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A Comparative Study on Biological Removal of Cr(VI) by *Fusarium Solani* under Different Modes of Operational Strategies

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Abstract

In the present work biological removal of Cr(VI) was carried out in fed batch mode of operation using the growing cells of *Fusarium solani* isolated from soil. The fed batch process was studied by constant volume pulse feeding (CVPF) and increasing volume pulse feeding (IVPF), and the effects of these operational strategies on biological performance were compared with a conventional batch process. Batch studies indicated the maximum specific Cr(VI) removal to be 71 mg/g at pH 5.0 and at 500 mg/l initial Cr(VI) ion concentration. In CVPF process the maximum specific Cr(VI) removal was found to be 35.51 mg/g and 25.15 mg/g as compared to 51.9 mg/g and 42.5 mg/g obtained in IVPF during first and second pulse feedings respectively in both the cases. These results were compared with the Cr(VI) removal obtained in earlier studies conducted by the present authors using the continuous mode of operation. The continuous mode of operation was found to be the best operational strategy in which the process could be operated for longer duration with maximum specific Cr(VI) removal of 62.27 mg/g. Nearly complete removal was obtained using single stage reactor at lower Cr(VI) concentrations up to 100 mg/l and a multi stage reactor at higher Cr(VI) concentration.

Keywords: Cr(VI), *Fusarium solani*, batch, fed batch and continuous operations

1. Introduction

Cr(VI) is one of the toxic heavy metals which are common pollutants of the environment [1]. Wastewaters containing Cr(VI) are generated in many industrial processes including chrome plating, metal cleaning and processing, wood preservation and alloy preparation etc., [2] and are often being discharged into natural environment with out getting any treatment especially from small and medium scale industries. This is of serious environmental concern as Cr(VI) persists indefinitely in the environment complicating its remediation. The persistent nature makes it accumulate in the food chain, which with time reaches harmful levels in living beings resulting in serious health hazards such as irritation in lungs and stomach, cancer in digestive tract, low growth rates in plants, death of animals, etc. Conventional methods for removing toxic Cr(VI) from wastewaters include chemical reduction followed by precipitation, electrochemical treatment, reverse osmosis and ion-exchange. However, such processes require high energy or large quantity of chemical reagents. Moreover, these methods are ineffective at lower concentration of metal ions present in large volume of wastewaters and generate large quantity of toxic sludges, disposal of which again causes secondary pollution [3]. Environment friendly processes, therefore, need to be developed to clean-up the environment without creating harmful waste by-products. Bioremediation involves

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potential application of microorganisms in removal of heavy metals and has been recognized as a potential alternative to the conventional methods for treatment of contaminated wastewaters [4].

The growing, resting and non-living cells of microorganisms are reported to remove Cr(VI) from aqueous solutions. However, most of the work to remove Cr(VI) have been carried out using non-living fungal cells and a very little information is available on use of growing and resting cells. The use of non-living cells has advantages over growing and resting cells due to the absence of both toxicity limitations and requirements of growth media and nutrients. Moreover, adsorbed metal can be easily desorbed and regenerated biomass can be reused. However, the most important limitation with non-living biomass is that biochemical cell energetic reactions are no longer continued as the cells are dried, whereas both growing and resting cells can be maintained biochemically active. Resting cells have the advantage that they require very low maintenance energy to remain biochemically active, whereas growing systems have the advantage over the non-living and resting cells that the simultaneous removal of metal is obtained during growth of the organism and separate biomass production processes e.g., cultivation, harvesting, drying, processing and storage can be avoided of course the major limitation of using growing systems for biosorption of metals is that cell growth is inhibited when the metal concentration is high. This problem can be overcome by the use of metal tolerant organism. The tolerance and removal capacities are the essential characteristics of growing biomass used in a metal removal process [5-17].

