European Journal of Biotechnology and Bioscience
Online ISSN: 2321-9122
www.biosciencejournals.com
Volume 3; Issue 10; October 2015; Page No. 20-25

Removal of insoluble inorganic compounds from aqueous suspensions by living and dead various fungal biomass

AM Saad

Microbial Chemistry Dept. National Research Centre, 33 Bohouth St., Dokki, Giza, Egypt.

Abstract

The bio-adsorption efficiency of insoluble compounds and particulates by various fungal biomass (live or heat killed) varied considerably among different fungal species. The biomass of *Rhizopus* spp. and *Mucor* spp. showed the highest bio-adsorption efficiency (80-100%) towards the tested compounds compared to other fungi. However, bio-adsorption percentage with *Aspergillus niger* and *Penicillium chrysogenum* was relatively low. In fact the statistical analysis (analysis of variance) indicate that the type of fungal biomass is the most important criteria affecting the bio-adsorption process. The result shows that, in addition to fungal species differentiation, there is also a significant fungal depended selectivity for the adsorbed compound. The status of the fungal biomass (live or killed) is a significant factor for efficiency of adsorption. The use of M. *rouxii* NRRL 1894 biomass in the form pelleted biomass for the removal of insoluble- inorganic compounds was less efficient than the dispersed biomass. The adsorption was found to be influenced by time and temperature. *Rhizopus* sp. adsorbed increasing amount of $CaCO_3$ with increasing length of exposure, also the removal% of $CaCO_3$ by *Rhizopus* sp. biomass was efficient within a temperature range of 20 °C to 40 °C. A decrease of $CaCO_3$ adsorption was observed at 10 °C.

Keywords: Fungi, Biomass, Bio-adsorption, Removal, Insoluble-inorganic compounds.

Introduction

A recent development in environmental biotechnology is the use of microbial biomass as biosorbents for heavy metals [1, 2]. The biosorption of metal ions and radionuclide by microbial biomass has been extensively studied [2, 3]. However, studies on the adsorption of insoluble inorganic compounds and particulates are particularly sparse [4, 5, 6]. The metal-bearing industrial effluents in many cases contain both insoluble-inorganic compounds as well as metal ions. Metal ions in water can occur naturally from anthropogenic sources and from leaching of ore deposits, which mainly include solid waste disposal and industrial effluents. The level of heavy metals in water system has substantially increased over time with rapid development of industrial activities [7]. Adsorption is one of the safest, easiest, and most cost-effective methods because it is widely used in effluent treatment processes [8]. The major advantages of biosorption are the use of inexpensive biosorbents and its high effectiveness in reducing the heavy metal ions [9]. Biosorption are particularly suitable to treat dilute heavy metal wastewater. Typical biosorbents can be derived from three sources as follows [10, 11]. 1- Nonliving biomass such as bark, lignin shrimp, krill, squid, and crab shell; 2- algal biomass; 3- microbial biomass for example, bacteria, fungi, and yeast. This paper reports on search for fungal strains which show enhanced adsorption properties for insoluble-inorganic compounds and particulates.

2. Materials and Methods

2.1 Microorganisms and culture conditions

Mucor sp. NRRL 8135, *Mucor rouxii* NRRL 1430, *Mucor rouxii* NRRL 1894, *Thermomucor indicae-seudaticae* NRRL 6429, and *Aspergillus niger* NRRL 595 were obtained from ARS culture collection (NRRL), Peoria, Illinios, USA. *Rhizopus* sp., *Rhizopus nigricans* and *Penicillium chrysogenum* were obtained from the Microbial Chemistery Department culture collection, NRC. *Mucor* spp. and *Rhizopus* spp. were maintained on PDA medium at 4 °C. *A. niger* and *Penicillium chrysogenum* were maintained on agar slants of YPG medium at 4 °C and subcultured every two weeks.

2.2 Media

2.2.1. Potato dextrose medium: contained (g/l), potato, 300; glucose monohydrate, 20 with or without agar, 20.

2.2.1. Yeast extract peptone glucose (YPG): contained (g/l), yeast extract, 10.0; peptone, 30.0 and glucose monohydrate, 40.0 with or without agar, 20.

