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CHROMIUM REMOVAL BY A HALOTOLERANT FUNGAL ISOLATE *PENICLLIUM SP*. ARIKSPF2 IN SUBMERGED AND IMMOBILIZED STATE

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ABSTRACT:
Chromium contributes potent heavy metal pollutant in ternary industries wastewater. It causes carcinogenic and mutagenic effect on living organisms. Due to cost effectiveness and several disadvantages of physicochemical methods, living microorganisms are used for chromium removal by biosorption. A salt tolerant fungal isolate was used to evaluate its potential for certain environmental remediation processes. Aim of this study was to determine the efficiency of chromium absorption by the fungal isolate both in submerged culture condition and after immobilization on different supports. Submerged culture showed 80 % removal of chromium upto 48 hours. The efficiency of chromium removal declined after immobilization of fungi on various supports.

Keywords: *Chromium removal, Halotolerantfungi, Immobilization*

[I] INTRODUCTION

Pollution is the introduction of contaminants into the natural environment that causes adverse change. Among the all pollution heavy metal pollution produced by heavy metals used in various industries and in very little amount by natural activities. Heavy metals such as chromium (III) (IV), Plutonium, mercury and lead produce mutagenic and carcinogenic effect on living organisms[13, 14].Various physicochemical methods such as reverse osmosis, electro-dialysis, ultrafiltration, ion-exchange, chemical precipitation, phytoremediation have been routinely used for removal of heavy metals[4, 5]. Biosorption of heavy metals by microorganisms has been successfully used by scientific community as the traditional methods suffer from several disadvantages such as cost effectiveness, no recovery of heavy metals[18].Several biological materials like fungi, bacteria, several plant materials were used for chromium removal.Due to presence of salt in higher amount in industrial wastewater removal of chromium by using microbes was introduced[8, 15]. These problems could be overcome by using microorganisms as many of them have the capacity to tolerate higher salts concentration [16].Other advantage was heavy metals could be recovered for further

use. By immobilizing the organism removal of heavy metals may be enhanced.

[II] MATERIALS AND METHODS

2.1. Effect of NaCl on growth of fungi: To check the salt tolerant capacity of isolate was allowed to grown in sterile potato dextrose broth (containing 200gl^{-1} potato (in fusion form), 20 g l^{-1} dextrose) with different concentration (0 M, 0.85M, 1.71M, 2.56M and 3.42M) of NaCl& was allowed to grown at 27°C at 100 rpm for 72 hrs on shaking incubator.Contents of the flask filtered and wet weight and dry weight (after drying at 60 °C) determined.

2.2. Chromium removal: To check the chromium removal efficiency of the fungal isolate, it was allowed to grow at condition dictated above, after proper grow was observed (72hrs) chromium salt, 400ppm (in single or split dose) was added, 2ml sample was withdrawn after specified time intervals from the flask, filtered and filtrate was taken in clean glass tube. Chromiumwas estimated (in 0.1 ml of filtrate) using spectrophotometer by diphenylcarbazide method at 540nm. Chromium removal was measured by following equation.

% Chromiumremoval = Initial absorbance – observed absorbance / Initial absorption x 100

2.3. Chromium removal by immobilized fungal mycelium: The isolate was allowed to grow in potato dextrose broth, after 72hrs, 2gm of sterile wooden chip were added and fungi were allowed to growth on wood chip substratum. After 72 hrs, the wood chips were separated and used in shaken flask and packed bed reactors for determining efficiency of chromium removal. Buffer (phosphate buffer, pH 6 and 0.5 M) containing chromium salt was used as reaction medium and reduction in chromiumcontent in solution was measured.Variations in the experiment were created by adding glucose(2 %), calcium chloride (800 ppm), sodium chloride (5 %) and other nutrients and effect on chromium removal by fungi was studied. Similarly, the fungal mycelium was immobilized in calcium alginate (7 %) gel and studies carried out.

[III] RESULTS AND DISCUSSIONS

3.1. Effect of NaCl concentration on fungal growth: Series of experiments were conducted to determine the effect of increasing NaCl (salt) concentration on growth of fungi and its ability to tolerate hypersaline environments and also to determine either the isolates were halophiles or halotolerant. The culture was inoculated in series of liquid medium with increasing salt concentration of NaCl and wet weight and dry weight were measured to determine the growth of isolate with respect to different salt concentration.

