Int.J.Curr.Microbiol.App.Sci (2016) *5*(5): 647-656

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 5 Number 5 (2016) pp. 647-656**
Journal homepage: http://www.ijcmas.com

Original Research Article http://dx.doi.org/10.20546/ijcmas.2016.505.065

Characterization and Bio-prospecting of Fungi for Ag(I), Au(III) and Pd(II) sorption

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Keywords

Screening, Fungi, Silver, Gold, Palladium, Sorption.

Article Info

Accepted:
18April 2016
Available Online:
10 May 2016

A B S T R A C T

Present work describes macroscopic and microscopic characterization of 41 fungi and enumerates their potential to sorb precious metals, namely; Ag(I), Au (III) and Pd(II). Fungal biomass included, 29 of *Aspergillus*, 5 from *Mucor*, 1 *Rhizopus* and 4 different unidentified micro-fungi and 2 mushrooms. Maximum and minimum biomass was produced by isolate SRD7 and isolate SRD17 respectively. Isolate SRD49, was the most efficient, while, SRD39 was the least efficient in sugar utilization. Number of fungi capable of Ag(I), Au(III) and Pd(II) sorption reduces as percent sorption increases. Overall, fungi had much variation in their precious metal sorption ability. Variation for precious metal sorption was in the order Ag(I)>Au(III)>Pd(II). Ag(I), Au(III) and Pd(II) sorption ranged between 6.88-79.48, 7.47- 56.74 and 22.66-40.3%, respectively.

Introduction

Mineral reserves in the earth's crust are limited but their uses are unlimited. Numerous uses of metals generate large volume of liquid wastes containing low amounts of metals. Release of heavy metals into the waste streams poses a threat of getting mixed with natural water bodies and entering the food chains. Heavy metals are toxic to flora, fauna as well as humans and can even be fatal. On the other hand, release of precious metals into the waste streams needs to be paid attention to, keeping in mind their monetary value and strategic uses.

The current trend is to reduce, reuse and recycle. Thus, there is a need to recover the metals from secondary sources or generated wastes. Biosorption is suitable solution for this purpose. It is an environmentally benign, economic and efficient technology for recovery of precious metals. Biosorption is the passive process of metal adsorption and sequestration by chemically charged

moiety or functional groups present on the biomass. Biosorption developed over 3 decades ago (Volesky, 1999) as a new, economic and efficient scientific approach for metal recovery, utilising metal sequestering and immobilising properties of non-viable biomass. This process could help to recover even small amounts of heavy metals from industrial effluents (Viera and Volesky, 2000; Gardea-Torresdey *et al.*, 2004). Although much work is done in this field, very few commercially viable products float in the market.

The living forms in nature are capable of doing many spectacular things, such as; extracting metals present in ores, cleaning the vast oils spills, providing plant nutrients in the soil, producing life-saving antibiotics, fabricating specialized nanoparticles and many more. Thus, they assist the humans to thrive and sustain in the ecosystem. Huge bulks of these available biomass still remain unexplored. This apart, fungi also are used in many industrial processes, therefore, are generated as wastes in large quantities due to loss in activity over a period of time, as a part of physiological changes. With this in view, characterization and bio-prospecting of selected fungal biomass was done as potential sorbents for precious metals.

Materials and Methods

Preparation of Media

Czepek dox broth (CDB) (g/L): sucrose 30.0; NaNO3 3.0; KH2PO4 1.0; KCl 0.5; MgSO4 0.5 and FeSO4 0.01; pH 7.0 was used for the cultivation of various fungal cultures used in the study. Czepek dox agar (CDA) had 30 g/L agar in addition to the constituents present in CDB.

Procurement and Cultivation of Fungal Biomass

Thirty nine fungal isolates were procured from the Department of Microbiology and Biogas Research Centre, Gujarat Vidyapith, Sadra, Gujarat, India. These fungal strains were isolated from different ecological niche around Sadra. Two mushrooms were collected from nature from the Gujarat University campus.