In the present study, different operational strategies were tried to achieve the maximum removal of Cr(VI) from the medium using *Fusarium solani* during its growth. The fed batch operational strategies (pulse feeding operation) aimed at maintaining availability of fresh nutrient medium and minimization of the inhibitory effect of the dissolved components in media. Pulse feeding was carried out in two modes of operation as constant volume pulse feeding (CVPF), i.e., intermittent feeding with simultaneous withdrawal of medium and increasing volume pulse feeding (IVPF), i.e., intermittent feeding without withdrawal of medium.

2. Materials and Methods

2.1. Microorganism and inoculum preparation

Fusarium solani isolated from soil was used in the present study [18], which was grown in 250 ml Erlenmeyer flask in a shaking incubator at 30° C and 180 rpm using media of the following composition (g/L): glucose, 10.0; K_2HP0_4, 0.5; NaCl, 1.0; $MgS0_4$, 0.1; NH_4N0_3, 0.5 and yeast extract, 5.0. The pH of the media was 6.0. An inoculum of 10% (v/v) of a 36 h old culture was used for the Cr(VI) removal studies.

2.2. Batch studies

Batch study was conducted in Erlenmeyer flask (250 ml) using media (100 ml) containing Cr(VI), to which a 10% (v/v) inoculum was added. The flask was incubated at 30° C and 180 rpm. The pH was adjusted to 5.0 using sulphuric acid. The process was monitored with time till the substrate limiting condition was reached. The samples were periodically withdrawn and centrifuged at 5000 rpm for 30 min and the supernatant liquid was separated and analyzed for residual Cr(VI) concentration and residual sugar concentration. The biomass collected by centrifugation was washed, dried and the dry weight of biomass was estimated gravimetrically. All experiments were performed in triplicates.

2.3. Fed batch studies

2.3.1. CVPF

This mode of operation consists of intermittent feeding with simultaneous withdrawal of medium. In a fed batch bioreactor (working volume-3 l) a 10% (v/v) inoculum concentration was added to the medium containing 500 mg/l initial Cr(VI) concentration at pH 5.0. The volume of the medium initially was kept at 3 l and the process of Cr(VI) removal was

monitored for 5 days when substrate was completely utilized. The first pulse feeding was performed on the 5th day. One litre of homogeneous medium was drawn out of the reactor and fresh medium (1 l) containing required quantity of Cr(VI) to maintain 500 mg/l Cr(VI) concentration was added to bring the volume to its original (3 l). On the 12th day next pulse feeding was performed and the process was monitored till 19th day.

2.3.2. IVPF

In IVPF mode of operation carried out in a fed batch bioreactor (working volume-3 l) under the conditions as described in case of CVPF, the volume of the medium initially was kept at 1 l and the process was monitored for 5 days when substrate was completely utilized. On the 5th day, 1 l fresh medium was added to the reactor and similar addition was done on the 12th day. The process was monitored till 19th day.

In both CVPF and IVPF operations, the liquid samples collected periodically were analyzed for residual Cr(VI) concentration and residual substrate concentration. The biomass collected was estimated gravimetrically. All experiments were performed in triplicates.

3. Assay Techniques

The residual Cr(VI) concentrations in the medium was determined spectrophotometrically (Sytronics UV-VIS spectrophotometer 117) at 540 nm using Di-phenyl carbazide as the complexing agent [19]. The sugar concentration in the medium was analyzed by di-nitro salicylic acid (DNS) method at 540 nm [20].

4. Results

4.1. Batch studies

Figures 1a and 1b show the changes in biomass concentration (gm of dry biomass weight per litre of liquid medium) of *Fusarium solani* and residual glucose concentration (g/l), respectively with time (h) in the medium at different initial Cr(VI) concentrations (0-5000 mg/l) and at initial medium pH 6.0. The figure 1a clearly shows that in the absence of Cr(VI) the lag period was 3 h followed by the exponential phase of growth (up to 30 h). The growth of the *Fusarium solani* reached stationary phase within 36 h.