The isolate was able to grow without NaCl in the medium, so the isolate is halotolerant and able to grow from 0.85M to 3.42M NaCl concentration, so it can be called halophilic halotolerant fungi. Isolate optimally grew at 1.71M NaCl according to table 3.1. Isolate were also allowed to grow on solid medium containing o to 3.42M NaCl concentration to determine optimum growth of fungi on solid medium The results were better as compared with Halophiles reported by Sehar and Hameed, (2011), showed optimum growth at 0.85M NaCl in medium.

3.2. Chromium removal by fungi

Experiments were undertaken to determine the whether the isolate remove chromium or not. Two type of experiment were done first was simple removal by isolate in single dose chromium addition and other split dose chromium addition. Table-1 shows the data for chromium removal.

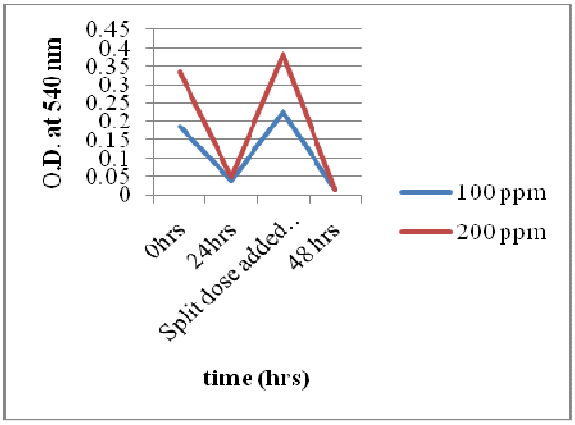
[Table-1]

Chromium concentrate-ion (ppm) added	O.D. at 540nm			
	0 hours	24 hours	Split dose of chromium after 24 hrs	48 hrs
100	0.188	0.04	0.228	0.015
200	0.336	0.049	0.385	0.014
400	0.757	0.239	Not added	0.06

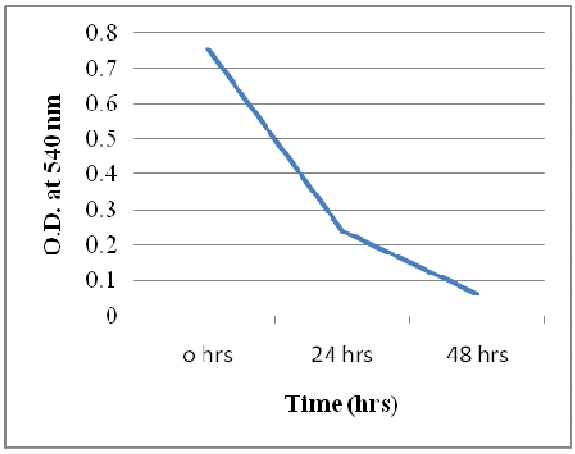
Table-1 Chromium removal by fungi in split dose

Isolate efficiently removedchromium from the medium at all tested concentration of chromium as shown in table-1 even after split dose addition.(graph-1.a). Nearly 400 ppm chromium was removed by isolate after 48 hrs as we can

see in (graph-1.b). andthe capacity of removing more than 400 ppm is obvious contrast to report of Meleigy and Osman, 2010 where longer incubation time was required to remove heavy metals. So we can say that isolate can be used for absolute removal of chromium.



Graph-1.a Chromium removal by fungi (slit dose addition)



Graph-1.b Chromium (400ppm) removal by fungi

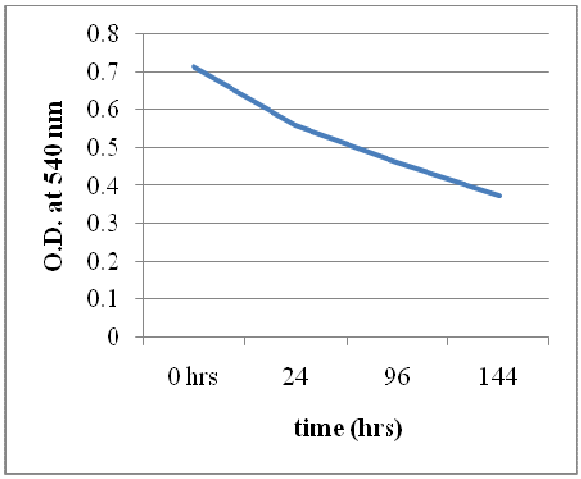
3.3 Chromium absorption by fungi immobilized on wood chip

Experiments were carried out check the effect of fungal immobilization on chromium removal. Isolates was immobilized on wooden chip and Chromium removal was estimated. Observation was amassed in table-2.