Mycelial plugs with 6 mm diameter were obtained from 96 h grown fungal cultures (all fungi except mushrooms) on CDA in a petri plate. One plug of each isolate was used to inoculate 0.1 L of pre-sterilized SDB in 0.5 L Erlenmeyer flasks. The inoculated flasks were incubated on an environmental shaker rotating at 130 rpm at 30±2 °C. The biomass was harvested after 96 h by filtration through a double layer muslin cloth.

All the fungal biomass, including, mushrooms (obtained from nature, Gujarat University campus, Ahmedabad, India) were washed thrice with sterile distilled water to remove the residual media constituents or unwanted materials. An excess of the water was removed by squeezing the biomass in the muslin cloth. The biomass was either directly weighed; as wet weight, or dried at 55±2°C till constant weight was obtained; as dry weight.

Characterization of Fungi

Macroscopic characteristics of the fungi were noted in terms of colony diameter, texture, colour of conidiophores, base colour, pigment produced, if any.

Microscopically observations were made with respect to presence of septa, rhizoids, shape of conidial head, vesicle, metulae, phialides and conidia for all fungi except the 2 mushrooms. Substrate utilization profile of these fungi was done by evaluating the residual sugar in the medium.

Preparation of Metal Stock Solutions

Analytical grade $AgNO_3$, $HAuCl_4 \cdot 3H_2O$ and $PdCl_2$ were used to prepare metal solutions. For each metal, 20 mM metal stock was prepared, which was diluted appropriately, to obtain the working solutions as per the experiment.

Batch Sorption Experiments

Glasswares used for all the sorption experiments were thoroughly washed with detergent followed by rinsing with double distilled water 3 times. These glassware were used for Au(III) and Pd(II) sorption. Additionally, in case of Ag(I), all glasswares were treated with 1% HNO3 for 24 h followed by rinsing with double distilled water 3 times. For all Ag(I), Au(III) and Pd(II) sorption experiments, the initial pH of the system was adjusted to pH 5.0, 2.0 and 2.0 respectively, using 1 M HCl and/or NaOH.

Before the addition of biomass to the system, sample was withdrawn, which was used to determine the initial metal concentration. Controls without the biomass were also run in parallel for all experiments. After the desired time period, aliquots were withdrawn; biomass was separated by filtering through double layer muslin cloth, followed by filtrate collection. The metal uptake was determined from the difference in the initial and residual metal concentration of the filtrate. The comparative evaluation of all the sorbents for precious metal removal capacity was carried out at 2 h contact time, using 0.2 L of 0.2 mM Ag(I) or Au(III) or Pd(II) solution in a 0.5 L Erlenmeyer flasks added with 5 g/L biomass, shaken at 130 rpm and 30±2°C.

Analytical Procedures

The total residual sugar in the medium was determined by the phenol-sulfuric acid method (DuBois *et al.*, 1956). The residual metal content of the filtrate obtained after separation of the biomass was determined by the standard method of atomic absorption spectroscopy (SL-243, Elico, India).

Results and Discussion

Glasswares to be used for Ag(I) sorption were pre-treated with dilute nitric acid to avoid adsorption of positively charged Ag(I) to negatively charged silicates on the surface of the glasswares. Glasswares for Au(III) and Pd(II) sorption did not require any acid pre-treatment, since, these metals are present as anions in an aqueous system.

Identification and Characterization of Fungal Biomass

Thirty fungal isolates included, 29 of *Aspergillus*, 5 from *Mucor*, 1 *Rhizopus* and 4 different unidentified fungi. The macroscopic and microscopic characteristics of these isolates are given in Table 1. The tentative taxonomic identification, dry wet, wet weight to dry weight ratio, total sugar utilization details of 39 fungal isolate are provided in Table 2. Isolate SRD7 showed maximum biomass production (19.62 g/L), while, isolate SRD17 had minimum dry weight (2.58 g/L) under the experimental conditions. Twenty four fungi had a biomass dry weight less than 5 g. Only 4 out of 39 fungi studied had a dry biomass greater than 10 g. Eleven fungal isolates showed biomass dry weight ranging from 5-10 g. An ideal sorbent should be effective in metal removal as well as easily available. The amount of biomass produced (dry weight) per unit volume of the medium by the isolate indicated the amount of biosorbents produced. For selection of fungi the high biomass productivity is one of the desirable criteria. Analysis of wet weight to dry weight ratio suggested that, *A.niger* SRD2

produced the fluffiest biomass in the broth medium, while *A. terreus* SRD7 was the most compactly grown biomass.