2.3 Inocula

Discs 4mm diameter were cut with a sterile cork borer from the edge of growing colonies and transferred to flasks containing PDA or YPG liquid medium (2 discs/ 50ml).

2.4 Growth conditions

The cultures were incubated either static or shaken (150rpm) at 28 °C for 3 days except *Thermomucor indicae-seudaticae* was incubated at 40 °C. The biomass was harvested by filtration, washed several times with distilled water and dried between two filter papers.

3. Ability of fungi to adsorb insoluble-inorganic compounds [4].

The precultured fungal biomass (5g wet weight; 0.39 g dry weight) either live or heat killed by boiling with distilled water for 15 min. was added to sterile distilled water (100 ml) in 250 ml flasks amended with sterile amounts of one of the following compound (0.7g): basic lead carbonate, calcium carbonate, calcium silicate, elemental sulphur and zinc dust. The flasks were incubated in duplicate for 18h, at 30 °C. By the end of the experiment the content of each flask was filtered through pre-weighed filter papers (Whatman No.1). The biomass was removed from the filter paper and the non-adhering insoluble compound was removed by washing the biomass several times with distilled water onto the filter paper which was then dried at 45 °C for 24h to constant weight. The amount of adsorbed compound by biomass was calculated by subtracting the weight of compound on the filter paper from the weight of compound initially added to the flask. Appropriate controls were prepared and treated simultaneously as the experiment.

3.1 Factors affecting bio-adsorption

The conditions affecting $CaCO_3$ bio-adsorption by fungal biomass was studied.

3.1.1 Equilibrium experiments

In order to determine the minimum time of equilibration for maximum adsorption of calcium carbonate (0.7% w/v) by *Rhizopus* sp. biomass (live and heat killed), several equilibrium experiments were conducted at different times 3,6,9,15,18 or 24h at 30 °C.

3.1.2 Effect of temperature

The effect of different temperatures (10, 20, 30 or 40 °C) on the adsorption of calcium carbonate during 6h period was investigated.

3.1.3 Effect of type of growth form on adsorption of insoluble inorganic compounds

The fungus *M. rouxii* NRRL 1894 was grown in pellet form in submerged culture or in mycelial form in surface culture. The experimental conditions were performed as described above in No.3.

4. Statistical analysis

For the experiments of insoluble-inorganic compounds, the experiments were conducted in duplicate; the analysis of variance was conducted according to the simple factorial design of experiment as described by [12].

5. Results and Discussion

5.1 Adsorption of insoluble-inorganic compounds by various fungi.

The biomass (live or heat killed) of eight species of fungi were separately added to aqueous suspensions of each of the following compounds at a concentration of 0.7% (w/v): basic lead carbonate, calcium carbonate, calcium silicate, elemental sulphur or zinc dust, and kept shaken for 18h at 30 °C. The adsorption percentages of these compounds are presented in Table (1). It is evident from these results that the amounts of adsorbed compounds (mg/g wet weight biomass) differ quite markedly among biomass of different fungal species.

Biomass of *Rhizopus* spp. and *Mucor* spp. were more efficient in adsorption than *Aspergillus niger* or *penicillium chrysogenum* biomass. Saad [5] found that the non-growing *Mucor rouxii* and *Rhizopus* sp. biomass effectively adsorbed insoluble-inorganic compounds when present as a mixture. In general the living biomass was superior in adsorption than the heat killed biomass. The adsorption percentage of insoluble-inorganic compounds and particulates by *Rhizopus* spp. and *Mucor* spp. biomass was 80-100 % in suspensions containing up to 0.7 % (w/v). However, bio-adsorption with *A. niger* and *P. chrysogenum* was relatively low. Similar finding had been reported by [4] who found that *A. niger*, *Fusarium solani* and *Penicillium notatum* adsorbed ochre much less effectively than did *Mucor flavus*. However, the result of the present work indicate that *M. rouxii* and *Rhizopus* sp., were more efficient in adsorption than *M. flavus* reported in the study of [4] with respect to the relative short period required for contact of the biomass and compounds as well as their relative higher efficiency in suspension containing increased amounts of compounds or particulates. Therefore, it is concluded that the taxonomic grouping of the fungus is an important factor which influences bio-adsorption. *Rhizopus* and *Mucor* are members of the order *Mucorales,* while *A. niger* and *P. chrysogenum* belong to *Ascomycetales*. The major macromolecular constituents of *Mucorales* cell walls are chitosan and chitin, while that of *Ascomycetales* are glucans and chitin [13]. Chitosan, which is distinctive for *Mucorales* has been implicated in the uptake of heavy metal ions [14].

