Brazilian Journal of Biological Sciences, 2018, v. 5, No. 9, p. 33-45.
ISSN 2358-2731
https://doi.org/10.21472/bjbs.050904

Enhanced biological removal of Cr(VI) in Continuous Stirred Tank Reactor (CSTR) using *Aspergillus* sp.

Mousumi Sen

Department of Applied Chemistry. Amity School of Engineering & Technology. New Delhi-110061. India. E-mail: mousumi1976@gmail.com.

Abstract. Biological removal of Cr(VI) from aqueous solution was studied in batch and continuous mode of operation using the growing *Aspergillus* sp. Continuous removal of Cr(VI) was studied in Continuous Stirred Tank Reactor (CSTR) maintaining the microorganism in living or biologically active state. In batch bioreactor, the growth and Cr(VI) removal by the organism were studied at different initial Cr(VI) concentration at pH 5.0. Whereas, in continuous mode of operation both single and two stage reactors were also studied for Cr(VI) removal. Batch studies indicated the maximum specific Cr(VI) removal to be 41.2 $mg.g^{-1}$ at pH 5.0 and at 500 mg L^{-1} initial Cr(VI) concentration. However, in continuous mode of operation, the maximum specific Cr(VI) removal was found to be 39.4 $mg.g^{-1}$ after first stage operation with an additional 39.32 $mg.g^{-1}$ obtained in the second stage operation. Hence, these results indicated that the continuous mode of operation could be the ideal operational strategy in which the process could be operated for longer duration with a enhanced Cr(VI) removal.

Keywords: Hexavalent chromium; Heavy metal pollutants; Batch and continuous operation; Specific growth rate; Two stage reactor.

Received
March 21, 2018

Accepted
April 27, 2018

Released
April 30, 2018

Full Text Article

ORCID

0000-0001-7004-0313
Mousumi Sen

Introduction

Cr(VI) is one of the major toxic heavy metal pollutants in the environment (Sen et al., 2007) and is frequently present in wastewaters coming from industries such as electroplating, chrome plating, metal cleaning and processing, wood preservation and alloy preparation etc., (Germain and Patterson, 1974). However, it is a natural tendency for small and medium scale industries to discharge the wastewaters into natural environment without giving any prior treatment before disposal. The persistent nature of Cr(VI) makes it accumulate in the food chain and with time reaches harmful levels in living beings resulting in serious health hazards such as irritation in lungs, death of animals, cancer in digestive tract, low growth rates in plants, etc. The conventional treatment techniques used for removal of

toxic Cr(VI) from wastewaters include chemical precipitation, oxidation-reduction, electrochemical treatment, reverse osmosis and ion-exchange etc. However, biosorption processes which uses microorganisms as biosorbent is advantageous over the conventional methods as it requires very low energy to operate the process and moreover, does not produce any chemical toxic sludge, disposal of which again causes secondary pollution (Sudha and Abraham, 2001). Environment friendly processes, therefore, need to be developed with the potential application of microorganisms in removal of heavy metals and thus been recognized as a potential alternative to the conventional methods for treatment of contaminated wastewaters (Muter et.al., 2001).

The growing, resting and non-living dry biomass of microorganisms have been reported to remove Cr(VI) from aqueous solutions (Llovera et.al., 1993; Muter et al., 2001; Srinath, et al., 2002). However, most of the work to remove Cr(VI) have been carried out using non-living dry biomass whereas, the use of growing and resting cells shows very scanty information. The use of non-living dry biomass has advantages over growing and resting cells as the former does not require any growth media and nutrients. Moreover, adsorbed metal can be easily desorbed and regenerated biomass can be further reused. However, the most important limitation with non-living dry biomass is that biochemical cell energetic reactions are going to be stopped as the cells are dried. The growing cells have the advantages over the non-living and resting cells that the simultaneous removal of metal is obtained during growth and the separate biomass production processes can be avoided. However, the major limitation of using growing cells is that cell growth is inhibited when high metal concentration is used. This problem can be overcome by using metal tolerant organism, which will not only grow in presence of metal but also capable of removing metals during its growth.

The aim of the present study is to find out the maximum removal of Cr(VI) from the media using *Aspergillus* sp. during its growth. Continuous removal of Cr(VI) was studied in Continuous Stirred Tank Reactor (CSTR) maintaining the microorganism in living or biologically active state. This will also help the system to treat larger quantities of wastewaters in a shorter duration of time moreover, the process can be continued for a longer duration of time as compared to the batch system.

Materials and methods

Microorganism and inoculum preparation

Aspergillus sp. (filamentous fungus) used in the present study was isolated from industrial wastewaters. The biomass was grown in 250 mL Erlenmeyer flask in a shaking incubator at 30 °C and 180 rpm. The media used for the growth having the following composition ($g.L^{-1}$): glucose, 10.0; NaCl, 1.0, K_2HP0_4, 0.5; NH_4N0_3, 0.5, $MgS0_4$, 0.1; and yeast extract, 5.0. The pH of the media was 6.0. A 10% (v/v) of a 30 h old culture was used as an inoculum for the Cr(VI) removal studies.

Batch studies

Batch cultivation for growing the fungi was conducted using Erlenmeyer flask (250 mL) with media (100 mL) containing Cr(VI) and a 10% (v/v) inoculums, on a rotary shaker (180 rpm) at pH 5.0 and temperature at 30 °C. The process of cultivation 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 centrifugation was done to separate the biomass from the liquid part. The collected biomass was

then washed with distilled water, dried and weight of dry biomass was measured gravimetrically.

Studies in continuous flow system

The continuous removal of Cr(VI) from the media was studied in a Continuous Stirred Tank Reactor (CSTR) of maximum 3 L working volume. A schematic diagram of the bioreactor assembly is shown in Figure 1. CSTR consists of a cylindrical glass reactor which is at inlet point was connected to a media holding tank through a peristaltic pump and the outlet of the system was connected to a liquid collection system. In order to maintain the optimum temperature of the media the reactor was heated externally using a heating tape connected to a temperature control module. The reactor was connected with a multipoint injection system at the bottom of the reactor to provide sufficient sterile air (aeration) and a magnetic stirrer was equipped to ensure effective mixing of the media component within the reactor. All equipments were autoclaved and aeration using sterile air was maintained in the reactor by allowing air to flow into the liquid system through air filter.