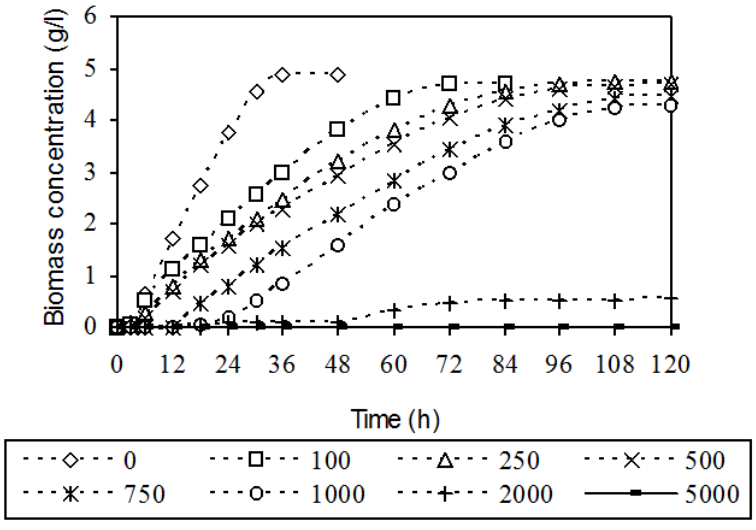

Fig. 1 (a) Change in biomass concentration of *Fusarium solani* with time at different initial Cr(VI) concentrations

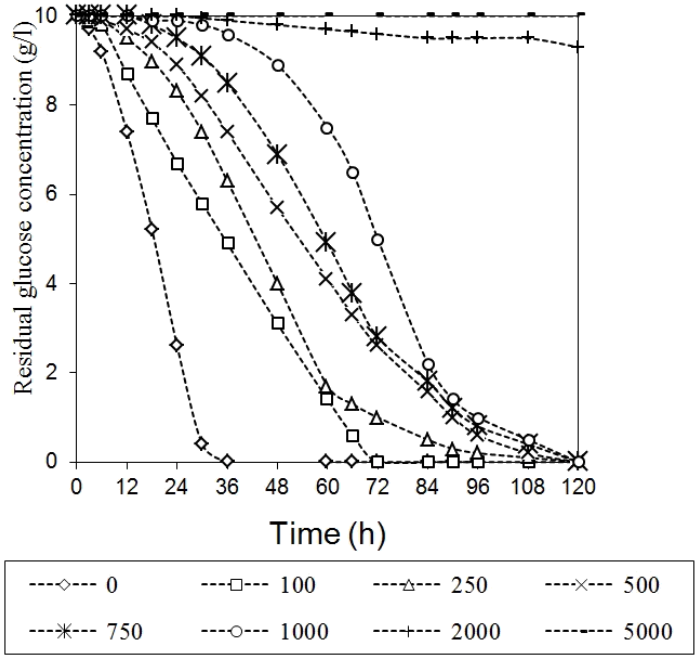

Fig. 1 (b) Change in residual glucose concentration with time at different initial Cr(VI) concentrations

The Figure 1b indicates complete utilization of glucose in 36 h of incubation period in the absence of Cr(VI) ion. In the presence of Cr(VI), the fungus was able to grow even at 2000 mg/l initial Cr(VI) concentration (Figure 1a). The lag period was found to be nearly the same up to 500 mg/l initial Cr(VI) ion concentration, beyond which the lag period increased with increase in initial Cr(VI) ion concentration up to 2000 mg/l.

The change in pH with time at different initial Cr(VI) concentrations (0-1000 mg/l) during the growth of *Fusarium solani* shown in Table 1. In the absence of Cr(VI) ion, pH decreased from an initial value of 6.0 to 4.8 in 15 h, i.e., the early phase of the exponential growth (Figure 1a) and then gradually increased to 5.6 in 36 h when glucose was completely utilized (Figure 1b).