The statistical analysis (analysis of variance) presented in Table (2) indicate that the type of fungal biomass (live or heat killed) is the most important criteria affecting the adsorption process (statistical f = 6.53**hs). The result show that, in addition to fungal species differentiation (f= 56.12**hs), there is also a significant fungal dependent selectivity for adsorbed compound (f = 33.31**hs). For almost all species of fungi, the quantity of basic lead carbonate or zinc dust adsorbed per gram biomass showed the highest value, while the least amounts were those of calcium silicate and elemental sulphur. Calcium carbonate adsorption showed an intermediate value. The adsorption process of lead carbonate, elemental sulphur or zinc dust from distilled water by fresh biomass of *Rhizopus sp*. is shown in Figures 1, 2, 3.

5.2. Factors affecting the adsorption of calcium carbonate by Rhizopus sp.

5.2.1. Time course of calcium carbonate adsorption

Time course of calcium carbonate adsorption by *Rhizopus* sp. biomass (live or heat killed) was examined (Figure 4). Several experiments at different equilibration times ranging from 3 to 24h were conducted. The results show that the living biomass of *Rhizopus* sp. adsorbed increasing amounts of calcium carbonate with increasing the period of exposure, culminating in adsorption values of 70.92% at 18h and 100% at 24h. The living biomass had a much higher capacity for calcium carbonate adsorption in comparison with the heat killed biomass, particularly during the period of initial rapid adsorption, where a 3-fold increase in adsorption rate was observed in the first 3h. It is to be noted that by increasing the period of contact between heat killed biomass and calcium carbonate > 18h., the adsorption percentage decreased (46%). The mechanism of adsorption has not been studied in detail. However, it was presumed to be related to surface charge on the mycelium [4]. The physical nature of particulate metal forms may be altered by the fungus prior to adsorption. For example, when *M. flavus* was grown with lead sulfide, particles of this compound were converted to a fine even suspension which was then completely adsorbed after one week [4].

5.2.2. Effect of temperature on calcium carbonate adsorption.

The calcium carbonate adsorption by *Rhizopus* sp. biomass (live or heat killed) was tested at temperatures ranging from 10 °C to 40 °C (Figure 5). The results indicate that calcium carbonate adsorption by *Rhizopus* sp. biomass was efficient within a temperature range of 20 °C to 40 °C. A decrease of calcium carbonate adsorption was observed at 10 °C. The living *Rhizopus* sp. biomass was more efficient in calcium carbonate adsorption than the heat killed one. Aksu *et al.* [15] reported that, temperature does not influence the biosorption processes in the range of 20-35 °C.

5.2.3. Effect of fungal growth form on adsorption of insoluble-inorganic compounds.

The biomass of *Mucor rouxii* NRRL 1894 obtained from shaken cultures was in pelleted form. Therefore, the efficiency of insoluble-inorganic compounds adsorption by dispersed biomass or pelleted biomass forms was tested. Data in Table (3) indicate that the dispered biomass form was far more efficient than the pelleted biomass. Although living dispersed biomass was more efficient in adsorption than the heat killed one. It is also observed that the heat killed pelleted biomass was relatively very slightly efficient than living pelleted biomass in adsorption.

