Research Journal of Chemical and Environmental Sciences
Res J. Chem. Environ. Sci. Vol 7 [2] April 2019: 63-71
Online ISSN 2321-1040
CODEN: RJCEA2 [USA]
©Academy for Environment and Life Sciences, INDIA
Website: www.aelsindia.com/rjces.htm

ORIGINAL ARTICLE

Bioremediation of Chromium from Synthetic Solution by using viable Microbial Cells and Consortium

Upma[1], Sumit Pal[2,3], Dilip Kumar S[3], Parimala Sai K[3], Vimala Y[3]
[1]Division of Dairy Microbiology, ICAR-National Dairy Research Institute, Karnal, Haryana
[2]CRDT, Indian Institute of Technology, New Delhi
[3]GITAM Institute of Science, GITAM University, Visakhapatnam, Andhra Pradesh
Corresponding author: sumitiitdelhi2013@gmail.com

ABSTRACT

Chromium is a toxic and carcinogenic heavy metal, the most prominent example of toxic chromium is hexavalent chromium (Cr VI). Chromium (VI) is a transition element that is used in tanning, metal finishing, petroleum, refining, iron and steel industries, inorganic chemical production and textile processing and pulp production. Heavy metal toxicity can result in damaged to blood composition kidney and liver .In the present work the chromium remediation ability of B.subtilis, P.putida, E.coli, A.flavus P.chrysosporium studied individually and in consortia. Among these organisms, P.chrysosporium (81.3%) and Co-culture of P.putida + B.subtilis (91.4%) and P.chrysosporium + A.flavus(93.8%) adsorbs chromium better than other organisms studied.

Keywords: *Bioremediation, Co-culture, Chromium, P.chrysosporium, B.Subtilis*

Received 21.02.2019 Accepted 28.03.2019

INTRODUCTION

The rate of environmental pollution keeps on increasing at an alarming rate due to the activities of man such as urbanization, technological advancement, unsafe agricultural practices and rapid industrialization which degrades the environment. With this the release of heavy metals is persistent due to their toxicity which poses a severe threat to organisms exposed to high levels of such pollutants [1, 2]. Heavy metals such as Pb, Cd, Cr, Zn, Se, Ni and As which are not useful to plants, are capable of reducing plant growth due to reduced photosynthetic activities, plant mineral nutrition, and reduced activity of essential enzymes [3, 4]. Heavy metals are cytotoxic at low concentrations and could lead to cancer in humans [5]. These toxic metals could accumulate in the body when consumed in contaminated food through the food chain and become health risks to living organisms [6]. In connection to treatment, Bioremediation is gradually being accepted as the standard practice for the restoration of heavy-metal-contaminated sites since it is more eco-friendly and cost effective compared to the conventional chemical and physical methods, which are often very expensive and ineffective when metal concentrations are low, in addition to producing significant amounts of toxic sludge [7, 8]. The ability of microorganisms to degrade pollutants depends on the suitability of environmental conditions for their growth and metabolism which include suitable temperature, pH, and moisture [9, 10]. In the present work the chromium remediation ability of *B.subtilis, P.putida, E.coli, A.flavus* and *P. chrysosporium* studied individually and as co-culture.

MATERIALS AND METHOD

A. Collection of Bacterial cells:

a. Bacillus subtilis**:** It is gram positive rod shaped bacterium commonly found in soil. It was collected from Department of Biotechnology, GITAM University. Then inoculated into sterilized nutrient broth and nutrient agar and incubated at 37° C for 24hrs.

Table 1 Biochemical Tests:

Name of Test	Result
Indole	Negative
Methyl red	Negative
Voges proskauer	Positive
Citrate utilization test	Negative
Catalase	Positive
Oxidase test	Negative
Nitrate reductase test	Positive

b. ***Escherichia. coli :*** *E.coli* is Gram negative rod shaped bacteria. It play an important role in the removal of toxic hexavalent chromium from the polluted site, *E.coli* was collected from Department of Biotechnology, GITAM University.. Then inoculated into sterilized nutrient broth and nutrient agar and incubated at 37° C for 24 hrs.

Table 2 Biochemical Tests:

Name of test	Result
Indole	Positive
Methyl red	Positive
Voges proskauer	Negative
Citrate utilization test	Negative
Oxidase	Negative
Nitrate reduction	Positive
Urease	Negative
Catalase	Positive

c. ***Pseudomonas putida:*** It is a gram negative rod shaped saprotrophic soil bacterium. *P.putida* was collected from Department of Biotechnology, GITAM University. Then inoculated into sterilized nutrient broth and nutrient agar and incubated at 37° C for 24 hrs.

Table 3 Biochemical Tests:

Name of test	Result
Indole	Negative
Methyl red	Negative
Voges proskauer	Negative
Citrate utilization test	Positive
Catalase	Positive
Oxidase	Positive
Nitrate reduction	Negative
Urease	Negative

All glassware used for experimental purpose were washed in 10% nitric acid to remove any possible interference by other metals and properly autoclaved to avoid any type of contaminations. The media was prepared accurately and autoclaved, after growth on agar, culture was microscopically observed and studied for the contamination by staining with gram's staining and methylene blue.