Results of sugar utilization (Table 2a) indicate that total sugar utilization ranged from 60.56 to 97.69%. Isolate SRD49, was the most efficient, while, SRD39 was the least efficient in sugar utilization. At the end of 96 h of incubation, these isolates had about 2 and 40% residual substrate in the medium respectively. Microbes differ in their metal resistance as well as metal uptake capacities (Gupta *et al.*, 2000). Moreover, substrate utilization is also an individual organism's characteristic and varies greatly. Variation in substrate utilization potential of these fungi may be correlated to their metabolic activity. Substrate utilization potential cannot be ignored, since it influences process economics

Screening of Biomass for Precious Metal Sorption

Silver, gold and palladium, sorption capacity of fungal biomass was classified into into 4 groups viz., A (<45%), B (45-60%), C (61-75%) and D (>75%).

As observed in Table 2b, six, fifteen, fourteen and six fungal biomass belonged to groups A-D for Ag(I) sorption. In case of Au(III) sorption, thirty nine and two fungi out of the total fungi screened, belonged to groups A and B, respectively. As noted, all the screened fungi belonged to group A for Pd(II) sorption. As observed, the number of fungal biomass capable of Ag(I), Au(III) and Pd(II) sorption reduces from groups A to D i.e. as % sorption increases. Fungi show more variation in their Ag(I) sorption potential compared to that for Au(III) and Pd(II) in that order.

As can be seen from data presented in Table 2a, Ag(I), Au(III) and Pd(II) sorption ranged between 6.88-79.48, 7.47- 56.74 and 22.66-40.3%, respectively. Maximum Ag(I), Au(III) and Pd(II) sorption was given by, A. oryzae SRD4, *A. terreus* SRD49 and *A. fumigatus* SRD20, respectively. *A. terreus* SRD7, *A. flavus* SRD36 and *A. terreus* SRD49 showed minimum Ag(I), Au(III) and Pd(II) sorption, respectively. Best genus for Ag(I), Au(III) and Pd(II) sorption were *A. niger*, *A. terreus* and *A. fumigatus*, respectively. Among the various fungi, *A. terreus* was found to be infereior for Ag(I) sorption, while, *A. flavus* was found to be inferior for Au(III) as well as Pd(II).

Within *Aspergillus* and *Mucor* genera, Ag(I), Au(III) and Pd(II) sorption ranged between 32.65-79.48 and 35-78.65, 5.47-54.74 and 26.14-38.96 and 22.18-40.30 and 35.41-37.67%, respectively. Both *Aspergillus* and *Mucor* genera, showed almost equal potential as well as variation for Ag(I) sorption. The *Aspergillus* genus showed more sorption as well as variation for Au(III) and Pd(II) than *Mucor* genus (*Mucor*aceae family). Within the *Aspergillus* genus, *A. fumigatus* showed much variability for Ag(I) and Pd(II) sorption, while, *A. niger* as well as *A. terreus* demonstrated much variation in Au(III) sorption potential. Although an increased number of isolates in each group could influence the variability it was not necessary. *A. fumigatus* group with 10 isolates showed less variability for Au(III) sorption than, *A. terreus* group with just 3 isolates.