The accumulation may be largely an adsorptive process, presumably involving organic ligands on the mycelium surface. The adsorption being sufficiently strong to withstand washing of the mycelium with water. The lower adsorption by heat killed mycelium may be due to an alternation by heat of metal binding ligands present on the fungus wall /or due to the weakening of physical strength of the mycelium that support the adsorption process. For use in industrial or technical operations, freely dispersed biomass has several disadvantages in that it may cause problems in the operation of reactors by blocking flow lines and the separation of biomass is difficult. The use of pelleted fungal biomass has proved capable of operating in continual flow systems in the recovery of heavy metals [16]. The result of present investigation show that pelleted biomass of *M. rouxii* was less efficient than the dispersed biomass. The diminished adsorption efficiency of the pelleted biomass may be related to decreased surface /volume ratio compared with dispersed form. This is supported by the finding that the heat killed pelleted biomass was slightly efficient in adsorption than the living-pelleted biomass. It was observed that heat killed pelleted biomass assumed a loose and fluffy texture that may result in increasing surface area to volume ratio.

Table 1: Comparison of bio-adsorption (%) of insoluble-inorganic compounds (0.7 %, w/v suspensions) by different fungal biomass. (Amounts of adsorbed compounds calculated as mg/g wet weight biomass are given in parentheses).

Fungal biomass	$2PbCO_3.pb(OH)_2$	$CaCo_3$	$CaSiO_4$	S°	Zinc dust
Rhizopus sp.	L 100 (140) K 100 (140)	89 (173) 71 (99)	82 (115) 74 (104)	93 (130) 78 (109)	100 (140) 100 (140)
Rhizopus nigricans	L 98 (137) K 98 (137)	86 (120) 85 (119)	67 (94) 40 (56)	73 (102) 62 (87)	98 (137) 96 (134)
Mucor rouxii NRRL 1894	L 99 (139) K 98 (137)	93 (130) 88 (123)	73 (102) 56 (78)	81 (113) 60 (84)	98 (137) 98 (137)
Mucor rouxii NRRL 1430	L 95 (133) K 95 (133)	86 (120) 65 (91)	69 (97) 62 (87)	61 (85) 51 (71)	91 (127) 88 (123)
Mucor sp. NRRL 8135	L 95 (133) K 75 (105)	58 (81) 49 (69)	64 (90) 50 (70)	62 (87) 49 (69)	91 (127) 95 (133)
Thermomucor indicae-seudaticae NRRL 6429	L 92 (129) K 83 (116)	56 (78) 82 (115)	73 (102) 62 (87)	53 (74) 44 (62)	100 (140) 100 (140)
Aspergillus niger NRRL 595	L 61(85) K 30 (42)	17 (24) 10 (14)	19 (27) 12 (17)	18 (25) 12 (17)	42 (59) 70 (98)
Penicillium chrysogenum	L 36 (50) K 37 (52)	30 (42) 36 (50)	45 (63) 22 (31)	31 (43) 4 (5.6)	34 (48) 61 (85)

The living biomass (L) or heat killed (K) (5g wet weight) added to 100 ml of distilled water containing 0.7%, w/v of the compound. The mixtures were kept shaken (150 rpm) for 18 h. at 30 °C.

Table 2: Analysis of variance (AN.O.VA) in the means percentages removal of different insoluble-inorganic compounds by eight fungal biomass either living or heat killed.

Source of variation	d.F	M.S.	F
Organisms	(a) 7	11229.47	56.12**
Insoluble-inorganic compounds	(b) 4	6664.20	33.31**
Status	(c) 1	1307.34	6.53**
Interaction	(ab) 28	265.23	1.33 N.S
Error	40	200.08	

** Highly significant

N.S not significant Status (c) Living or heat killed biomass.

Fig 1: Adsorption of lead carbonate from distilled water by fresh biomass of *Rhizopus* sp. (d). Water containing lead carbonate, but lacking biomass (C).

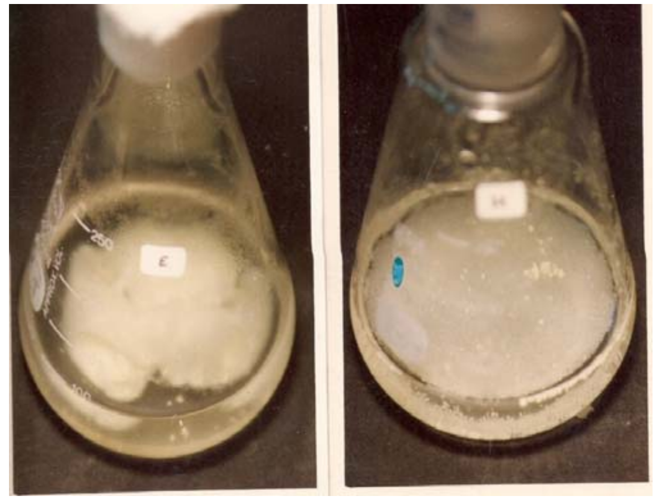

Fig 2: Adsorption of elemental sulphur from distilled water by fresh biomass of *Rhizopus* sp. (E). Water containing elemental sulphur, but lacking biomass (H).