B. Collection of fungal cells:

a. ***Phanerochaete chrysosporium***: *Phanerochaete chrysporium* is a white rot fungi that are able to degrade lignin to carbon dioxide this is achieved, in part, by lignin peroxidases. White rot fungi have been used in bioremediation efforts to break down potentially harmful chemicals in soil and water. *Phanerochaete chrysosporium* MTCC 787 is collected from MTCC, IMTECH, Chandigarh, India in lyophilized from and rehydrated by using a Pasteur pipette to add 1 ml sterile water to the freeze-dried pellet then drawn up the entire contents into the pipette and transferred to a test tube with about 5ml sterile water. Let the fungus rehydrated for a minimum of 2 hours. Then transferred to sterilized malt extract broth and malt extract agar and incubated at the recommended temperature guided by the MTCC Chandigarh, India.

b. ***Aspergillus flavus:*** *A. flavus* is a common fungus, it is a common mold in the environment and can cause storage problems in stored grains. It has been collected from the Department of FST, GITAM University.

Preparation of Chromium solution:

282.4mg of potassium dichromate was added in 100 ml of distilled water and sterilized at standard condition [11]. The concentration of the Cr (VI) was 1000ppm in the stock solution, 0.1ml of stock solution was added in the 0.9 ml of the medium broth, then the final concentration of Cr (VI) was become 100 ppm in the test tube. [12]

Preparation of reagents and chemicals for the Di-phenyl carbazide test:

Di-phenyl carbazide solution:

1.0 gm of 1-5 Di-phenyl carbazide was added in 200ml of acetone and stored in sterilized and dried brown coloured bottle [11].

Sulphuric acid solution:

For the preparation of 100ml of 1:1 sulphuric acid solution, 50ml of sulphuric acid was added in the 50ml of distilled water.

Orthophosporic acid.

Methodology of Di-phenyl carbazide test:

1ml of supernatant was added with 1ml of sulphuric acid solution with 60 µl of orthoposporic acid in a sterilized conical flask containing 50ml of distilled water and remained undulate for 5mins. After 5 mins optical density was measured of solution by spectrophotometer.

Bioremediation of chromium in synthetic solution by viable bacterial cells at different pH:

In this process the loop full cultures of *Bacillus subtilis*, *Pseudomonas putida*, *Escherichia coli* were taken in the test tubes containing the basal salt liquid medium (1ml) prepared distilled water and (0.1ml) of chromium solution was added and maintained at different pH 2,4,7 and 9 with another test tubes as control. Then these test tubes were incubated at room temperature 37°C for 24hrs. After 24hrs the test tubes centrifuged at 5000rpm for 14mins and then supernatant taken for analysis by Di-phenyl carbazide method for chromium and absorbance was taken at 540 nm.

Bioremediation of chromium in synthetic solutions by viable bacterial consortium at different pH:

In this process co-cultures were prepared by taking loop full cultures as follows:

- *Escherichia coil + Pseudomonas putida*
- *Escherichia coli + Bacillus subtilis*
- *Bacillus subtilis + Pseudomonas putida*

All these co-cultures were mixed in a separate test tubes containing the basal salt liquid medium (1ml) prepared with distilled water and 0.1ml of chromium solution in it , and different pH 2, 4, 7 and 9 with another test tubes as control and incubated at room temperature for 24 hrs. After 24 hrs., test tubes were centrifuged at 5000 rpm for 14 minutes and supernatant taken for the analysis by Di-phenyl carbazide method for chromium and absorbance was taken at 540nm.

Bioremediation of chromium in synthetic solution by viable fungal mycelial cells at different pH:

In this process the loop full cultures of *Phanerochaete chrysosporium* and *Aspergillus flavus* were taken in the test tubes containing the basal salt liquid medium (1ml) prepared distilled water and (0.1ml) of chromium solution was added and maintained at different pH 2,4,7 and 9 and another test tubes as control. Then these test tubes were incubated at room temperature 37°C for 72hrs. After 72hrs the test tubes centrifuged at 5000 rpm for 14mins and then supernatant taken for analysis by Di-phenyl carbazide method for chromium and absorbance was taken at 540nm.

Bioremediation of chromium in synthetic solution by consortium of viable fungal mycelia cells at different pH:

In this process the co-cultures were prepared by taking loop full cultures of *P. chrysosporium* and *A. flavus* were taken in the test tubes containing the basal salt liquid medium (1ml) prepared distilled water and (0.1ml) of chromium solution was added and maintained at different pH 2,4,7 and 9 with another test tubes as control. Then these test tubes were incubated at room temperature 37°C for 72hrs. After 72 hrs the test tubes centrifuged at 5000 rpm for 14 mins and then supernatant taken for analysis by Di-phenyl carbazide method for chromium and absorbance was taken at 540 nm.

