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Original Article

Fluoride Removal from water by Sorbing on Plant and Fungal Biomass

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Abstract

The capacity of microbial biomass to remove hazardous element from aqueous solution even after being killed has been well documented. Such material could be used to decontaminate waste water originating from industries involved mining, refining, nuclear fuel processing etc. The present study identified saw dust, *Aspergillus penicilloides* and *Mucor racemosus* as potential biomaterial for calcium and fluoride removal. Maximum Ca^{2+} sorption was observed in saw dust however *Aspergillus penicilloides* and *Mucor racemosus* provide themselves as the better agent for f^- removal after Ca^{2+} treatment. This proves that the cations on the surface of biomass may be used to removal anions that are not usually removed as the cell envelops carry negative charges or their surface. Biosorption can therefore provide a technical answer to control pollution due to permissible, illegal and accidental discharge and hence may become an essential implement for environmental protection.

Keywords*: Biomass, Biosorption, *Aspergillus penicilloides*, *Mucor racemosus

Introduction

There has been an ever increasing global concern about the fluoride anion, an excess of which can inflict numerous toxic effects on many biological systems (Bhatnagar and Bhatnagar 2000; Pushink and Miller 1990; Tsezos and Bell 1989) .Microalgal biomass has been extensively studied as a biosorbent for various heavy metals (Holan, Volesby and Prasetyo 1993; Wilde and Benemann 1993) and hazardous substances (Liu and Wu 1993; Tsezos Bell 1989) however being negatively charged surface it cannot absorb sufficient anions. Phosphate supply could modify flocculation and adsorption behavior in growing *Corynebacterium glutamicum* (Buch, Mozes, Wandrey and Rouxhet 1998). Fluoride sorption using a technique to pretreat surface with Ca^{2+} (Bhatnagar, Bhatnagar and Jha 2002). Biosorption removal of fluoride if possible, shall become an alternative or adjunct method the conventional technology of fluoride removal. In view of this, in the proposed study, fungal biomass and saw dust were used to pretreat with cations and find if fluoride removal may be affected using this technique.

Materials and Methods

Aspergillus penicilloides and *Mucor racemosus* were grown in Asthana and Hawker's (AH) medium-A (Asthana and Hawker 1936). The pH of medium was adjusted to 7.0 $\pm$ 0.1 with 0.1N NaOH or 0.1N HCl. Aseptically 1 ml culture from the growing phase was inoculated in 20-25 ml of AH medium in 100 ml Erlenmeyer flask and incubated for 2-3 days at 34 $\pm$ 1°C temperature.

Asthana and Hawker (AH) medium-A (Asthana and Hawker 1936)

D Glucose	5.00g
KNO_3	3.50g
KH_2PO_4	1.75g
$MgSO_4\,7H_2O$	0.75g
Distilled Water	1000ml

Harvesting the biomass

The biomass was obtained by centrifugation of growing culture at 8,000 r.p.m for 15 minutes followed by washing with double distilled water, recentrifugation and finely drying at 60º C for 72 hours in an incubator. It was than crushed using mortar and pestle to obtain a uniform powder. culture tube, washed thoroughly in running tap water before transplanting into small polycups containing mixture of sterilized sand and garden soil and irrigated with 1/10 diluted liquid MS medium and covered with poly bags for in vitro hardening. After hardening in polycups they were subsequently transferred to 15 cm diameter pots containing, sand and compost (2:1:1) and maintained under mist irrigation. Then they were shifted for field planting. Experiments were performed with a minimum of 15 replicates for initiation and were repeated at least twice.

Calcium biomass analysis

0.1 g of dried biomass or filtered wood powder was suspended in test tubes containing 10 ml of aqueous $CaCl_2$ solution in the concentration varying from 30 to 100 μg ml^{-1} and allowed to stand for 5 hours after putting it mixed for 5 minutes on rotor. Before analysis the samples were centrifuged at 5000 r.p.m. for 15 minutes and the supernatant was collected and to determine calcium using Systronics Flame Photometer 128.