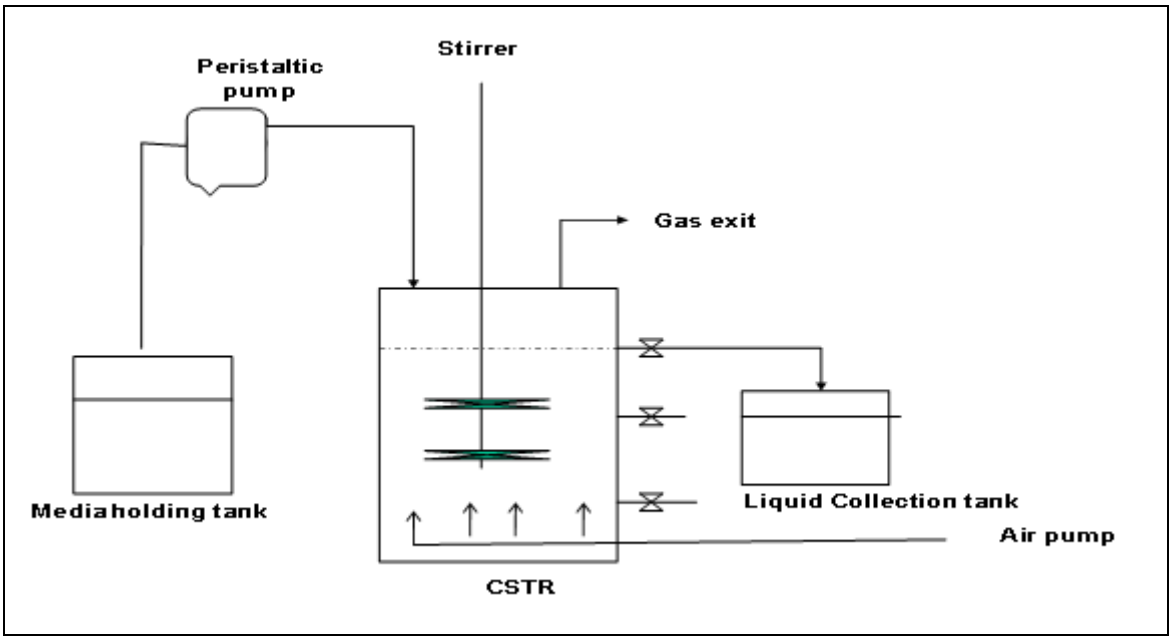

Figure 1. Schematic diagram of the bioreactor (CSTR) assembly.

The reactor initially was operated in the batch mode using 3 L media, containing Cr(VI) and nutrient components under aerated and agitated conditions at a temperature of 30 °C, 10% (v/v) inoculum concentration and at pH 5.0. After getting sufficient amount of biologically active cell (*Aspergillus* sp). the process was operated in the continuous mode by feeding the media into the reactor through the peristaltic pump maintaining the desired dilution rate. The reactor run in continuous mode of operation under steady-state condition was monitored for 20 days. The liquid sample was collected and the residual Cr(VI) concentration, residual sugar concentration were periodically analysed The biomass obtained was estimated gravimetrically.

A systematic study was carried out in a single stage bioreactor in continuous mode of operation using media at different dilution rates (0.01-0.07 h^{-1}) and different initial Cr(VI) concentrations(50-500 $mg.L^{-1}$). The optimum dilution rate was determined under steady state condition in order to get maximum removal of Cr(VI) at a given initial Cr(VI) concentration.

In a single stage reactor, as complete removal of Cr(VI) at higher Cr(VI) concentration (100 $mg.L^{-1}$) was not obtained, therefore, to get nearly complete removal at higher concentration, a two stage continuous bioreactor was need to be conducted at a dilution rate 0.01 h^{-1} , pH 5.0 and initial metal concentration 500 $mg.L^{-1}$.

The second stage reactor was initially operated in the batch mode to get the sufficient amount of biologically active cell (*Aspergillus* sp). Now the liquid obtained from the first stage bioreactor under steady state condition was continuously filtered through a mesh (300 μm), and then growth nutrient were added and fed into the second stage reactor through a peristaltic pump. The liquid samples were periodically collected from both the reactors and analysed for residual Cr(VI) concentration, residual substrate concentration and the biomass.

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 (DPC) as the complexing agent (APHA, 1989). The sugar concentration in the medium was analysed by di-nitrosalicylic acid (DNS) method at 540 nm (Millar, 1959).

Results and Discussion

Batch studies

Figures 2 and 3 show the changes in biomass concentration ($g.L^{-1}$) of *Aspergillus* sp. and residual glucose concentration ($g.L^{-1}$), respectively, with growth period (h) of the fungus in the media at different initial Cr(VI) concentrations (0-2,000 $mg.L^{-1}$) and at pH 5.0. The Figure 2 clearly shows that when the media does not contain any Cr(VI), the lag period was 3 h, followed by the exponential phase of growth upto 24 h. The stationary phase of growth was reached within 28 h of growth period.

The Figure 3 indicates complete utilization of glucose in 30 h of incubation period in the absence of Cr(VI). However, the fungus was able to grow even in the presence of higher Cr(VI) concentration i.e., at 1000 $mg.L^{-1}$ (Figure 2). The lag period was found to be marginally increased upto 500 $mg.L^{-1}$ initial Cr(VI) concentration, beyond which it increased significantly with increase in initial Cr(VI) concentration upto 1,000 $mg.L^{-1}$. Both the growth rate (Figure 2) and the glucose utilization rate (Figure 3) by the *Aspergillus* sp. were found to be decreased with increase in initial Cr(VI) concentration ranging from 0-1,000 $mg.L^{-1}$ although glucose was found to be completely utilized in every case.