Calculations

The percentage removal of chromium from fortified solutions was calculated. Percentage removal of chromium is equal to 100-(A/B×100). Where A is optical density of test solution (containing fungus) and B is optical density of control solution. [12]

RESULT AND DISCUSSION

Bioremediation/ Bio-sorption Study

In this study chromium was bio remediated by using the living cells of bacterial and fungal cells as individual and as co-culture and some selected changes has been observed.

Bioremediation of chromium in synthetic solution by viable bacterial cells at different pH:

The percentage removal of chromium from the synthetic solution by *E.coli* was 46.9 % at pH 2, 32.2% at pH 4, 27.9% at pH 7, 34.7% at pH 9.(Figure 1) Among all pH, the removal of chromium shows best at acidic pH 2 may be due to the presence of more hydrogen ions in the solution which promotes the adsorption of metal from solution.

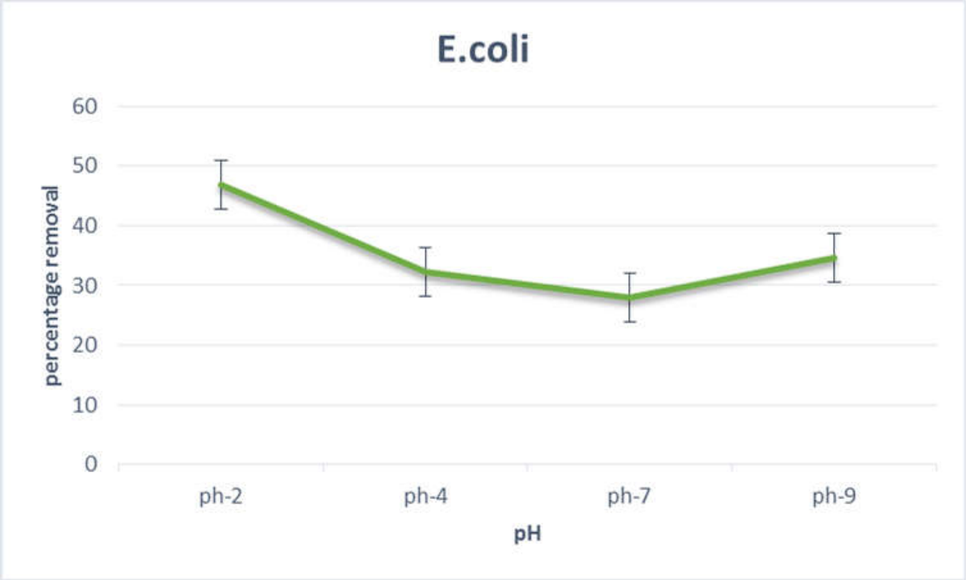

Figure 1 Percentage removal of chromium from the synthetic solution by *E.coli* at different pH

The percentage removal of chromium from the synthetic solution by *B.subtilis* was 33.8% at pH 2, 36.1% at pH 4, 32.1% at pH 7, 37.8% at pH 9. (Figure 2) Among all pH , the removal of chromium shows best at basic pH 9 because of the presence of more OH in the solution which helps in the metal adsortion. From the above findings it was proved that microbial biosorption of heavy metals from aqueous solutions depends on properties of adsorbent and molecules of adsorbate transfer from the solution to the solid phase. It has been also reported that biosorption capacities for heavy metals are strongly pH sensitive and that adsorption increases as solution pH increases [13]. The low bioaccumulation capacity at low pH is attributed to the competition of hydrogen ion with the metal ion on the sorption site. Therefore, in case of biosorption percentage removal efficiency of metal ion increases with increase in its alkalinity i.e. pH range from 2-9 is due to the relationship of bioaccumulation with the number of negative charges and which depends on dissociation of functional groups. With increase in pH beyond nine, the chromium removal rate decreased, which might be due to the osmotic changes. [14]

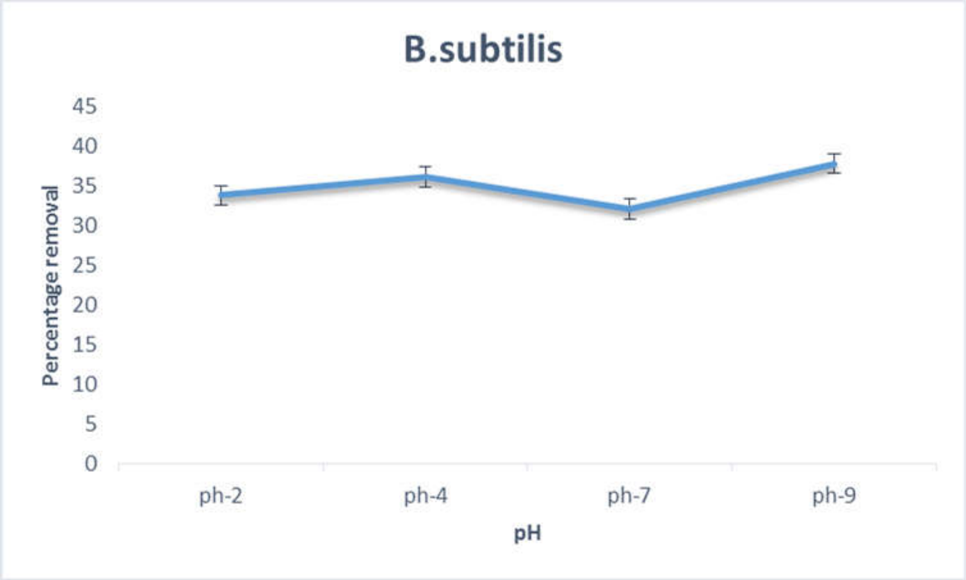

Figure 2 Percentage removal of chromium from the synthetic solution by *B.subtilis* at different pH