Table 1 Change in pH at different time of growth of *Fusarium solani* at different initial Cr(VI) concentrations

Initial Cr(VI) conc. (mg/l)	0	100	250	500	750	1000
Time (h)	pH values					
0	6.0	6.0	6.0	6.0	6.0	6.0
6	5.2	5.8	5.8	5.8	5.8	5.9
12	4.9	5.5	5.6	5.6	5.8	5.8
15	4.8	5.4	5.5	5.5	5.7	5.7
18	5.0	5.3	5.85	5.4	5.7	5.7
24	5.1	5.1	5.3	5.3	5.7	5.7
36	5.6	5.3	5.5	5.2	5.4	5.4
48	--	5.5	5.9	5.1	5.2	5.2
72	--	6.5	6.0	6.3	5.4	5.7
96	--	--	6.2	6.4	5.9	5.9
120	--	--	6.5	6.5	6.5	6.5

A similar trend was observed in the presence of Cr(VI) ions at all the concentrations, i.e., pH decreased from an initial value of 6.0 to 5.1-5.3 in the early phase of the exponential growth and then gradually increased to 6.5 when glucose was completely utilized.

Batch studies on Cr(VI) removal indicated maximum specific Cr(VI) removal at pH 5.0 using growing cells of *Fusarium solani* at 500 mg/l initial Cr(VI) concentration. At the same concentration about 64% removal was observed, although nearly complete removal was obtained at lower concentration up to 100 mg/l. A similar trend was observed in continuous mode of operation. At 500 mg/l concentration, Cr(VI) removal was only to the extent of 62.27 mg/g which was found to be 54.8% of the initial 500 mg/l concentration. As it was not possible to obtain complete removal at higher Cr(VI) concentrations, alternate strategies were tried to operate the process in a fed batch reactor in two different modes of pulse feeding operations i.e., CVPF & IVPF. Enhanced Cr(VI) removal was expected in fed batch operation as compared to the batch due to the availability of fresh medium containing nutrients and the dilution of the medium minimizing the inhibitory effect of the dissolved components in the medium.

Figure 2 shows the changes in residual Cr(VI) concentration in the presence of *Fusarium solani* with time in constant volume pulse feeding (CVPF) mode of operation at 500 mg/l initial Cr(VI) concentration and at pH 5.0. In CVPF, the volume of the medium (containing 500 mg/l Cr(VI) concentration) initially was kept at 3 l and the process was monitored for 5 days. It appears from the figure that the total residual Cr(VI) concentration in the presence of *Fusarium solani* decreased to 190 mg/l on 5th day when glucose was completely utilized. This indicates that 310 mg/l of Cr(VI) was removed biologically from the medium. The specific Cr(VI) removal was 68.89 mg/g, which was found to be nearly the same as the value (71 mg/g) obtained in batch operation. On the 5th day, 1L of homogeneous medium was removed from the reactor and fresh media (1 l) was added to bring the volume to its original 3 l. This fresh 1 l media contained the required quantities of nutrients and the calculated quantity of Cr(VI) required to maintain the Cr(VI) concentration at 500 mg/l. This process was monitored till 12th day and this was done to ensure the fresh nutrient availability to the *Fusarium solani* and dilution of the medium. On the 10th day the biological removal of Cr(VI) was found to be 138.5 mg/l and which remained constant till 12th day when 12% glucose till remain unutilized and the specific Cr(VI) removal was found to be 35.51 mg/g. Similarly, second pulse feeding was done on the 12th day and the process was monitored till 19th day. On the 17th day, the total removal was found to be 80.5 mg/l which remained constant till 19th day and 50% glucose remained unutilized. The specific Cr(VI) removal was 25.12 mg/g.