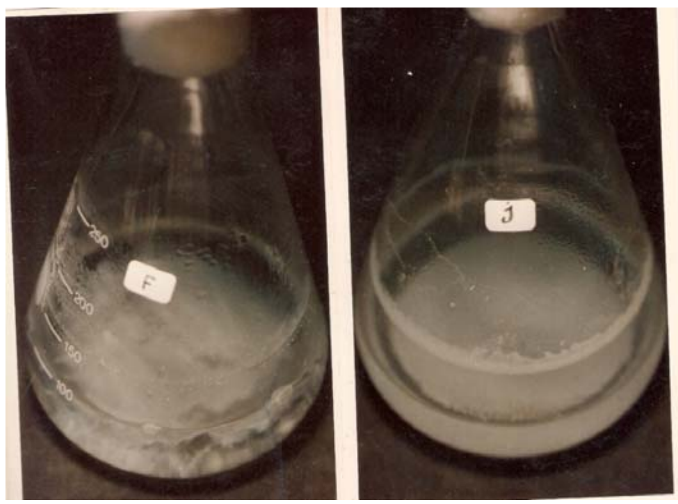

Fig 3: Adsorption of zinc dust from distilled water by fresh biomass of *Rhizopus* sp. (F). Water containing zinc dust, but lacking biomass (J).

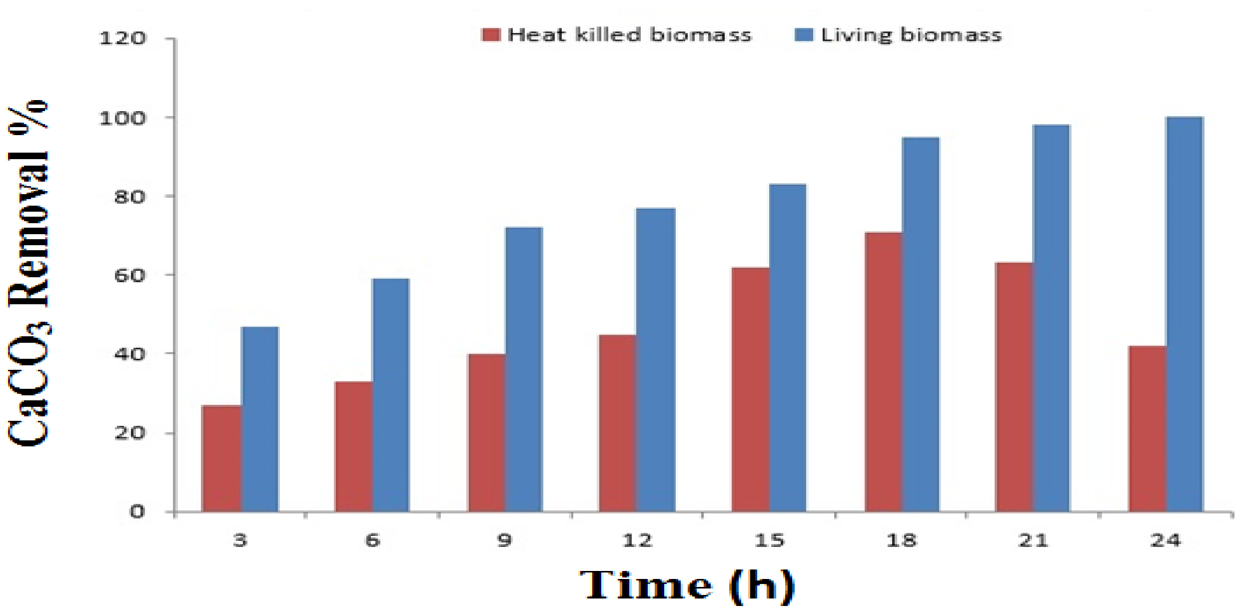

Fig 4: Effect of time on the adsorption of $CaCO_3$ by living or heat killed *Rhizopus* sp. Biomass (5.0g wet weight= 0.39g dry weight) from $CaCO_3$ suspension (100 ml containing $CaCO_3$ 0.7% (w/v) were incubated shaken (150 rpm) at 30 °C.