The percentage removal of chromium from the synthetic solution by *P.putida* was 59.8% at pH 2, 47.1% at pH 4, 50.8% at pH 7, 51.7% at pH 9. (Figure 3) Among all pH , the removal of chromium shows best at acidic pH 2 because of the presence of more hydrogen ions in the solution.

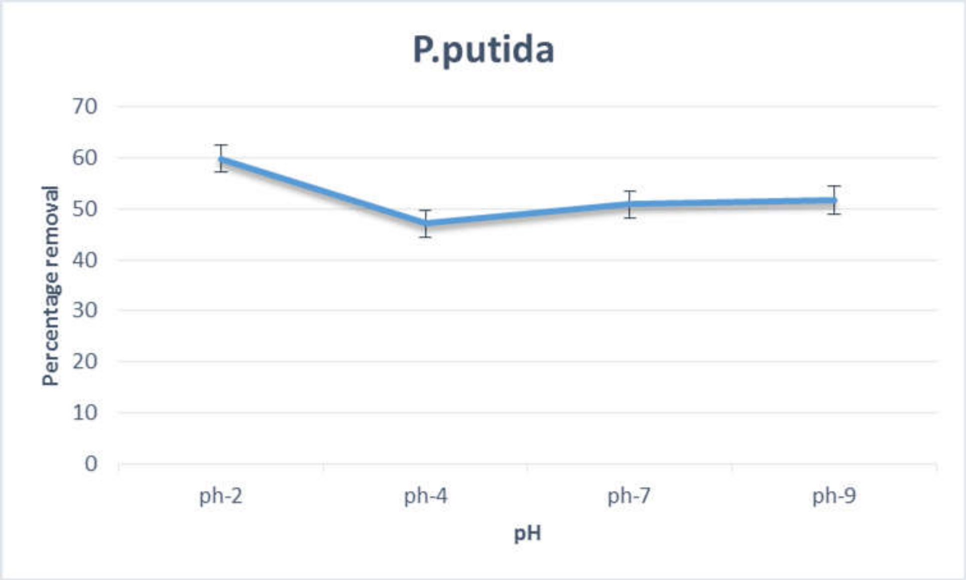

Figure 3 Percentage removal of chromium from the synthetic solution by *P.putida* at different pH

Bioremediation of chromium in synthetic solution by viable fungal mycelial cells at different pH:
The percentage removal of chromium from the synthetic solution by *A.flavus* was 91.2% at pH 2, 13.3% at pH 4, 26.9% at pH 7, 7.6% at pH 9. (Figure 4) Similar results were shown by *Aspergillus foetidus* it removed 97% chromium at pH 7 [15]. In one of earlier studies *Aspergillus sp N2* was able to reduce 20% Cr (VI) from solution at acidic pH while on same acidic pH *Penicillium sp N3* was reduce 93% of Cr (VI) , the same study was repeated in case of neutral pH with both the species and this time *Aspergillus* sp. N2 reduced 74% of Cr (VI) and *Penicillium sp N3* removed only 35% Cr (VI) [18]. The reason behind this results is that the percentage removal of chromium at high pH is depends upon the negative charge present on the fungal surface [17] due to the functional groups which helpful in the binding of cation Cr (VI) up to the optimum range of pH while at lower pH, due to the protonation of binding site resulting from high concentration of proton, negative charge intensity on the site is reduced which results in the reduction or inhibition for the binding of metal ion [14].