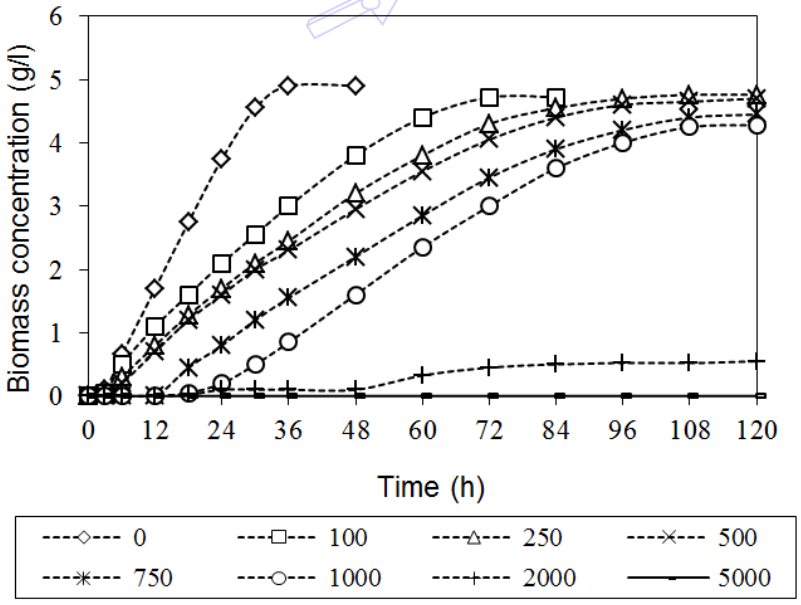

Fig. 2 Changes in residual Cr(VI) concentration with time using medium in constant volume pulse feeding (CVPF) mode of operation

Figure 3 shows the changes in residual Cr(VI) concentration with time in increasing volume pulse feeding (IVPF) mode of operation at 500 mg/l initial Cr(VI) ion concentration and at pH 5.0.

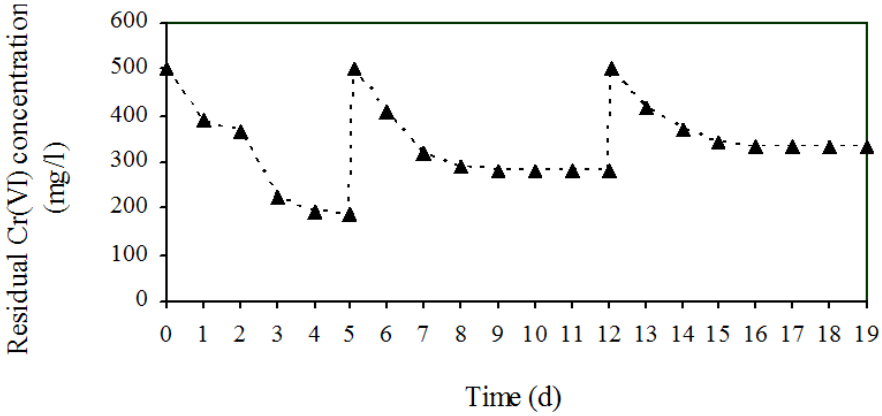

Fig. 3 Changes in residual Cr(VI) concentration with time using sterile growth medium in increasing volume pulse feeding (IVPF) mode of operation

In IVPF, the volume of the media (containing 500 mg/l Cr(VI) concentration) initially was kept at 1l and the process was monitored for 5 days when the glucose was completely utilized. The similar specific Cr(VI) removal of 68.8 mg/g was observed in 5 days. The first pulse feeding was done on the 5th day by adding 1l fresh media and the process was monitored till 12th day. On the 10th day the biological removal of Cr(VI) in the presence of *Fusarium solani* was 218 mg/l and remained constant up to 12th day and the specific Cr(VI) removal was found to be 51.9 mg/g. Similarly, second pulse feeding was done on the 12th day and the removal was found to be decreased to 166 mg/l on the 17th day and remained constant upto 19th day when glucose was found to be 13 % unutilized. The specific Cr(VI) removal was 42.5 mg/g.