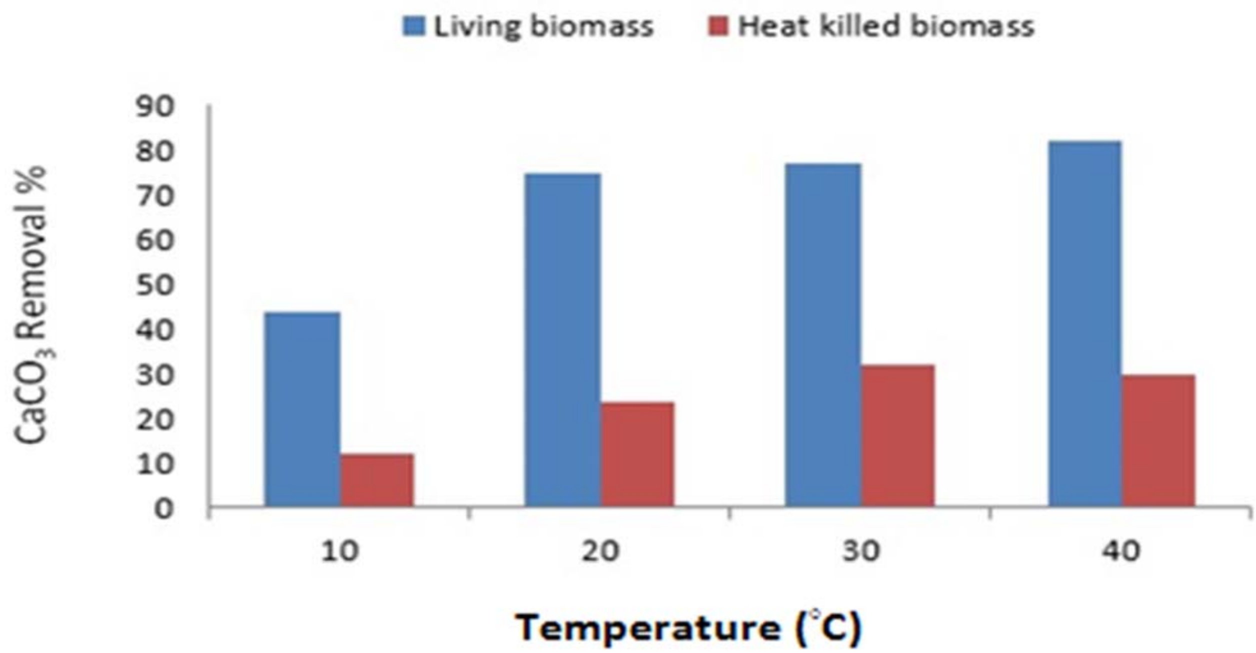

Fig 5: Effect of temperature on the adsorption of CaCO3 by living or heat killed *Rhizopus* sp. biomass. For experimental conditions, see footnote to Fig. 4, except the flasks were incubated for 6h.

Table 3: Effect of type of growth form on adsorption (%) of insoluble-inorganic compounds by *Mucor rouxii* NRRL 1894*.

Compound % (w/v)in suspension		Dispersed biomass form		Pelleted form	
		L	K	L	K
$2PbCO_3.Pb(OH)_2$	0.9	97 (174)	87 (156)	17 (30)	30 (54)
$CaCO_3$	0.7	93 (130)	88 (123)	25 (35)	37 (51)
$CaSiO_4$	0.7	73 (102)	56 (78)	31 (43)	35 (49)
S°	0.7	81 (113)	60 (84)	48 (67)	50 (71)
Zinc dust	1.0	97 (194)	88 (176)	42 (84)	51 (102)

* Figures presented in parentheses indicate adsorbed compound mg/g wet weight of biomass. For experimental conditions, see footnote in Table 1.