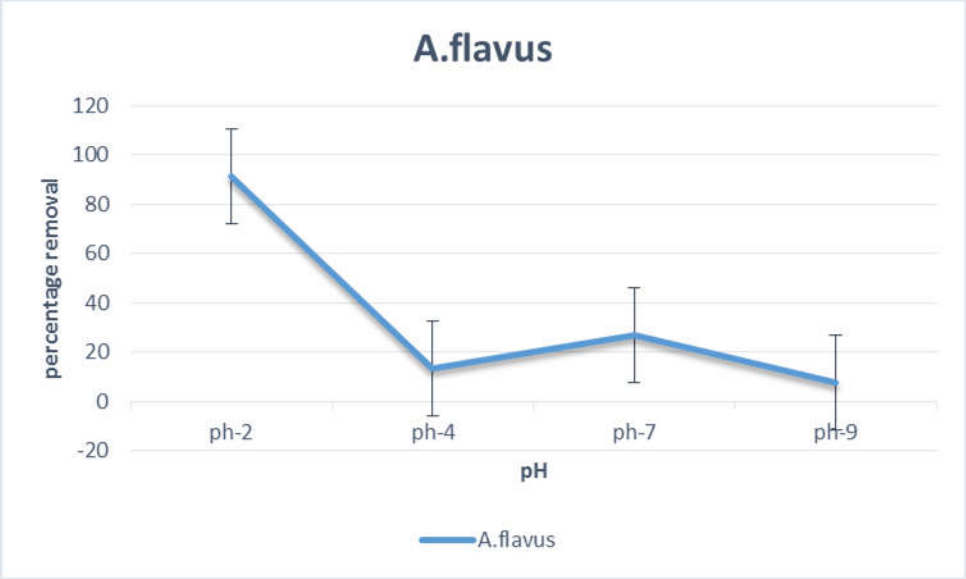

Figure 4 Percentage removal of chromium from the synthetic solution by *A. flavus* at different pH

The percentage removal of chromium from the synthetic solution by *P.chrysosporium* was 79.2% at pH 2, 31.1% at pH 4, 34.5% at pH 7, 81.3% at pH 9. (Figure 5) Same results were shown in a study where biosorbent cells adsorbs more chromium from synthetic solutions then the viable cells of *P.chrysosporium*. [14].

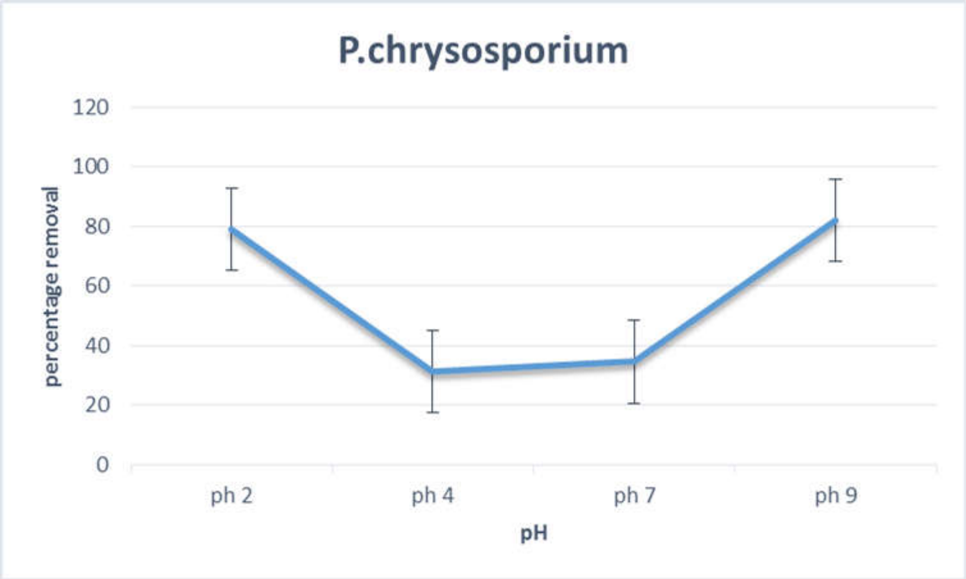

Figure 5 Percentage removal of chromium from the synthetic solution by *P.chrysosporium* at different pH

Bioremediation of chromium in synthetic solutions by viable bacterial consortium at different pH:
The co-cultures of *E.coli + P.putida* shows the percentage removal of chromium from the synthetic solution 80.9% at pH 2, 92.2% at 4 ,53.7% at pH 7, 89.1% at pH 9. (Figure 6) On other study the percentage removal of chromium from synthetic solution by co-cultures of *E.coli + B.subtilis* was 79.9% at pH 2, 67% at pH 4, 54.2% at pH 7, 79.2% at pH 9. (Figure 7) In third study, the percentage removal of chromium from synthetic solution by co-cultures of *P.putida+ B.subtilis* was 69.1% at pH 2, 91.4% at pH 4, 52.3% at pH 7, 63% at pH 9. (Figure 8)
The ratio of adsorption of chromium is higher in co-culture than the individual microorganism.