The decreased growth with increase in initial Cr(VI) concentration from 0 to 500 $mg.L^{-1}$ has also been reported with *Candida utilis* (Muter et al., 2001). About 95.5% decrease in biomass concentration has been reported with *C. utilis* as compared to only 4.5% decrease obtained in the present study conducted with *Aspergillus* sp. when Cr(VI) concentration was increased from 0 to 500 $mg.L^{-1}$. These results clearly

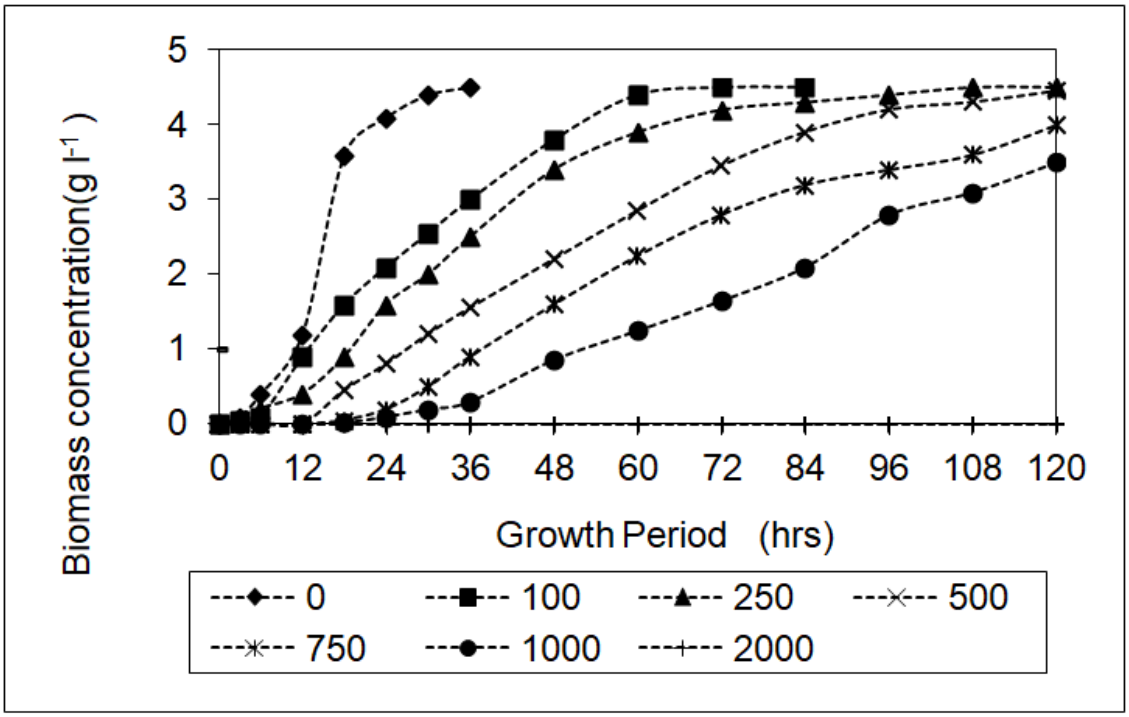

Figure 2. Change in biomass concentration of *Aspergillus* sp. with time at different initial Cr(VI) concentrations (0-2,000 mg.L^{-1}) in batch mode of operation.

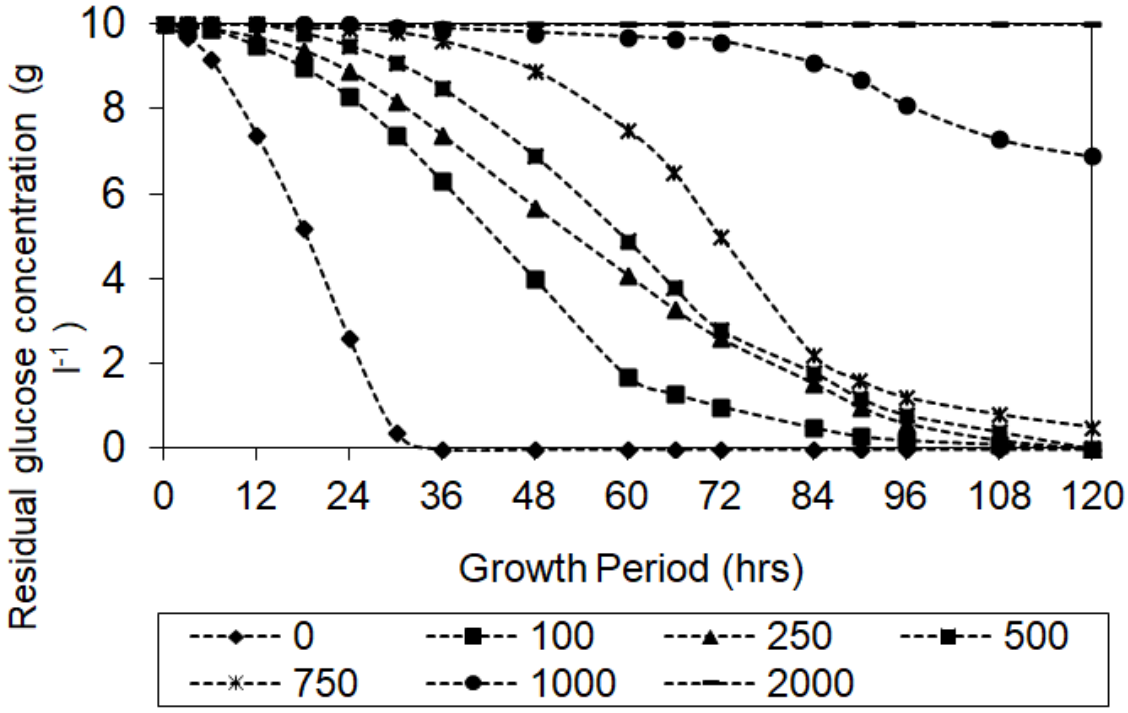

Figure 3. Change in residual glucose concentration with time at different initial Cr(VI) concentrations(0-2,000 mg.L^{-1}) in batch mode of operation.

indicate that *Aspergillus* sp. isolated in the laboratory and used by the present author can tolerate high concentration of Cr(VI) as compared to *C. utilis* reported in the literature.

The specific growth rate (μ) of the organism can be calculated by using the growth equation given by Dursun et al. (2003).

$$\mu = \frac{1}{x}\frac{dx}{dt} \quad (1)$$

Integrating, equation (1) can be expressed as:

$$ln\ \frac{x}{xi} = \mu\ (t_x - t_{xi}) \quad (2)$$

where x_i and x are the biomass concentrations at the beginning (t_{xi}) and at the end of the exponential phase (t_x), respectively, at time t_{xi} and t_x.