Table 2 shows a comparison of Cr(VI) removal in different modes of operations at 500 mg/l initial Cr(VI) concentration and at pH 5.0 [21-28].

Table 2 Comparison of Cr(VI) removal in batch, CVPF, IVPF and continuous operation

Mode of operation		Conc. of Cr(VI) removal (mg/l)	Cr(VI) remove (%)	Rate of Cr(VI) removal (mg/l/d)	Specific Cr(VI) removal (mg/g)
Batch		320	64	64	71.1
CVPF	(0-5 d)	310	62	62	68.89
	1st pulse feed(6-12d)	138.5	27.7	19.7	35.51
	2nd pulse feeding (13-19 d)	80.5	16.1	11.5	25.15
IVPF	(0-5 d)	310	62	62	68.89
	1st pulse feed (6-12d)	218	43.6	31.1	51.9
	2nd pulse feeding (13-19 d)	166	33.2	23.7	42.5
Continuous	Single stage	274	54.8	65.76	62.27
Multi stage	First stage	274	84 (over all)	65.76	62.27
	Second stage	146		29.59	33

5. Discussion

The growth rate of the *Fusarium solani* (Fig. 1(a)) and the glucose utilization rate (Fig. 1(b)) were found to be decreased with increase in initial Cr(VI) concentration from 0-1000 mg/l although glucose was found to be utilized completely in each case.

The decreased growth of other organisms, e.g., *Candida utilis* with increase in initial Cr(VI) concentration from 0 to 500 mg/l has also been reported [29]. About 95.5% decrease in biomass concentration of *Candida utilis* was observed as

compared to only 4% decrease obtained in the present study conducted with *Fusarium solani* when Cr(VI) ion concentration was increased from 0 to 500 mg/l. These results clearly indicate that *Fusarium solani* can tolerate high concentration of Cr(VI) as compared to other organisms.

The decrease in pH with time at different initial Cr(VI) concentration shown in Table 1 perhaps due to the formation of pyruvate and hydrogen ions during the growth of the *Fusarium solani*. An increase in pH could be due to the conservation of the available hydrogen ions by the coenzymes (produced in Tricarboxylic Acid Cycle) to form the reduced coenzymes needed for the oxidation of the Acetyl-CoA (obtained from the degradation of pyruvate) to CO_2 and H_2O via Tricarboxylic Acid Cycle.

5.1. CVPF

In CVPF operation, the *Fusarium solani* possibly could not adjust to the fluctuating growth environment due to the pulse feeding. Another possibility could be removal of viable cells during withdrawal of medium. This resulted in unutilisation of glucose and reduced growth causing reduced Cr(VI) removal. Therefore, by adopting CVPF strategy as no further enhancement was observed in Cr(VI) removal after first and second pulse feeding on the 5^{th} and the12^{th} day , the CVPF operation was discontinued after 19^{th} day.

5.2. IVPF

Although significant removal of Cr(VI) was obtained in IVPF mode as compared to CVPF mode after first and second pulse feeding, the values were still found to be lowered than the values obtained in the batch mode. Therefore, the process could not be operated further under fed batch condition.

Table 2 shows a comparison of Cr(VI) removal obtained in the present study using CVPF and IVPF mode of operations with the data obtained from the earlier studies using the same *Fusarium solani* under batch and continuous mode of operations. In the batch mode, the Cr(VI) removal was 64%. In CVPF mode, the percentage of Cr(VI) removal drastically decreased after each feeding leading to discontinuation of the process after 19^{th} day. A similar trend was also observed in IVPF operation. In continuous mode of operation, Cr(VI) removal was found to be 84% using a two- stage reactor. Out of the above strategies, the continuous mode was found to be the best operational strategy for continuous removal of Cr(VI) as the process could be operated successfully for a long time in continuous mode with higher Cr(VI) removal as compared to other operational strategies. The overall removal rate (mg/l/d) of Cr(VI) with batch operation was 3.1 times more than the rate obtained after first pulse feeding and 5.4 times after second pulse feeding in CVPF operation. The removal rate in batch operation is also found to be 2 times and 2.6 times more than the rate obtained after first and second pulse feeding in IVPF operation; on the contrary, in the continuous mode operation the overall removal rate was found to be higher than the batch operation in a single stage bioreactor, whereas in the second stage operation the rate of Cr(VI) removal was found to be decreased.