6. Conclusion

Biomass of fungi might be used on industrial scale to remove insoluble particles from solutions, a process which would be economically most attractive for the removal of small amounts of valuable particulates from a large volume of water. As early as Williams [17] showed that fungi could adsorb gold from colloidal solution, although this ability seems not to have been exploited industrially. Recently Nouri *et al.* [7] reported that metal ions in water can occur naturally from anthropogenic sources and from leaching of ore deposits, which mainly include solid waste disposal and industrial effluents.

7. References

1. Volesky B. biosorbent materials, Biotech. Bioeng. Symp 1986; 16:121-126.
2. Gadd GM. Accumulation of metals by microorganisms and algae, In: Biotech, "Special Microbial Processes", (Rehm, H, -J., ed.) VCH Verlags-gesellschaft Weinheim 1988; 6b:401-433.
3. Shumate SE, Stranberg GW. Accumulation of metals by microbial cells, In: Comprehensive Biotechnol., (Moo-Yung, M., C. N. Robinson and J.A. Howell, eds.), pergamon Press, New York 1985; 4:235-247.
4. Wainwright M, Grayston SJ, de Jong P. Adsorption of insoluble compounds by mycelium of the fungus Mucor flavus, Enzyme Microb. Technol 1986; 8:597-600.
5. Saad AM. Biosorption of soluble and insoluble-inorganic compounds by non-trained and cobalt- trained *Mucor rouxii* NRRL 1894 and *Rhizopus* sp. Biomass, Eur. J Biotech and Biosci. 2014; 2(5):21-26.
6. Saad AM. Factors affecting cobalt uptake by cobalt-trained *Mucor rouxii* NRRL 1894 biomass, Eur. J Biotech and Biosci. 2015; 3(3):1-6.
7. Nouri AH, Mahvi AA, Jahed GR, Ahmedpour E. Investingation of heavy metals in groundwater, Pakistan J Biolog Sci. 2006; 9(3):377-384.
8. Balkose D, Baltacioglu H. Adsorption of heavy metal cations from aqueous solutions by wool fibers, J Chem Technol and Biotechnol. 1992; 54(4):393-397.
9. Zwain HM, Vakili M, Dahlan I. Waste material adsorbents for Zinc removal from waste water: A comprehensive review, Int. J Chem Eng. 2014, 13. Article ID347912,
10. Apiratikul R, Pavasant P. Batch and column studies of biosorption of heavy metals by *Caulerpa lentillifera*, Biores. Technol 2008; 99(8):2766-2777.
11. Huang F, dang Z, Guo CL, Lu GN, Gu RR, Liu HJ *et al*. Biosorption of Cd(II) by live and dead cells of *Bacillus*

cereus RC-1 isolated from cadmium contaminated soil, Colloids Surf B Biointerfaces 2013; 107:11-18.

12. Steel RCD, Torrie JH. Priniciples and procedures of statistics with special reference to the Biological Sciences, McGraw-Hill CO., Inc., New, York, Toronto and London, 1960.
13. Bartnicki-Garcia S. Cell wall chemistry, morphogenesis and taxonomy of fungi, Ann. Rev. Microbiol 1986; 22:87-108.
14. Muzzarelli RAA, Tanfani F, Gianfranco S. Chelating, Film forming, and coagulating ability of the chitosan-glucan complex from *Asperillus niger* industrial wastes, Biotech. Bioeng 1980; 22:885-896.
15. Aksu Z, Sag Y, Kutsal T. The biosorption of copper by C. *vulgaris* and Z. ramigera, Environ. Technol 1992; 13:579-586.
16. De Rome L, Gaad GM. Use of pelleted and immobilized yeast and fungal biomass for heavy metal and radionuclide recovery, J Industrial Microbiol. 1991; 7:97-104.
17. Williams M. Ann. Bot. 1918; 32-531, c.f., Wainwright, M., Grayston, S. J and de Jong, P. Adsorption of insoluble compounds by mycelium of the fungus Mucor flavus, Enzyme Microb. Technol 1986; 8:597-600.