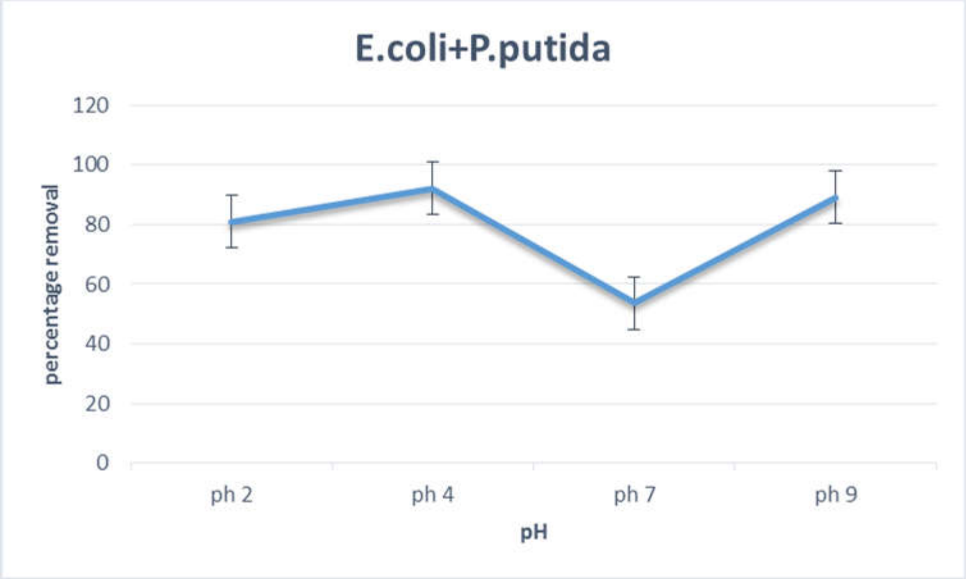

Figure 6 Percentage removal of chromium from the synthetic solution by *E.coli+P.Putida* at different pH

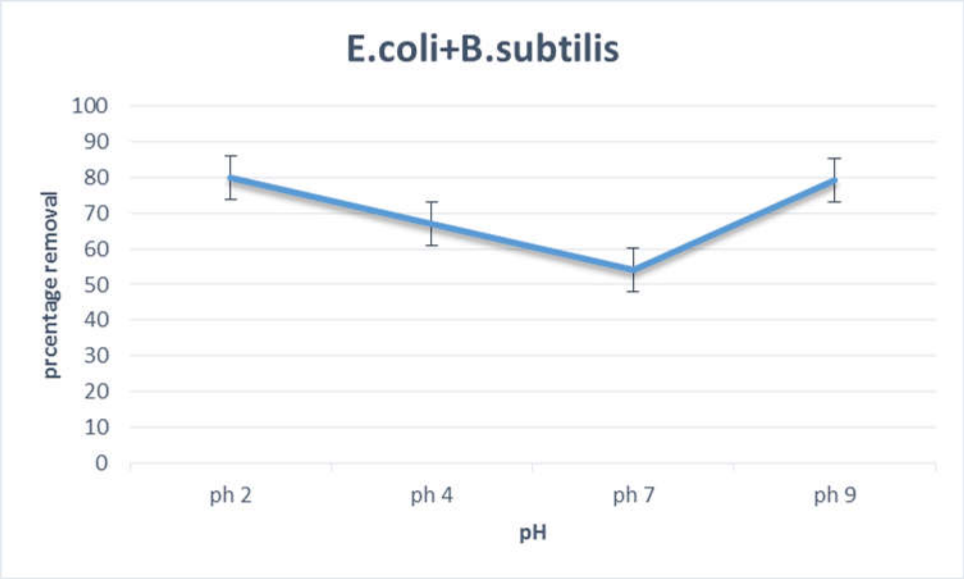

Figure 7 Percentage removal of chromium from the synthetic solution by *E.coli+B.subtilis* at different pH

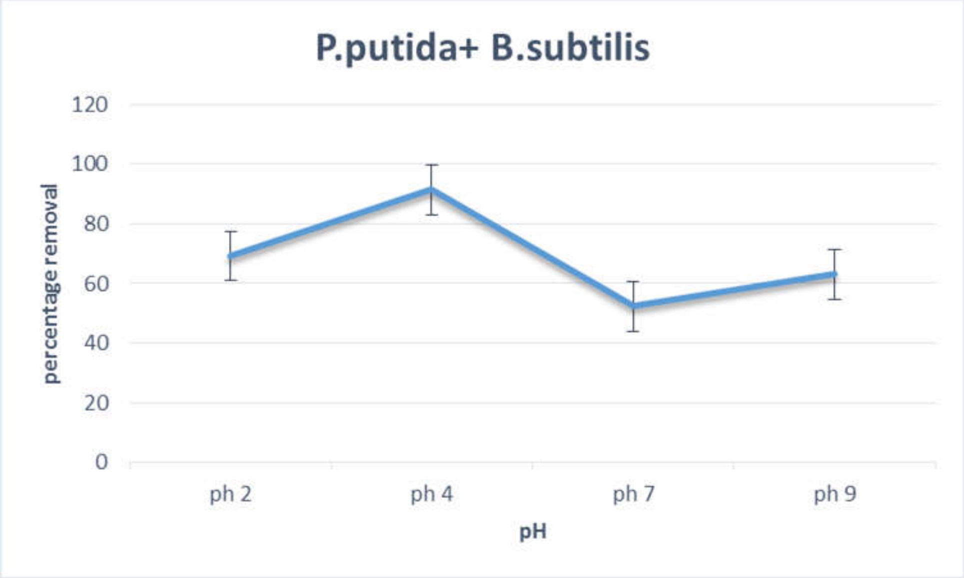

Figure 8 Percentage removal of chromium from the synthetic solution by *P.putida+B.subtilis* at different pH

Bioremediation of chromium in synthetic solution by consortium of viable fungal mycelia cells at different pH:

The co-cultures of *P.chrysosporium + A.flavus* the percentage removal of chromium from synthetic solution was 93.8% at pH 2, 8.3% at pH 4, 42.3% at pH 7, 16.9% at pH 9. (Figure 9) The ration of adsorption of chromium is high in fungal consortium and it shows good results in co-cultures.