6. Conclusion

While comparing all the above strategies it was observed that the process of Cr(VI) removal in a single stage bioreactor can be continued for long time at higher removal rate. The multistage operation appears to have an advantage that enhanced Cr(VI) removal can be obtained and the process can be continued for a long time. A single stage reactor would be effective at lower concentration, whereas a multi stage reactor system shall be needed at higher concentration in higher removal of Cr(VI) in continuous mode.

References

[1] B. R. Sudha and E. Abraham, "Biosorption of Cr(VI) from aqueous solution by Rhizopus nigrificans," Bioresource Technology, vol. 79, no. 1, pp. 73-81, Aug. 2001.

[2] T. Srinath, T. Verma, P. W. Ramteka, and S. K. Garg, "Chromium (VI) biosorption and bioaccumulation by chromate resistant bacteria," Chemophere, vol. 48, no. 4, pp. 427- 435, 2001.

[3] K. Komari, P. Wang, K.Toda, and H. Ohyake, "Factors affecting chromate reduction in Enterobactor cloacae strain HOI," Appl. Microbial Biotec. vol. 31, pp. 567-570, 1989.

[4] M. Faisal and S. Hassan, "Microbial conversion of Cr (VI) into Cr (III) in industrial effluent," African Journal of Biotechnology, vol. 3, no. 11, pp. 610-617, 2004.

[5] Z. Aksu and D. Akpinar, "Competitive biosorption of phenol and chromium (VI) from binary mixture onto dried anaerobic activate sludge," Biochemical Engineering Journal, vol. 7, no. 3, pp. 183-193, 2001.

[6] B. Volesky, Biosorption and biosorbents in Biosorption of heavy metals. Boca Raton: CRC press Inc., 1990.

[7] S. Llovera, R. Bonet, M. D. Simon-Pujol, and F. Congregado, "Effect of culture medium ions on chromate reduction by resting cells of Agrobacterium radiobacter," Appl. Microbial. Biotechnol, vol. 39, pp. 424-426, 1993.

[8] J. Campos, M. Martinez-Pacheco, and C .Cervantes, "Hexavalent chromium reduction by a chromate resistant Bacillus Sp. Strain. Antonie Van Leeuwenhoek," vol. 68, pp. 203-208, 1995.

[9] P. Pattanapipitpaisal, N. L. Brown, and L. E. Macaskie, "Chromate reduction by Microbacterium liquefaciens immobilized in polyvinyl alcohol," Biotech Letter, vol. 23, pp. 41-43, 2001.

[10] B. Barkhordar and M. Ghiasseddin, "Comparision of Langmuir and Freundlich equilibriums in Cr, Cu and Ni adsorption by sargassum," Iranian Journal of Environmental Health Science and Engineering, vol. 1, no.2, pp. 58-64, 2004.

[11] G. W. Stratten, "Review in environmental Toxicology (ed. Hodson E.)," Elsiever, Amsterdam, pp. 85-94, 1987.

[12] O. Muter, A. Patmalnieks, and A. Rapoport, "Interrelations of the yeast *Candida utilis* and Cr (VI): metal reduction and its distribution in the cell and medium," Process Biochemistry, vol. 36, no. 10, pp. 963-970, 2001.

[13] T. Srinath, T. Verma, P. W. Ramteka, and S. K. Garg, "Chromium (VI) biosorption and bioaccumulation by chromate resistant bacteria," Chemophere, vol. 48, no. 4, pp.427-435, July 2002.

