das N, Vimala R, Karthika P. Biosorption of heavy metals-an overview. *Ind. J. Biotechnol.* 2008; 7: 159-169.
7. Gulay B, Sema B, Yakup AM. Biosorption of heavy metal ions on immobilized white-rot fungus *Trametes versicolor*. *J. Hazard. Mater. B* 2003; 101: 285-300.
8. Hassen A, Saidi N, Cherif M, Boudabous A. Resistance of environmental bacteria to heavy metal. *Bioresour. Technol.* 1998; 64: 7-15.
9. Hemambika B, Rani MJ, Kannan VR. Biosorption of heavy metals by immobilized and dead fungal cells: A comparative assessment. *J. Eco. Nat. Envir.* 2011; 3(5): 168-175.
10. Hussein H., Farag S., Moawad H. (2003) Isolation and characterisation of *Pseudomonas* resistant to heavy metals contaminants. *Arab J. Biotechnol.*, 7, 13-22.
11. Ozer A, Gürbüz G, Çalimli A, Körbahti BK. 2009 Biosorption of copper(II) ion on *Enteromorpha prolifera*: application of response surface methodology (RSM). *J. Chem. Eng.* 2011; 146: 377-387.
12. Pal TK, Bhattacharyya S, Basumajumdar A. Cellular distribution of bioaccumulated toxic heavy metals in *Aspergillus niger* and *Rhizopus arrhizus*. *Int. J. Pharm. Biol. Sci.* 2010; 1(2): 1-6.
13. Parameswari E, Lakshmanan A, Thilagavathi T. Biosorption and metal tolerance potential of filamentous fungi isolated from metal polluted

- ecosystem. *Electron. J. Environ. Agric. Food Chem.* 2010; 9(4): 664-671.
14. Park D, Yun YS, Jo JH, Park JM. Mechanism of hexavalent chromium removal by dead fungal biomass of *Aspergillus niger*. *Water Research* 2005; 39: 533-240.
 15. Plackett RL, Burman JP. The design of optimum multifactorial experiments. *Biometrika* 1946; 33: 305- 325.
 16. Rajendran A, Sundaramurthy AB, Thangavelu V. Statistical Evaluation of Medium Components Using Plackett-Burman Experimental Design and Kinetic Modeling of Lipase Production by *Bacillus sphaericus*. *Chem. Biochem. Eng. Q.* 2007; 21: 181-188.
 17. Rani G, Harapriya M. Microbial biomass: An economical alternative for removal of heavy metals from wastewater. *Indian J. Exp. Biol.* 2003; 41: 945-966.
 18. Rania MAA, El Hanafy AA, Abd El-Latif S, El-Assar SA, Fadel MS. Ligninolytic Oxidative System of Fungi Egyptian Isolates and Their Applications in the Decolorization of Industrial Dyes. 2013; 27(6): 4269-4275.
 19. Shankar C, Sridevi D, Joonhong P, Dexilin M, Thamaraiselvi K. Biosorption of chromium and nickel by heavy metal resistant fungal and bacterial isolates. *J. Hazard. Mater.* 2007; 146: 270-277.
 20. Tian-Wei T, Hu B, Haijia S. Adsorption of Ni²⁺ on amine-modified mycelium of *Penicillium chrysogenum*. *Enz. Microb. Technol.* 2004; 35: 508-513.
 21. Tunali S, Çabuk A, Akar T. Removal of lead and copper ions from aqueous solutions by bacterial strain isolated from soil. *J. Chem. Eng.* 2006; 15: 203-211.
 22. Veglio F, Beolchini F. Removal of metals by biosorption: a review. *Hydrometallurgy* 1997; 44: 301-316.
 23. Wang J, Chen C. Biosorption of heavy metals by *Saccharomyces cerevisiae*: A review. *Biotechnol. Adv.* 2006; 24: 427-451.
 24. Xiao X, Luo SL, Zeng GM, Wan WZY, Chen L. Biosorption of cadmium by endophytic fungus (EF) *Microsphaeropsis* sp LSE10 isolated from cadmium hyperaccumulator *Solanum nigrum* L. *Bioresour. Technol.* 2010; 101: 1668-1674.
 25. Yakup AM, Gulay B, Meltem Y, Sema B, Omer G. Biosorption of Hg²⁺, Cd²⁺, and Zn²⁺ by Ca-alginate and immobilized wood-rotting fungus *Funalia troglia*. *J. Hazard. Mater. B* 2004; 109: 191-199.
 26. Yan G, Viraraghavan T. Heavy metal removal from aqueous solution by fungus *Mucor rouxii*. *Water Res.* 2003; 37: 4486-4496.
 27. Yilmaz M, Tay T, Kivanc M, Turk H. Removal of copper II ions from aqueous solution by a lactic acid bacterium. *Brazilian J. Chem. Eng.* 2010; 27: 309-314.