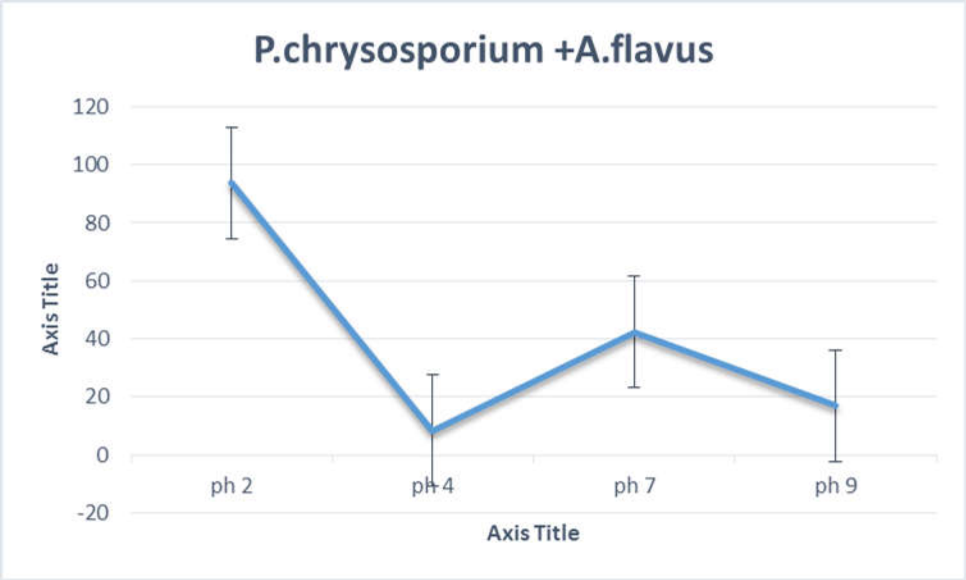

Figure 9 Percentage removal of chromium from the synthetic solution by *P. chrysosporium+A.flavus* at different pH

Table 4 Compared result obtained from this study with the similar earlier study.

Microorganism	**Initial Conc. of Cr (VI)**	**Percentage removal of Chromium**	**References**
Marine Aspergillus niger	400 mgL-1	95.00	[19]
Aspergillus niger	30 mgL-1	91.03	[20]
Aspergillus sydoni	30 mgL-1	87.95	[20]
Penicillium janthinellum	30 mgL -1	86.61	[20]
Aspergillus niger	10 mgL-1	63.00	[15]
Pseudomonas putida	100 mgL-1	95.68	[3]
Aspergillus niger	500 mgL-1	75.00	[16]
Bacillus cereus strain XMCr-6	100 mgL-1	Complete reduction after 48 Hrs.	[3]
Aspergillus foetidus	5 mgL-1	97	[21]
Bacillus cereus GIDM20	52 mgL-1	85	[3]
Aspergillus species	100 mgL-1	92	[22]
Phanerochaete chrysosporium	100 mgL-1	48.6	[23]
Enterobacter cloacae B2-DHA	1000 mgmL-1	81	[3]
Bacillus subtilis	100 mgL-1	95.19	[3]
Phanerochaete chrysosporium MTCC787 (Dried Cells)	100 mgL-1	99.7	[18]
Phanerochaete chrysosporium MTCC787(Dried Cells)	100 mgL-1	99.84 (with Tween 80 soln.)	[18]
P.putida + *B.subtilis* (Viable cells)	100 mgL-1	91.4	Present study
P.chrysosporium + *A.flavus*(Viable cells)	100 mgL-1	93.8	Present study
P. chrysosporium (Viable cells)	100 mgL-1	81.3	Present study

CONCLUSION

In present study we found that absorption of chromium from synthetic solutions by using different microorganisms with different methodology is that the co-culture shows better adsorption then individual microbial cells. Co-culture of *P.putida* + *B.subtilis*(91.4%) and *P.chrysosporium* + *A.flavus* (93.8%) shows better adsorption at acidic pH of solution while in individual *P.chrysosporium* (81.3%) shows better adsorption at basic pH. So, we can suggest that co-culture of microorganism is better option for the removal of chromium from contaminated sites and water bodies.

ACKNOWLEDGEMENTS

The authors are thankful to the Department of Microbiology, Department of Biochemistry and Department of Biotechnology, GITAM Institute of Science for providing the laboratory facilities and their constant support.