[14] K. S. Selvaraj, S. Manonmai, and S. Pattabhi, "Removal of hexavalent chromium using distillery sludge," Bioresource Technology, vol. 89, pp. 207-211, 2003.

[15] S. S. Middleton, R. B. Latmani, M. R. Mackey, M. H. Ellisman, M. B. Tebor, and C. S. Criddle, "Cometabolism of Cr(VI) by *Shewanella Oncendensis* MR-1 produced cell associated reduced Chromium and inhibit growth," Wiley InterScience, 2003.

[16] A.Y. Dursan, G. Ulsu, Y. Cuci, and Z. Aksu, "Bioaccumulation of cupper(II), lead(II) and chromium(VI) by growing *Aspergillus niger*," Process Biochemistry, vol. 38, no. 10, pp. 1647-1651, 2003.

[17] J. M. Panacastro, F. M. Jeronimo, E. Garcia, and F. R. O. Canizares-Villanueva, "Heavy metals removal by the micro algae *Sceneunus* in crassatulus in continuous cultures," Bioresource Technology, vol. 94, no. 2, pp. 219-222, 2004.

[18] M. Sen and M. G. Dastidar, "Hexavalent chromium reduction and its distribution in the cell and medium by chromium resistant *Fusarium solani*," International Journal of Eng. And Tech. Innovation, vol. 3, no. 1, pp. 01-09, 2013.

[19] Standards methods for the examination of water and wastewater, 17th ed. American Public Health Association (APHA), pp. 157 -162, 1989.

[20] G. L. Millar, "Use of di-nitro salicyclic acid reagent for determination of reducing sugar," Anal chem., vol. 31, pp. 426-428, 1959.

[21] M. Sen and M. G. Dastidar, "Biosorption of Cr (VI) by resting cells of Aspergillus sp.," Iran. J. Environ.Health. Sci. Eng., vol. 4, pp. 9-12, 2007.

[22] M. Sen, M. G. Dastidar, and P. K. Roychoudhury, "Biosorption of Cr(VI) using fungal strain of Fusarium sp.," American Society of Civil Engineers (ASCE), Practice Periodical of Hazardous, Toxic, and Radioactive Waste Management, vol. 9, pp. 147-151, 2005.

[23] M. Sen, M. G. Dastidar, and P. K. Roychoudhury, "Biological removal of Cr (VI) using an isolated fungal strain water and waste water perspectives of developing countries (WAPDEC)," Water Association, pp. 1163-1169, 2005.

[24] M. Sen, M. G. Dastidar, and P. K. Roychoudhury, "Comparative studies of batch and continuous biological treatment of Cr (VI) using *Fusarium solani*," Enzyme and Microbial Technology, vol. 41, pp. 51-56, 2007.

[25] M. Sen and M. G. Dastidar, "Adsorption- desorption studies on Cr(VI) using non-living fungal biomass," Asian Journal of Chemistry, vol. 22, pp. 2331-2338, 2010.

[26] M. Sen and M. G. Dastidar, "Biosorption of Cr(VI) by resting cells of *Fusarium solani*," Iran. J. Environ. Health. Sci. Eng., vol. 8, pp. 153-158, 2011.

[27] M. Sen, "A Comparative study on Biosorption of Cr(VI) by *Fusarium solani* under different growth conditions," Open Journal of Applied Sciences, vol. 2, pp. 146-152, 2012.

[28] M. Sen, M. G. Dastidar, and P. K. Roychoudhury, "Biosorption of Zinc and Nickel from wastewaters using non living cells of *Fusarium solani*," International Journal of Chemtech Applications., vol. 2, pp. 63-70, 2013.

[29] O. Muter, A. Patmalnieks, and A. Rapoport, "Interrelations of the yeast *Candida utilis* and Cr (VI): metal reduction and its distribution in the cell and medium," Process Biochemistry, vol. 36, pp. 963-970, 2001.