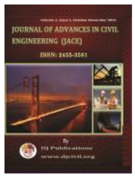
Journal of Advances in Civil Engineering, Vol. 1(1) 2015, pp. 25-32



RESEARCH ARTICLE

# Separation of Hexavalent Chromium, Chemical Oxygen Demand and Sulfate from Artificial Wastewater for Biogenic Sulfide Corrosion

*P.M Suthar[1]

[1]Assistant Engineer, Water Resources Organization, Public Works Department, Tamil Nadu, India.

Received-21 September 2015, Revised-20 October 2015, Accepted-29 October 2015, Published-15 November 2015

## ABSTRACT

The aim of this work is to judge the possibility of using culture in mixed retrieval of toxic and carcinogenic hexavalent chromium mesophilic sulfate-reducing bacteria (SRB). In order to treat it effectively hexavalent chromium containing wastewater and sulfate-reducing bacteria culture was embraced to 60 mg/L of hexavalent chromium and maintained through repeated sub-culturing to increase the sulfate-reducing bacteria growth and activity. Experiments in module biosorption were carried out in vials of glass serum by sulfate-reducing bacteria cultured, fulfilling 83.1% of hexavalent chromium , 77.9% sulfate, 86.7% chemical oxygen demand removals under the following optimized circumstances, pH 7, 7 days of hydraulic retention time, 37 ◦ C temperature and 50 mg/L hexavalent chromium initial concentration. Moreover experiments in sorption were conducted on artificial wastewater under conditions for optimal operational performance and resulted in 89.2% hexavalent chromium, 81.9% chemical oxygen demand and 95.3% sulfate reduction from wastewater simulated. This work results in contribution to a better comprehension of metal uptake by biogenic sulfides and would be beneficial in the potential biosorbents development that possess hexavalent chromium uptake for high capacities from environments in aqueous environments.

**Keywords**: PH 7, Hydraulic retention time, Biogenic sulfides, Artificial wastewater, Sulfate.

## 1. INTRODUCTION

Chromium can exist in several oxidation states ranging from chromium (II) chloride to hexavalent chromium in natural environment among which trivalent chromium (III) and hexavalent chromium (VI) are the most dominant species of chromium in the environment. [1] The Cr (VI) compounds are used in metallurgical and chrome plating industries for chrome alloy and chromium metal production. It is used as an oxidizing agent and in the production of other chromium compounds in chemical industry. About 80–90% of leather tanning process utilizes chromium chemicals out of which 40% of chromium used is discharged in the effluent as Cr (VI) and Cr (III) [2]. Cr (III) salts are used less widely, yet are being employed in photography, textile dyeing, ceramics and glass industry. Cr (III) is required in trace amount for living organisms as it decreases body fat, cholesterol and triglyceride levels, activating enzyme reactions, and increasing the muscle mass. Cr (VI) is not only highly toxic to all forms of living organisms but also mutagenic and carcinogenic in humans and animals [3]. The U.S. Environmental Protection Agency (EPA) has classified Cr (VI) as a Group ‘A’ human carcinogen. So due to its high toxicity, stringent regulations are applied on the discharge of Cr (VI) to surface water. The EPA has set a limit of 100 g Cr(III) and 50 g Cr (VI)/L for drinking water and a limit of ≤0.05 mg/L [4] for its discharge into surface water, while total chromium, including Cr (III), Cr (VI) and its other forms, should be lower than 2 mg/L [5]. So treatment of wastewater is one of the growing concerns in environmental cleaning. Conventional methods for treatment of metal contaminated wastewater are restricted, because of technical or economical constraints [6]. Therefore, recent studies have concentrated on the development of low cost processes. The use of microorganisms including bacteria, fungi, algae and yeast has gained much more

*Corresponding author. Tel.: +918903706036

Email address: djjames201507@gmail.com (P.M.Suthar)