Figure 4 show the values of μ, calculated during the exponential phase of growth of *Aspergillus* sp. (Figure 2) in the absence as well as in the presence of different initial concentrations of Cr(VI). A rapid decrease in μ from 0.103 h^{-1} to 0.01 h^{-1} was observed with the increase in Cr(VI) concentration from 0-1,000 $mg.L^{-1}$. Dursun et al. (2003) has been reported a similar trend of decrease in μ, from 0.075 to 0.039 h^{-1} by increasing the initial Cr(VI) concentration from 25 to 50 mg L^{-1} while growing *Aspergillus* sp. (Dursan et al., 2003).

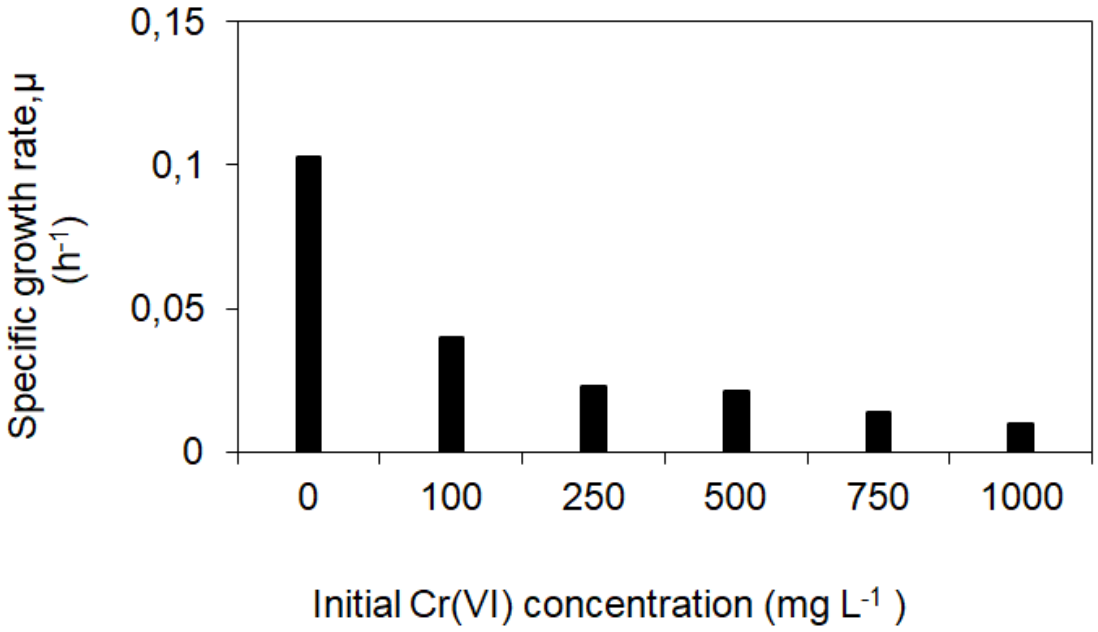

Figure 4. Specific growth rates of *Aspergillus* sp. at different initial Cr(VI) concentrations (0-1,000 $mg.L^{-1}$) in batch mode of operation.

Figure 5 shows the specific Cr(VI) removal ($mg.g^{-1}$) by *Aspergillus* sp. at different initial Cr(VI) concentrations (100-1,000 $mg.L^{-1}$) in batch mode of operation. The specific Cr(VI) removal was increased from 13.34 to 41.2 $mg.g^{-1}$ by increasing the initial Cr(VI) concentrations from 100 to 500 $mg.L^{-1}$ which could be due to the availability of more and more Cr(VI) for

bioaccumulation by *Aspergillus* sp. However, beyond 500 mg.L^{-1} concentration, the specific Cr(VI) removal decreased. This can be explained by the reduced cell growth and reduction in availability of binding sites at higher Cr(VI) concentrations. Moreover, we cannot ruled out the another possibility that accessibility of these binding sites on cell surface is hindered by the presence of excessive Cr(VI) in the media at higher concentration.

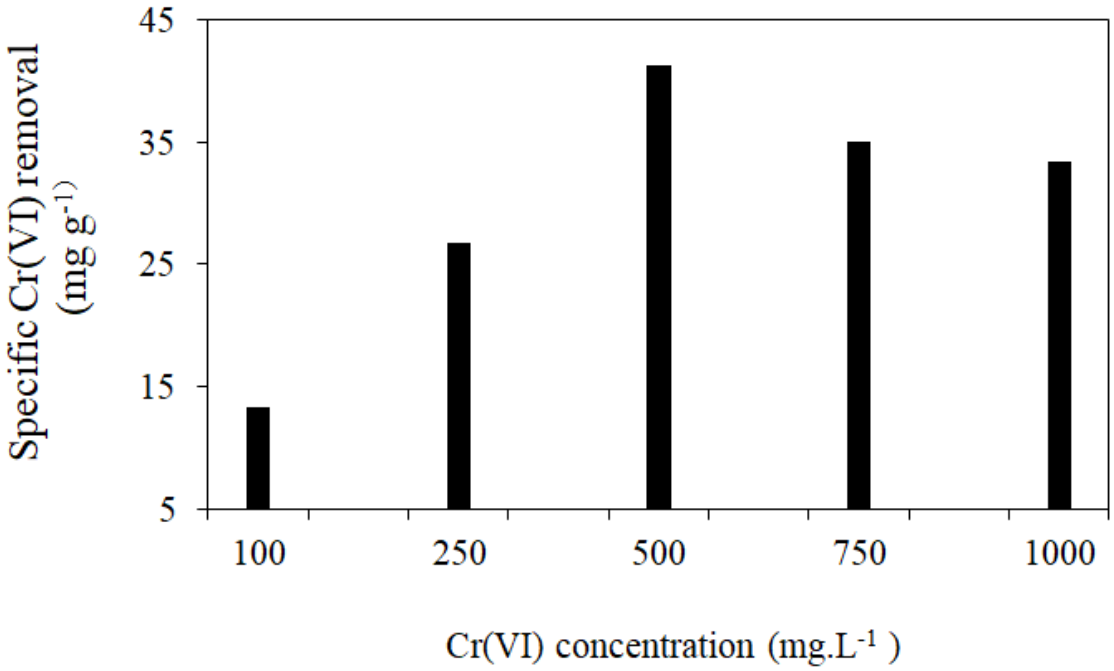

Figure 5. Specific Cr(VI) removal (mg g^{-1}) at different initial Cr(VI) concentrations (100-1,000 mg L^{-1}) in batch mode of operation.