REFERENCES

1. Sankhla, M. S., Kumari, M., Nandan, M., Kumar, R., & Agrawal, P. (2016). Heavy metals contamination in water and their hazardous effect on human health—a review. *International Journal of Current Microbiology and Applied Sciences*, *5*(10), 759-766.
2. Ayangbenro, A., & Babalola, O. (2017). A new strategy for heavy metal polluted environments: a review of microbial biosorbents. *International journal of environmental research and public health*, *14*(1), 94.
3. Ojuederie, O., & Babalola, O. (2017). Microbial and plant-assisted bioremediation of heavy metal polluted environments: A review. *International journal of environmental research and public health*, *14*(12), 1504.
4. Pal, S., Patel, N., Malik, A., & Singh, D. K. (2015). Heavy metal health risk assessment and microbial menaces via dietary intake of vegetables collected from Delhi and national capital regions peri urban area, India. *Journal of Food, Agriculture & Environment*, *13*(2), 83.
5. Alimba, C. G., Dhillon, V., Bakare, A. A., & Fenech, M. (2016). Genotoxicity and cytotoxicity of chromium, copper, manganese and lead, and their mixture in WIL2-NS human B lymphoblastoid cells is enhanced by folate depletion. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, *798*, 35-47.
6. Pal, S., & Vimala, Y. (2012). Comparative study on bioremediation of chromium in fortified solutions by viable, biosorbent and immobilised cells of Phanerochaete chrysosporium-MTCC787. *International Journal of Microbiology Research*, *4*(6), 240.
7. Sherameti, I., & Varma, A. (2015). *Heavy metal contamination of soils*. Berlin: Springer.
8. Bharagava, R. N. (2017). *Environmental Pollutants and their Bioremediation Approaches*. CRC Press.
9. Jacob, J. M., Karthik, C., Saratale, R. G., Kumar, S. S., Prabakar, D., Kadirvelu, K., & Pugazhendhi, A. (2018). Biological approaches to tackle heavy metal pollution: A survey of literature. *Journal of environmental management*, *217*, 56-70.
10. Pal, S., Patel, N., Malik, A., & Singh, D. K. (2016). Microbial menaces and their biodiversity present in crops irrigated with polluted Yamuna water in peri-urban agriculture area of Delhi and National Capital regions, India. *Journal of Pure and Applied Microbiology*, *10*(3), 2113-2120.
11. APHA, AWWA and WEF (1999) Standard Methods for the Examination of Water and Wastewater 20th Ed.
12. Pal, S., Bahuk, S., & Vimala, Y. (2016). Biosorption of Chromium from Fortified Solution Using Biodross. *Nature Environment and Pollution Technology*, *15*(3), 911.
13. Muya, F. N., Sunday, C. E., Baker, P., & Iwuoha, E. (2016). Environmental remediation of heavy metal ions from aqueous solution through hydrogel adsorption: a critical review. *Water Science and Technology*, *73*(5), 983-992.
14. Pal, S., & Vimala, Y. (2011). Bioremediation of chromium from fortified solutions by Phanerochaete chrysosporium (MTCC 787). *Journal of Bioremediation and Biodegradation*, *2*, 1-5.
15. Prasenjit B, Sumathi S (2005) Uptake of chromium by *Aspergillus foetidus*. J Mater Cycles Waste Manag 7 : 88-92.
16. Fukuda T, Ishino Y, Ogawa A, Tsutsumi K, Morita H (2008) Cr(VI) reduction from contaminated soils by Aspergillus sp. N2 and Penicillium sp. N3 isolated from chromium deposits . J Gen Appl Microbiol 54: 295-303.
17. Pundir, R., Chary, G. H. V. C., & Dastidar, M. G. (2018). Application of Taguchi method for optimizing the process parameters for the removal of copper and nickel by growing Aspergillus sp. *Water resources and industry*, *20*, 83-92.
18. Pal, S., & Vimala, Y. (2012). Bioremediation and decolorization of Distillery effluent by novel Microbial Consortium. *European Journal of Experimental Biology*, *2*(3), 496-504.
19. Arslan G, Pehlivan E (2008) Uptake of Cr+3 from aqueous solution by lignite based humic acids. Bioresource Technology 16: 7597-7605.
20. Churchill SA, Walters TV, Churchill PF (1996) Sorption of heavy metals by prepared bacterial cell surfaces. J Environ Eng 121: 706.
21. Friis N, Myers-keith P (1998) Biosorption of uranium and lead by *Streptomyces longwoodensis*. Biotechnol Bioeng 28: 21-28.
22. Yan G, Viraraghavan T (2003) Heavy metal removal from aqueous solution by fungus *Mucor rouxii*. Water Res 37: 4486-4496.
23. Zhang L, Zhao L, Yu YU, Chen (1998) CZ Removal of lead from aqueous solution by non-living *Rhizopus nigricans.* Water Res 32: 1437-1444.

CITE THIS ARTICLE

Upma, Sumit Pal, Dillep Kumar S, Parimala Sai K, Vimala Y. Bioremediation of Chromium from Synthetic Solution by using viable Microbial Cells and Consortium. Res. J. Chem. Env. Sci. Vol 7 [2] April 2019. 63-71