Fluoride treatment

Fresh biomass (0.1g) was treated as above with calcium chloride concentration at which maximum biosorption was obtained in the previous experiment. It was again separated by centrifugation and dried as earlier. 0.1 g of the dried material was suspended in centrifuge tubes containing 0-50 μg ml^{-1} (viz. 0, 15, 25, 50 μg ml^{-1}) fluoride, for 5 hour. After the treatment cells were separated by centrifugation. Analysis of residual fluoride in the supernatant was estimated using SPADNS method (Eaton *et al*., 1995). *Systronics* 118 UV-VIS Spectrophotometer was used for the purpose.

Results and Discussion

Saw dust and dead and dried biomass of *Aspergillus penicilloides* and *Mucor racemosus* were treated with aqueous solution of calcium chloride in the range of 30-100 μg ml^{-1} for a period of 5 hours. The Saw dust and *Mucor racemosus* sorbed maximal calcium at 50 μg ml^{-1}.While *Aspergillus* sp. display maxima at 60 μg ml^{-1} (Table 1 & Fig.1, 2, 3)

The uptake increased linearly as a function of calcium concentration till this concentration in each of the treatment, but the fall after this stage did not show any pattern.

To determine the effect of pretreatment of biomass with Ca^{2+}, the saw dust and *Mucor racemosus* biomass were first exposed to 50 μg Ca^{2+} ml^{-1} for 5hours and *Aspergillus penicilloides* with 60 μg Ca^{2+} ml^{-1} for 5 hours. Following this the Ca^{2+} treated biomass and saw dust were kept in varying concentrations of fluoride (viz. 0, 15, 25, 50 μg F^- ml^{-1}). Biomass without any prior treatment was used as control (Table 2).

From a solution of 50 μg F^- ml^{-1}, *Aspergillus penicilloides* could remove as much as 14 μg F^- ml^{-1} while *Mucor racemosus* removed 6 μg F^- ml^{-1} only on the other hand saw dust could remove only 2.5 μg F^- ml^{-1}.

In case of controls which were not treated earlier with Ca^{2+} removal was nil in most cases except 15 μg F^- ml^{-1} for saw dust and 50 μg F^- ml^{-1} for *Aspergillus penicilloides* proving the hypothesis that Ca^{2+} biosorption before fluoride treatment enhanced the rate of anion removal. Earlier reported (Bhatnagar , Bhatnagar and Jha 2002) 10 mg F^- l^{-1} removed by cyanobacterium *Anabana fertilissima* and 15 mg F^- l^{-1} *Chlorococcum humicola*. In comparison to this the present study showed that fungal biomass might be a better option for F^- removal after Ca^{2+} sorption. The difference in the abilities of plant (saw dust) and fungal biomass may be explained on the basis of their chemistry.

The structural polysaccharide cellulose in the most abundant natural polymer found in the world. Found in cell wall of plants, cellulose is one of the principle component provide physical structure and strength. Cellulose with β-(1,4) glycosidic linkage can adopt a fully extended conformation with alternating 180° flips of the glucose units. Ca^{2+} ions from a dimmer complex with cellulose by interacting with 6-OH (Hydroxyl) group of upper layer of cellulose and 6-OH (Hydroxyl) group of lower layer layer of cellulose. Surface attachment of Ca^{2+} remains free to interact with anions. Therefore cellulose of saw dist shows maximum biosorption of Ca^{2+} but low biosorption of fluoride comparing chitin structure of fungal biomass (Garret and Grisham 1995) .Both fungi show better fluoride biosorption than the saw dust.

Table 1 - Calcium biosorption by Saw dust and *Aspergillus penicilloides* and *Mucor racemosus*

Treatment ($\mu g\ Ca^{2+}\ ml^{1}$)	Control ($\mu g\ Ca^{2+}\ ml^{-1}$)	Saw Dust		*Aspergillus penicilloides*		*Mucor racemosus*	
		Concentration (After 5 hour)	Removal ($\mu g\ ml^{-1}$)	Concentration (After 5 hour)	Removal ($\mu g\ ml^{-1}$)	Concentration (After 5 hour)	Removal ($\mu g\ ml^{-1}$)
30	31.41	27.66	3.75	29.33	2.08	26.85	4.85
40	40.43	33.3	7.13	36.66	3.77	35.8	4.63
50	50.00	42.73	7.27	44.06	5.94	44.83	5.17
60	59.05	52.8	6.25	54.4	4.65	53.13	5.92
70	71.15	66.1	5.05	66.16	4.99	66.16	4.99
80	79.36	71.1	2.26	76.76	2.6	75.4	3.96
90	92.58	88.1	4.48	89.3	3.28	88.27	4.31
100	96.88	89.93	6.95	93.33	3.55	95.85	1.85

Each experiment was performed with 10 replicates and was repeated thrice.