A similar trend has been reported in specific Cr(VI) removal with increase in Cr(VI) concentrations using C. *utilis* (Muter et al., 2001; Dursan et al., 2003; Shugaba et al., 2012). Further, the removal of Cr(VI) is expected to be occurred initially by bioaccumulation followed by the enzymatic reduction of Cr(VI) into Cr(III) in the media (Borst-Paweles, 1981; Gharib and Gadd, 1998; Peña-Castro et al., 2004). Intracellular reduction of Cr(VI) into Cr(III) within the cells has also been reported by using bacterial cell of *Shewanella oneidenisis* MR-1 (Middleton et al., 2003). Hence, in the present work, the reduced enzymatic reduction of Cr(VI) which causes decreased removal of Cr(VI) in the media at higher Cr(VI) concentrations could be due to the reduced cell growth and hence, cannot be ruled out.

Continuous studies

The continuous studies were carried out in Continuous Stirred Tank Reactor (CSTR) with working volume of 3 L using different Cr(VI) concentrations (50-500 mg.L^{-1}), different dilution rates (0.01-0.07 h^{-1}) and pH 5.0. During the transient state operation of the reactor, immediately following the commencement of continuous feed, the residual Cr(VI) concentration initially increased. This situation was continued

until the reactor condition was stabilized and achieved a steady state condition indicated by the constant value of residual Cr(VI) in the media.

In each study, the dilution rates were maintained much below the specific growth rates of the *Aspergillus* sp. in order to avoid wash out conditions. However, wash out condition was observed when an experiment was conducted at 50 $mg.L^{-1}$ initial Cr(VI) concentration maintaining the dilution rate higher than the specific growth rate.

Figure 6 shows the change in residual Cr(VI) concentration with time at three different dilution rates 0.07, 0.05 and 0.03 h^{-1} and at 50 $mg.L^{-1}$ initial Cr(VI) concentration. In the presence of *Aspergillus* sp. the residual Cr(VI) concentration decreased as the dilution rate decreased. At 0.07 h^{-1} dilution rate which is higher than the specific growth rate (0.065 h^{-1}) of *Aspergillus* sp. at 50 $mg.L^{-1}$ initial Cr(VI) concentration, no significant removal of Cr(VI) was obtained under steady state condition, which clearly indicates the cell wash out condition. This is also supported by the negligible biomass concentration (0.65 $g.L^{-1}$) and incomplete utilization of glucose of *Aspergillus* sp. under steady state condition. Although, at dilution rate 0.05 h^{-1} (longer residence time) the Cr(VI) removal was found to be 29 $mg.L^{-1}$, but glucose still remain unutilized. This indicates further lowering of dilution rate in order to have enhanced Cr(VI) removal and off course complete glucose utilization for biomass growth. Thus, at a dilution rate 0.03 h^{-1} the glucose was found to be completely utilized and the maximum removal of Cr(VI) was observed to be 38 $mg.L^{-1}$.

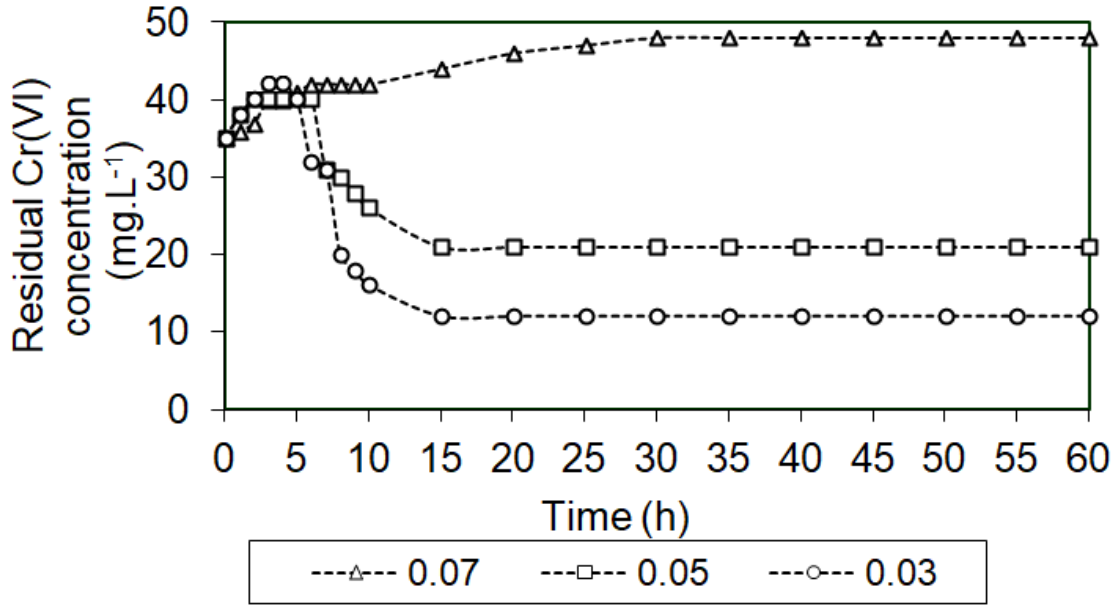

Figure 6. Change in residual Cr(VI) concentration with time at different dilution rates (0.03-0.07 h^{-1}) at 50 $mg.L^{-1}$ initial Cr(VI) concentration and in continuous mode of operation.

As glucose was completely utilized at this dilution rate, the Cr(VI) removal studies were not carried out at a dilution rate lower than 0.03 h^{-1}. A maximum Cr(VI) removal (55 $mg.L^{-1}$) was observed at 100 $mg.L^{-1}$

concentration by operating the process at lower dilution rate, 0.01 h^{-1} (Figure 7) and hence the glucose was completely utilized. Also at higher Cr(VI) concentrations 200, 300, 400 and 500 $mg.L^{-1}$, glucose was found to be completely utilized at dilution rate 0.01 h^{-1} and Cr(VI) removal was in the range of 99-156 $mg.L^{-1}$ (Figure 8). As glucose was completely utilized at 0.01 h^{-1} at all the concentrations further studies were not carried out at a dilution rate lower than 0.01 h^{-1}.