Table.1 Macroscopic and Microscopic Characteristics of the Fungi

Sr. No.	Fungal isolate	Tentative identification	Colony characteristic			Microscopic characteristic								
			Colony Dia-meter (mm)	Type of growth	Color of conidiophores	Base Color	Pigment	Septate/ Aseptate	Conidial head	Vesicle	Metulae	Phialides	Conidia	Comments
1	SRD3	*A. fumigatus*	26	Powdery	Greyish green smoky	White	No pigment	Septate	Compactly columnar	Dome shaped	Absent	Uniserate	Basipetal, globose to oval	NA
2	SRD9	*A. fumigatus*	12	Powdery	Greyish green smoky	White	No pigment	Septate	Compactly columnar	Dome shaped	Absent	Uniserate	Basipetal, globose to oval	NA
3	SRD11	*A. fumigatus*	20	Powdery	Greyish green smoky	White	No pigment	Septate	Compactly columnar	Dome shaped	Absent	Uniserate	Basipetal, globose to oval	NA
4	SRD14	*A. fumigatus*	26	Powdery	Greyish green smoky	White	No pigment	Septate	Compactly columnar	Dome shaped	Absent	Uniserate	Basipetal, globose to oval	NA
5	SRD16	*A. fumigatus*	17	Powdery	Greyish green smoky	White	No pigment	Septate	Compactly columnar	Dome shaped	Absent	Uniserate	Basipetal, globose to oval	NA
6	SRD19	*A. fumigatus*	12	Powdery	Greyish green smoky	White	No pigment	Septate	Compactly columnar	Dome shaped	Absent	Uniserate	Basipetal, globose to oval	NA
7	SRD20	*A. fumigatus*	33	Powdery	Greyish green smoky	White	No pigment	Septate	Compactly columnar	Dome shaped	Absent	Uniserate	Basipetal, globose to oval	NA
8	SRD21	*A. fumigatus*	31	Powdery	Greyish green smoky	White	No pigment	Septate	Compactly columnar	Dome shaped	Absent	Uniserate	Basipetal, globose to oval	NA
9	SRD22	*A. fumigatus*	12	Powdery	Greyish green smoky	White	No pigment	Septate	Compactly columnar	Dome shaped	Absent	Uniserate	Basipetal, globose to oval	NA
10	SRD23	*A. fumigatus*	36	Powdery	Greyish green smoky	White	No pigment	Septate	Compactly columnar	Dome shaped	Absent	Uniserate	Basipetal, globose to oval	NA
11	SRD2	*A. niger*	15	Powdery	Brownish black	White	No pigment	Septate	Globose, radiate	Globose	varying length, uniform size	Uniform	Globose,spiked	NA
12	SRD18	*A. niger*	20	Powdery	Black	White with yellow centre	No pigment	Septate	Globose, radiate	Globose	varying length, uniform size	Uniform	Globose,spiked	NA
13	SRD24	*A. niger*	16	Pwdery	Brownish black	White	No pigment	Septate	Globose, radiate	Globose	varying length,	Uniform	Globose,spiked	NA

										uniform size				
14	SRD25	*A. niger*	29	Pow dery	Brownish black	White	Whitish yellow diffusible	Septate	Globose, radiate	Globose	varying length, uniform size	Uniform	Globose,spi ked	NA
15	SRD26	*A. niger*	21	Pow dery	Blackish brown	White	No pigment	Septate	Globose, radiate	Globose	varying length, uniform size	Uniform	Globose,spi ked	NA
16	SRD27	*A. niger*	14	Pow dery	Brownish black	White with yellow centre	No pigment	Septate	Globose, radiate	Globose	varying length, uniform size	Uniform	Globose,spi ked	NA
17	SRD29	*A. niger*	8	Pow dery	Brownish black	White	Yellow diffusible	Septate	Globose, radiate	Globose	varying length, uniform size	Uniform	Globose, spiked	NA
18	SRD30	*A. niger*	13	Powdery	Black	White with yellow centre	No pigment	Septate	Globose, radiate	Globose	varying length,uniform size	Uniform	Globose,sp iked	NA
19	SRD33	*A. niger*	13	Powdery	Brownish black	White with yellow centre	Whitish Yellow diffusible	Septate	Globose, radiate	Globose	Closely-packed	Uniform	Globose,sp iked	NA
20	SRD1	*A. oryzae*	9	Powdery	Dirty green	White	Mustard yellow	Septate	Radiate	Globose	Closely-packed	Biserate	Basepetal, globose and oval	NA
21	SRD4	*A. oryzae*		Powdery floccose	Brownish green	Yelllowish brown	No pigment	Septate	Radiate	Globose	Closely-packed	Biserate	Basepetal, globose and oval	NA
22	SRD13	*A. oryzae*	26	Floccose / Fluffy	Greenish white	Creamish white	No pigment	Septate	Globose, Radiate	Crown-like		Biserate	Basepetal, globose and oval	NA
23	SRD36	*A. flavus*	15	Floccose powdery	Green with white at the centre	White	No pigment	Septate	Columnr	Dome-shaped	Closely packed	Biserate	Acropetal, globose	NA
24	SRD37	*A. flavus*	16	Powdery	Green with white at the centre	White	No pigment	Septate	Columnar	Dome-shaped	Closely-packed	Biserate	Acropetal, globose	NA
25	SRD5	*A. terreus*	12	Powdery	Yelllowish ochre	Lemon yellow	Diffusible brownish yellow	Septate	Columnar	Dome-shaped	Closely-packed	Biserate	Acropetal, globose	NA
26	SRD49	*A. terreus*	15	Powdery	Buff to yellow	Cream	Brownish yellow diffusible	Septate	Columnar	Dome-shaped	Closely-packed	Biserate	Acropetal, globose	NA
27	SRD7	*A. terreus*	17	Powdery	Yelllowish ochre	Buff	Diffusible yellow	Septate	Clumnar	Crown-like	Closely-packed	Biserate	Acropetal, globose	NA
28	SRD10	*A. nidulans*	15	Powdery	Green with white at the centre	Cream	No pigment	Septate	Radiate	Globose			Acropetal, Oval	NA
29	SRD44	*A. glaucus*	23	Fluffy	light brown	Creamish white	No pigment	Septate	Radiate to loosely columnar	Globose	Absent	Uniserat e	Ellipsoidal to round	Numerou s thin walled cleitothec ia