Table -2: Fluoride removal by Saw dust and *Aspergillus penicilloides* and *Mucor raracemosus*

Fluoride concentration	15 $\mu g\ F\ ml^{-1}$			25 $\mu g\ F\ ml^{-1}$			50 $\mu g\ F\ ml^{-1}$		
	Saw dust	*A. penicilloides*	*M. racemosus*	Saw dust	*A. penicilloides*	*M. racemosus*	Saw dust	*A. penicilloides*	*M. racemosus*
Control ($\mu g\ ml^{-1}$)	14.28	14.28	14.28	23.75	25	24	50	50	50
(Calcium Pretreated)	13.14	13.57	13.14	21.25	21	18.75	47.5	36	44
Removal ($\mu g\ F\ ml^{-1}$)	1.14	0.71	1.14	2.5	4	5.25	2.5	14	6
(No Pretreatment)	13.14	14.28	14.28	23.75	25	24	50	46	50
Removal ($\mu g\ F\ ml^{-1}$)	1.14	0	0	0	0	0	0	4	0

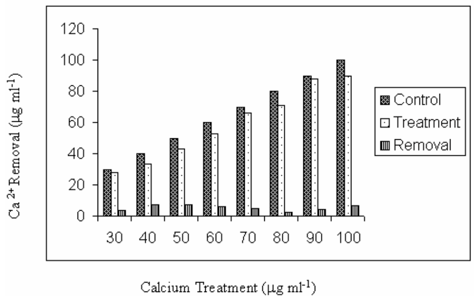

Fig.1: Calcium Removal by Saw dust

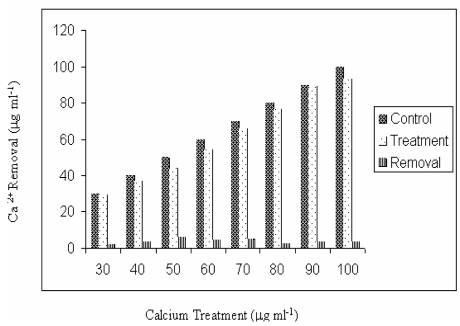

Fig.2: Calcium removal by *Aspergillus penicilloides* biomass

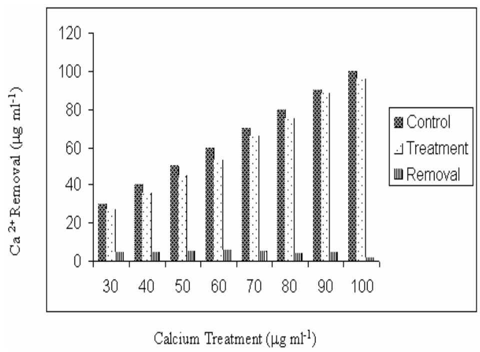

Fig.3: Calcium removal by *Mucor racemosus* biomass

Conclusion

The capacity of microbial biomass to remove hazardous element from aqueous solution even after being killed has been well documented. Such material could be used to decontaminate waste water originating from industries involved mining, refining, nuclear fuel processing etc.

The present study identified saw dust, *Aspergillus penicilloides* (Divsion-*Mycota*) and *Mucor racemosus* (Division-*Mycota*) as potential biomaterial for calcium and fluoride removal.Maximum Ca^{2+} sorption was observed in saw dust however *Aspergillus penicilloides* and *Mucor racemosus* provide themselves as the better agent for f^- removal after Ca^{2+} treatment. This proves that the cations on the surface of biomass may be used to removal anions that are not usually removed as the cell envelops carry negative charges or their surface.Biosorption can therefore provide a technical answer to control pollution due to permissible, illegal and accidental discharge and hence may become an essential implement for environmental protection.

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