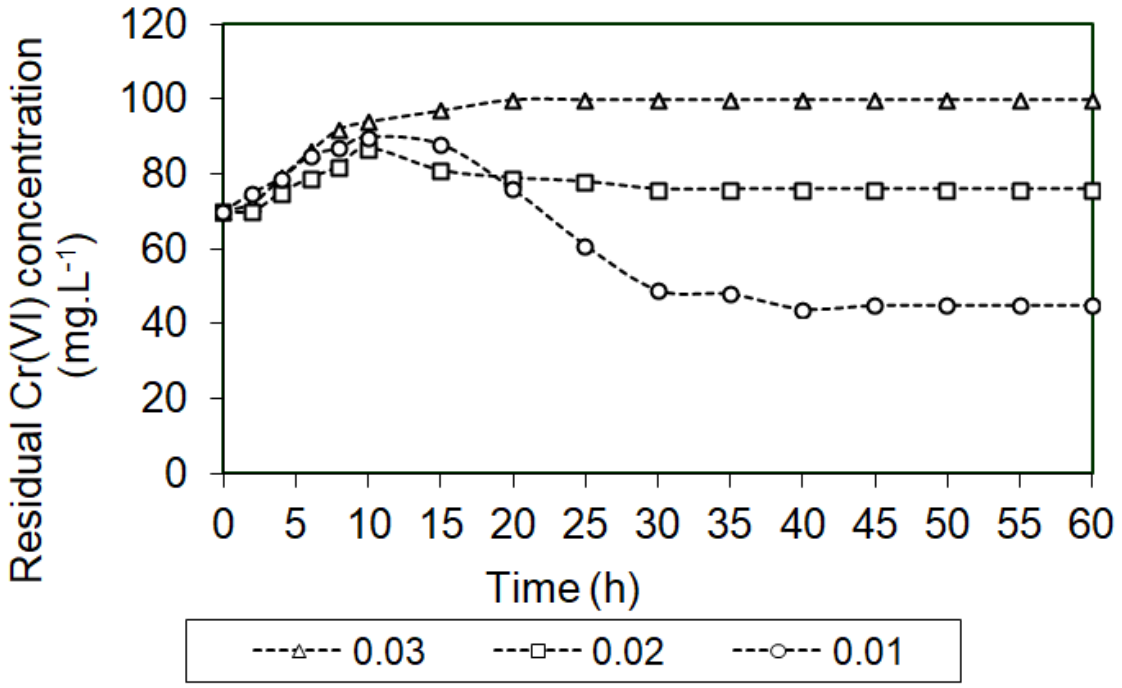

Figure 7. Change in residual Cr(VI) concentration with time at different dilution rates (0.01-0.03 h^{-1}) at 100 $mg.L^{-1}$ initial Cr(VI) concentration and in continuous mode of operation.

The specific Cr(VI) removal ($mg.g^{-1}$) at different initial Cr(VI) concentration (50-500 $mg.L^{-1}$), in continuous mode of operation is shown in Figure 9. The figure clearly indicates that the specific Cr(VI) removal increased with increase in Cr(VI) concentration upto 500 $mg.L^{-1}$ initial Cr(VI) concentration. The specific Cr(VI) removal obtained at 500 $mg.L^{-1}$ Cr(VI) concentration was found to be 39.4 $mg.g^{-1}$ as compared to 41.2 $mg.g^{-1}$ obtained in batch operation.

A continuous stirred tank reactor shall have the benefit over the batch bioreactors as it can treat a larger quantity of effluent in a shorter duration of time, moreover, the system and is ideally suited to apply where the microorganisms can be maintained in living or biochemically active state and the cell population can be adjusted to a steady environment to achieve a state of balanced growth which is however found to be difficult to obtain in batch bioreactors.

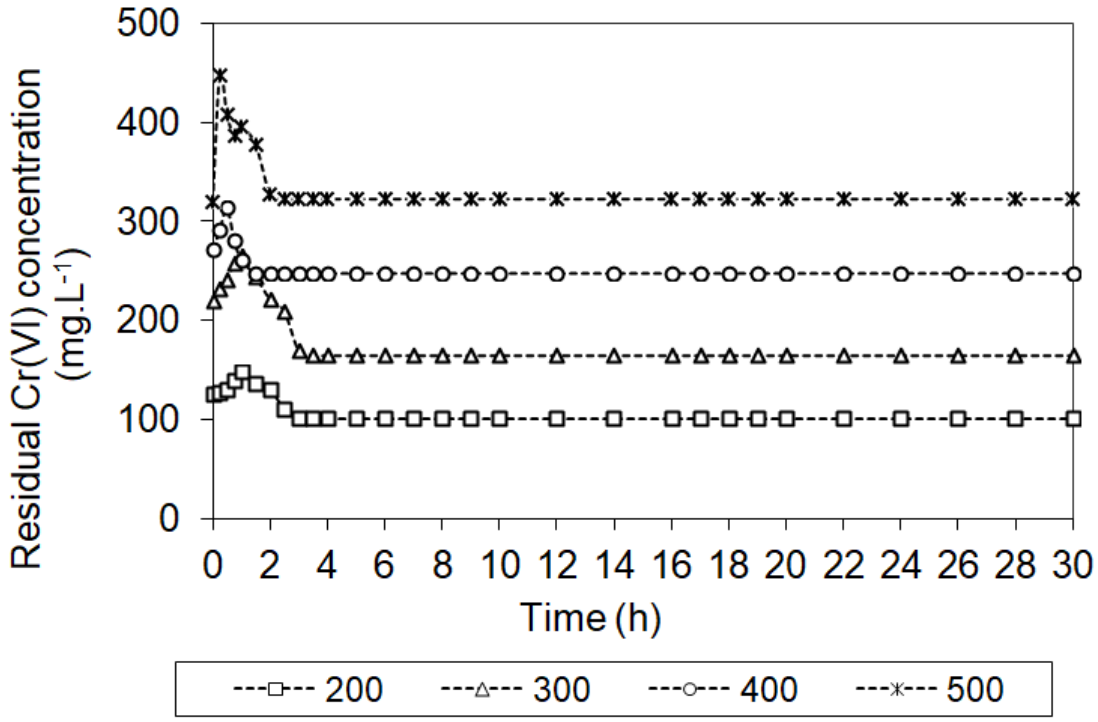

Figure 8. Change in residual Cr(VI) concentration with time at 0.01 h^{-1} dilution rate at 200, 300, 400 and 500 $mg.L^{-1}$ initial Cr(VI) concentration and in continuous mode of operation.