30	SRD6	*Mucor* sp.	Covers entire plate	Cottony	Grey heads	White	No pigment	Aseptate						Rhizoid absent
31	SRD15	*Mucor* sp.	Covers entire plate	Cottony	Grey heads	Pinkish white	No pigment	Aseptate						Rhizoid absent
32	SRD34	*Mucor* sp.	Covers entire plate	Cottony	Grey heads	White	No pigment	Aseptate						Rhizoid absent
33	SRD45	*Mucor* sp.	Covers entire plate	Cottony	Grey heads	White	No pigment	Aseptate						Rhizoid absent
34	SRD48	*Mucor* sp.	Covers entire plate	Cottony	Grey heads	White	Diffusible light creamish yellow	Aseptate						Rhizoid absent
35	SRD17	*Rhizopus* sp.	Covers entire plate	Cottony	Grey heads	White	Diffusible light creamish yellow	Aseptate						Rhizoid present
36	SRD8	Unidentified		Multi-coloured				Septate	Globose, radiate	Globose	varying length, uniform size	Uniform	Globose,spiked	NA
37	SRD28	Unidentified		Brown	Brown									NA
38	SRD31	Unidentified		Olive green	Olive green	White	NP							NA
39	SRD39	Unidentified	Covers entire plate	Chalky, hard	White	White	NP	Aseptate						NA

NP: No pigment produced, NA: Not applicable

Table.2A Dry weight, Wet weight to Dry Weight Ratio, Sugar Utilization Profile and Metal Sorption of the Fungi