Incubation time	O.D.at 540 nm
0 hrs	0.716
24	0.56
96	0.461
144	0.373

Chromium concentration = 400ppm

Table-2 Effect of fungal immobilization on chromium removal



Graph-2 Chromium removal by fungi immobilized on wood chips with respect to time

Isolate removed chromium when immobilized on wooden chip but the time taken to remove chromium was to long as observed in graph-2, so further optimization will be needed.

3.4. Chromium removal by column packed with immobilised fungi on wood chips

Series of cycle of chromium removal by column containing immobilized fungi were carried out and estimation was done to check the removal of chromium. Observations are depicted in table-3.

Cycle	O.D. at 540 nm	Cycle	O.D. at 540 nm
1	0.675	10	0.746
2	0.898	11	1.199
3	0.882	12	0.578
4	0.778	13	0.678
5	0.802	14	0.571
6	1.26	15	0.594
7	0.867	16	0.584
8	0.845	17	0.572
9	1.063	18	0.544

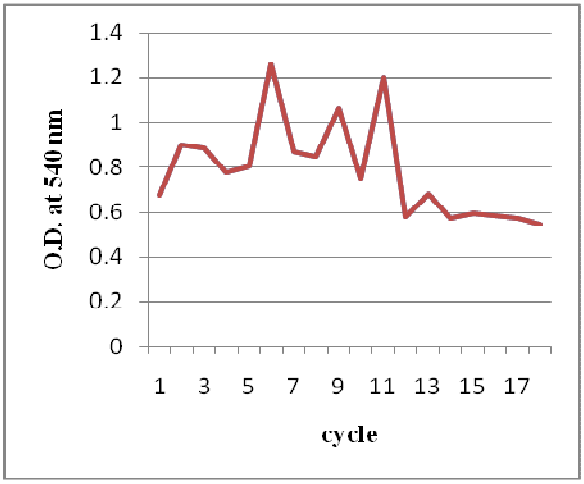
Table-3 Chromium absorption by immobilized fungi on wooden chip

Isolate removed chromium as observed in graph-3. But removal rate was slow, so further optimization is needed.

3.5. Chromium removal by fungi immobilized on sodium alginate beads

Series of flask system was prepared to check the chromium absorption capacity of beads prepared

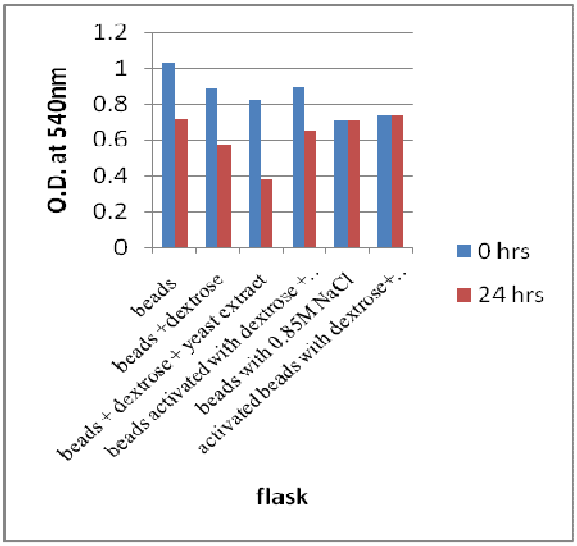
by immobilizing the fungal spore and ability to remove Cr was checked in differentconditions. Observations were amassed in table-4.



Graph-3chromium removal by continuous recycling through column

Experimental variants	O.D.at 540nm 0 hrs	O.D. at 540nm 24 hrs
beads	1.031	0.714
beads +dextrose	0.882	0.578
beads + dextrose + yeast extract	0.829	0.381
beads activated with dextrose + yeast extract	0.889	0.665
beads with 0.85M NaCl	0.705	0.705
activated beads with dextrose+ yeast extract + 0.85M NaCl	0.735	0.739

Table-4 chromium removal by fungi immobilised in Sodium alginate beads



Graph-4 Removal of chromium by fungi immobilised in sodium alginate beads.

Beads of fungal spore removed chromium efficiently in presence of dextrose and yeast extract compared to chromium removal only with beads as observed in graph-4. It is obvious from the graph that presence of dextrose and yeast extract in the reaction medium improved removal of chromium by fungal mycelium immobilized in calcium alginate. This indicates the importance of metabolic activity for chromium removal by the isolate.

IV. CONCLUSIONS

The fungi showed efficient removal of chromium in submerged fermentation conditions as well as after immobilization by surface growth on woodchips and entrapment in calcium alginate gel. Presence of nutrients in the reaction medium during chromium removal by immobilized fungi greatly improved the activity indicating importance of metabolic activity during chromium removal.

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