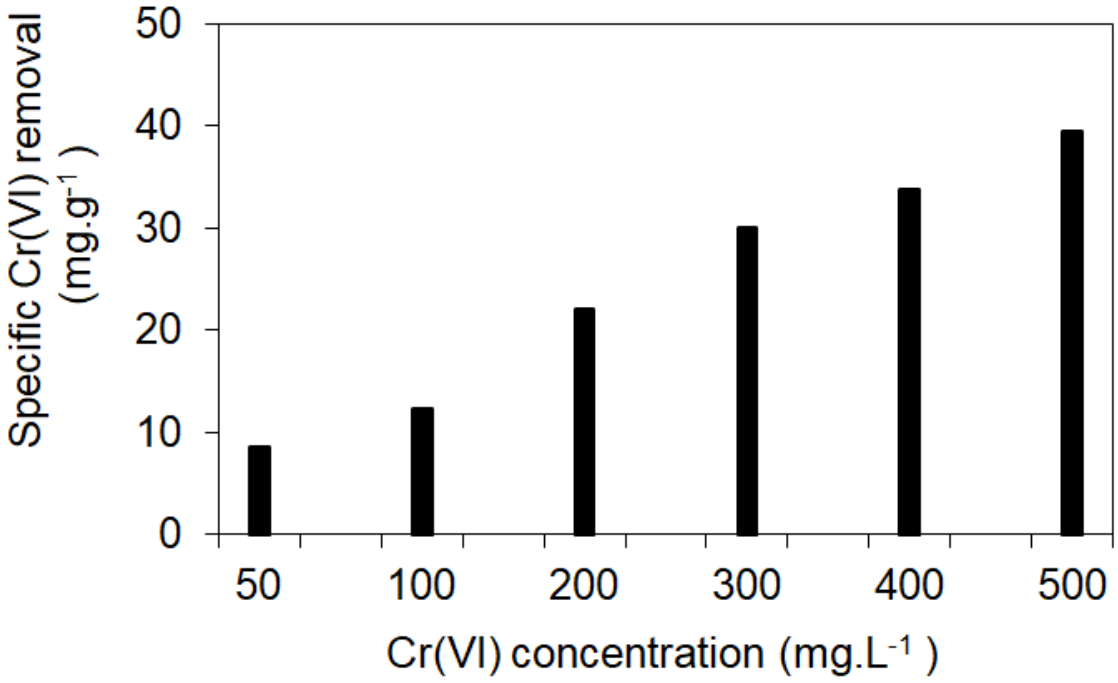

Figure 9. Specific Cr(VI) removal ($mg.g^{-1}$) at different initial Cr(VI) concentrations (50-500 $mg.L^{-1}$) in continuous mode of operation.

In continuous mode of operation, using a single stage bioreactor significant removal of Cr(VI) at higher Cr(VI) concentrations was not obtained although glucose was found to be completely utilized. Therefore, an enhanced Cr(VI) removal at higher Cr(VI) concentrations could be possible either by increasing the glucose concentration in the media or by using two stage bioreactor. The increased glucose concentration was expected to increase the biomass concentration of *Aspergillus* sp. resulting in enhanced Cr(VI) removal. However, increased biomass concentration, could increase cell to cell interaction which may result in the unavailability of binding sites, thus reducing Cr(VI) removal. On the other hand, two stage operation although require more numbers of CSTRs, the total quantity of glucose and nutrients would remain the same as that required for a single stage operational strategy and hence, more effective Cr(VI) removal could be possible due to the lower biomass concentration of *Aspergillus* sp. in each reactor.

Figure 10 shows the change in residual Cr(VI) concentration with time in a two stage continuous bioreactor at 0.01 h^{-1} dilution rate and at pH 5.0. In the first stage reactor, the total residual Cr(VI) concentration decreased to 323 $mg.L^{-1}$ starting from an initial Cr(VI) concentration of 500 $mg.L^{-1}$ under steady state condition. In the second stage reactor, the total residual Cr(VI) concentration further decreased to 148 $mg.L^{-1}$ starting from an initial 323 $mg.L^{-1}$ Cr(VI) concentration under steady state condition. The total Cr(VI) removal after second stage operation was found to be 352 $mg.L^{-1}$ (70.4%), as compared to 35.4% obtained in a single stage continuous bioreactor. The specific Cr(VI) removal of 39.4 $mg.g^{-1}$ was obtained after first stage operation with an additional 39.32 $mg.g^{-1}$ obtained in the second stage operation.

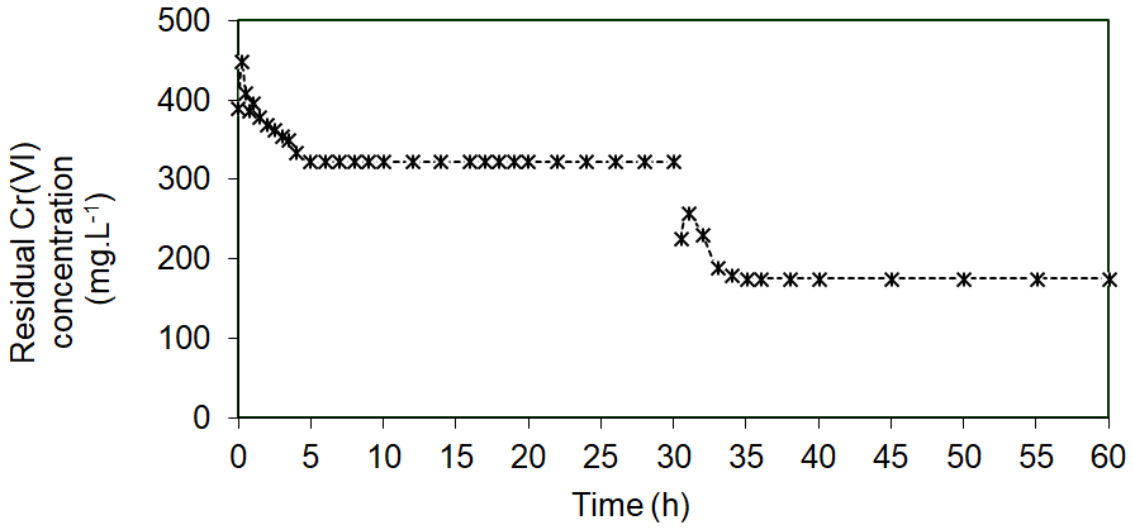

Figure 10. Change in residual Cr(VI) ion concentration with time in a two stage continuous bioreactor.