Sr. No.	Fungal isolate code	Dry weight (g/L)	Wet weight: dry weight	Sugar Utilization (%)	Metal sorption (%)		
					Ag(I)	Au(III)	Pd(II)
1.	SRD3	7.37	16.84	94.02	54.22	16.98	
2.	SRD9	5.20	10.38	74.65	79.19	22.62	
3.	SRD11	5.51	17.05	73.86	70.27	21.04	
4.	SRD14	5.82	6.65	80.67	45.9	11.37	29.45
5.	SRD16	4.71	18.38	94.87	74.24	15.50	37.26
6.	SRD19	4.21	24.37	79.26	56.6	14.60	
7.	SRD20	4.86	7.55	88.74	63.93	18.93	40.30
8.	SRD21	8.23	11.48	82.35	68.69	12.30	29.36
9.	SRD22	9.08	15.70	75.23	55.21	12.22	27.38
10.	SRD23	14.75	5.48	87.41	36.19	8.94	26.70
11.	SRD2	3.27	50.42	93.06	73.31	27.20	
12.	SRD18	3.55	48.04	93.76	60.36	18.12	
13.	SRD24	4.34	33.70	92.63	71.71	7.44	
14.	SRD25	3.01	26.81	93.57	57.97	24.76	
15.	SRD26	4.03	23.02	93.82	74.5	28.54	
16.	SRD27	3.02	27.95	92.37	66.73	31.51	
17.	SRD29	3.26	48.60	92.53	70.12	16.72	
18.	SRD30	4.71	21.13	93.69	63.15	12.84	
19.	SRD33	3.65	32.72	93.73	52.59	14.22	
20.	SRD1	9.80	7.93	96.08	51.84	25.01	35.41
21.	SRD4	4.41	38.50	93.75	79.48	22.57	
22.	SRD13	6.84	5.29	94.39	56.6	14.96	29.36
23.	SRD36	4.43	37.46	93.76	59.36	5.47	24.20
24.	SRD37	4.23	44.92	93.43	67.73	9.30	
25.	SRD5	17.20	3.46	93.76	49.07	30.65	39.45
26.	SRD7	19.62	2.22	88.11	32.65	34.95	
27.	SRD49	6.22	25.16	97.69	58.76	54.74	22.18
28.	SRD10	3.72	25.12	92.89	75.30	17.62	
29.	SRD44	7.07	8.53	94.57	58.78	37.84	38.72
30.	SRD6	2.67	3.61	92.58	35.00	26.14	
31.	SRD15	2.76	2.57	93.99	50.65	38.96	
32.	SRD34	2.94	3.04	93.62	57.39	34.54	
33.	SRD45	3.05	3.84	90.18	46.10	38.70	
34.	SRD48	4.01	7.19	90.55	78.65	28.11	37.67
35.	SRD17	2.58	16.11	90.50	76.99	33.59	35.41
36.	SRD8	2.61	21.53	91.85	68.23	20.18	
37.	SRD28	4.74	30.26	92.57	73.86	22.02	
38.	SRD31	5.51	3.77	83.00	79.68	14.05	
39.	SRD39	10.21	5.41	60.56	42.53	8.06	32.83

Standard deviation: ±2.5%

Table.2B Comparitive Precious Metal Removal amongVarious Biomass.

Metal	Number of fungi			
	Range of % metal sorption			
	<45(A)	45-60(B)	61-75(C)	>75(D)
Ag(I)	6	15	14	6
Au(III)	39	2	0	0
Pd(II)	17	0	0	0

Overall, fungi had much variation in their precious metal sorption ability, which may be attributed to (1) differences in the metal binding abilities of different organisms; (2) difference in the cell wall composition of the isolates from different taxonomic groups (families, genera as well as species) (Gupta *et al.*, 2000, Pethkar *et al.*, 2001); (3) difference in the ecological niche from which these fungi were isolated, which may have influenced their cellular make up (Shah, 2000, Shukla, 2012) and (4) difference in the growth rate of these fungi, which in turn would influence their physiological state and cellular make up (Farkas, 1979; Mehta and Gaur, 2005).

It can be concluded that fungi show much variation in their sugar utilization as well as precious metal sorption potential. Among the 41 fungi screened, A. oryzae SRD4, *A. terreus* SRD49 and *A. fumigatus* SRD20 were the most potential sorbents for Ag(I), Au(III) and Pd(II), respectively. In general, Ag(I), Au(III) and Pd(II) sorption ability of *Aspergillus* species was in the decreasing order as A. oryzae> *A. fumigatus*> *A. niger*> *A. flavus*>*A. terreus*, *A. terreus*> *A. niger*> A. oryzae> *A. fumigatus*> *A. flavus* and *A. fumigatus*> *A. terreus*> A. oryzae> *A. flavus*. Overall trend of precious metal sorption among fungi was in the order Ag(I) > Pd(II) > Au(III).

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How to cite this article:

Kruti R. Shah, Srinivas M. Duggirala, Devayani R. Tipre and Shailesh R. Dave. 2016. Characterization and Bio-prospecting of Fungi for Ag(I), Au(III) and Pd(II) sorption. *Int.J.Curr.Microbiol.App.Sci.*5(5): 647-656.
doi: http://dx.doi.org/10.20546/ijcmas.2016.505.065