From the above results, it showed that staged manner operation, e.g., using a two stage reactor in continuous mode operation resulted in about 70.4% removal of Cr(VI) using *Aspergillus* sp. as compared to 37.08% in batch mode operation at the same 500 $mg.L^{-1}$ initial concentration of Cr(VI). The staged manner operation, therefore, appears to be the better operational strategy as compared to the batch mode of operation as the process could be operated for a long time with higher Cr(VI) removal.

Conclusion

The removal of Cr(VI) from aqueous media using *Aspergillus* sp. is a complex phenomenon involving Cr(VI) adsorption and removal on the cell biomass. These necessitates the development of suitable operational strategy for effective removal of Cr(VI) from the media. It was observed in the present study that residence time, a very important parameter, played an significant role in Cr(VI) removal using *Aspergillus* sp. However, by manipulating the residence time properly the rate of removal of Cr(VI) can be enhanced in continuous mode of operation. However, the restriction imposed on the batch process, can be overcome by operating the process in a continuous mode with two stage bioreactor and at an optimum dilution rate. Although, no attempt has been made to optimize the dilution rate theoretically it was observed that an empirical optimization of dilution rate is a function of initial Cr(VI) concentration. The above studies showed that the staged manner operation in continuous mode be a better operational strategy for enhanced Cr(VI) removal at higher Cr(VI) concentration as compared to the batch mode of operation at the same initial Cr(VI) concentration and at optimized dilution rate.

Acknowledgement

The author is grateful to Centre for Energy Studies (CES) and Department of Biochemical Engineering and Biotechnology (DBEB), Indian Institute of Technology (IIT), Delhi, for providing the necessary laboratory facilities. The author is also thankful to Amity School of Engineering and Technology, Delhi, for providing the necessary computer facilities.

Conflict of interest statement

Author declares that they have no conflict of interests.

References

APHA - American Public Health Association. **Standards methods for the examination of water and wastewater**. 17. ed. Washington: American Public Health Association, 1989.

Borst-Paweles, G. W. F. H. Ion transport in yeast. **Biochimica et Biophysica Acta (BBA) - Reviews on Biomembranes**, v. 650, no. 2/3, p. 88-127, 1981. https://doi.org/10.1016/0304-4157(81)90002-2

Dursan, A. Y.; Ulsu, G.; Cuci, Y.; Aksu, Z. Bioaccumulation of copper(II), lead(II) and chromium(VI) by growing *Aspergillus niger*. **Process Biochemistry**, v. 38, no. 12, p. 1647-1651, 2003. https://doi.org/10.1016/S0032-9592(02)00075-4

Germain, L. E.; Patterson, E. Plating and cyanide wastes. **Journal of the Water Pollution Control Federation**, v. 46, p. 1301-1315, 1974.

Gharib, M. M.; Gadd, G. M. Evidence for the involvement of vacuolar activity in metal(loid) tolerance: vacuolar-lacking and -defective mutants of Saccharomyces cerevisiae display higher sensitivity to chromate, tellurite and selenite. **Biometals**, v. 11, no. 2, p. 101-106, 1998. https://doi.org/10.1023/A:1009221810760

Llovera, S.; Bonet, R.; Simon-Pujol, M. D.; Congregado, F. Effect of culture medium ions on chromate reduction by resting cells of *Agrobacterium radiobacter.* **Applied**

Microbiology and Biotechnology, v. 39, p. 424-426, 1993. https://doi.org/10.1007/BF00192105

Middleton, S. S.; Latmani, R. B.; Mackey, M. R.; Ellisman, M. H.; Tebo, B. M.; Criddle, C. S. Cometabolism of Cr(VI) by *Shewanella oneidensis* MR-1 produces cell-associated reduced chromium and inhibits growth. **Biotechnolology and Bioengineering**, v. 83, no. 6, p. 627-637, 2003. https://doi.org/10.1002/bit.10725

Millar, G. L. Use of dinitrosalicylic acid reagent for determination of reducing sugar. **Analytical Chemistry**, v. 31, no. 3, p. 426-428, 1959. https://doi.org/10.1021/ac60147a030

Muter, O.; Patmalnieks, A.; Rapoport, A. Interrelations of the yeast *candida utilis* and Cr(VI): metal reduction and its distribution in the cell and medium. **Process Biochemistry**, v. 36, no. 10, p. 963-970, 2001. https://doi.org/10.1016/S0032-9592(01)00136-4

Peña-Castro, J. M.; Martínez-Jerónimo, F.; Esparza-García, F.; Cañizares-Villanueva, R. O. Heavy metals removal by the microalga *Scenedesmus incrassatulus* in continuous cultures. **Bioresource Technology**, v. 94, p. 219-222, 2004. https://doi.org/10.1016/j.biortech.2003.12.005

Sen, M.; Dastidar, M. G.; Roychoudhury, P. K. Comparative studies of batch and continuous biological treatment of Cr(VI) using *Fusarium solani*. **Enzyme and Microbial Technology**, v. 41, no. 1/2, p. 51-56, 2007. https://doi.org/10.1016/j.enzmictec.2006.11.021

Shugaba, A.; Buba, F.; Kolo, B. G.; Nok, A. J.; Ameh, D. A.; Lori, J. A. uptake and reduction of hexavalent chromium by *Aspergillus niger* and *Aspergillus parasiticus*. **Journal of Petroleum & Environmental Biotechnology**, v. 3, no. 3, p. 1-8, 2012. http://doi.org/10.4172/2157-7463.1000119

Srinath, T.; Verma, T.; Ramteke, P. W.; Garg, S. K. Chromium(VI) biosorption and bioaccumulation by chromate resistant bacteria. **Chemophere**, v. 48, no. 4, p. 427-435, 2002. https://doi.org/10.1016/S0045-6535(02)00089-9

Sudha Bai, R.; Abraham, T. E. Biosorption of Cr(VI) from aqueous solution by *Rhizopus nigrificans*. **Bioresource Technology**, v. 79, no. 1, p. 73-81, 2001. https://doi.org/10.1016/S0960-8524(00)00107-3