Double blind peer review under responsibility of DJ Publications

http://dx.doi.org/10.18831/djcivil.org/2015011005

attention in recent years since they carry a wide range of binding sites for heavy metal ions [7]. Cr (VI) reduction by biogenic sulfides is one of the major approaches used for in form [8]. The best way to reduce the mobility of heavy metals is to transform them into insoluble compounds such as sulfides, which are of more stable forms. It has been reported that certain heavy metals such as Cr (VI) may be reduced under sulfate reducing conditions [9]. Sulfate-reducing bacteria (SRB) under anaerobic conditions oxidize simple organic compounds (such as acetic acid and lactic acid) by utilizing sulfate as an electron acceptor and generate hydrogen sulfide. Hydrogen sulfide reacts with heavy metal ions to form insoluble metal sulfides that can be easily separated from solution. The potential advantages of metal precipitation by biogenic sulfides include the production of lower sludge volumes and products with lower solubility as compared to hydroxide precipitation [10]. In addition, valuable metals can be recovered from metal sulfide sludges [11]. Several studies have reported process optimization, organic carbon (electron donor) consumption coupled with sulfate reduction, hydrogen production and the efficiency of metal precipitation in the biological treatment with biogenic sulfide [12][13]. In the proposed work optimization of the varying pH effect, temperature, metal dose and time of incubation is performed so as to reach hexavalent chromium high removal efficiency by (SRB). Hence this effort offers conditions of optimum parameters for Cr (VI) efficient removal using sulfate-reducing bacteria consortium. These optimized parameters were further applied to biogenic sulfides inquiry efficiency produced from bacterial consortia of sulfate-reducing in chromium (VI) separation, chemical oxygen demand and sulfate to treat a small scale bioreactor simulated wastewater.

## 2. MATERIALS AND APPROACHES

### 2.1. Development media and conditions

In all experiments, an alteration postgate development medium was used. The Postgate growth medium composition used was as follows (g/L): KH2PO4 0.6; Na2SO4 1.1; NH4Cl 3.0; CaCl2 0.07; FeSO4 0.006; sodium citrate 0.4; yeast extract 0.2; sodium lactate 16 mL; Resazurin 0.2%. In the dark all incubations were done at 38◦C. Analytical detoxification of Cr (VI) containing wastewater. Cr (VI) can be reduced in biological treatment processes to Cr (III), which is almost insoluble and less-toxic grade chemicals and solutions were prepared with sterile deionized water.

### 2.2. Inoculums source

The SRB mixed culture used in the present study was obtained from the sample of sludge collected from the sewage treatment plant anaerobic digester sludge. The screened sludge of one gram was inoculated in a bioreactor which is of small scale (1500 mL) containing 900 mL sequestrum selective medium. The Postgate isolation media structure was as (g/L): $Na_2SO_4$ 2.0; $KH_2PO_4$ 0.6; $NH_4Cl$ 2.0; $CaCl_2$ 0.8; $FeSO_4$ 0.2; yeast extract 2.0; agar 16.0; acid in mercaptoacetic 0.2; acid of ascorbic 0.2; ethanol (absolute) 4.6 mL; and Resazurin 0.2%. Reactor was kept in accordance with the growth of anaerobic bacteria in anaerobic conditions for a 7 day period at 38 °C till the media color changed to black. After anaerobic incubation of 7 days, the isolate inoculum of 300 mL was relocated into 900 mL contraception and another 1500 mL reactor was used in postgate growth medium. The postgate growth medium is selected partially for sulfate-reducing bacteria, with a lactate of sodium as donor of electron and source of carbon. The generation of H2S and medium blackening were observed as symptoms of positive feedback for the sulfate-reducing bacteria presence.

### 2.3. Experiments for batch

Systems of batch experiments were conducted under anaerobic conditions. For all the experiments vials of glass serum (125 mL) were used. Modified postgate medium of 80 mL with pH 7 was added in the batch tests vials. After inoculation, the bottles were sealed with stoppers of butyl rubber and aluminum crimp seals, and incubated at a temperature of 38 ◦C under the condition of static environment. Oxygen was replaced from the phase in gaseous form with nitrogen. Bacteria used in experiments for batch were closed serum cultivated in vials using SRB fixed procedures. Afterwards sealed vials and bacteria of 5 mL inoculum cultivated in the medium of postgate was added by a syringe which is sterile. The colour change from yellowish to colourless indicated the consortium growth. All tests were performed with duplicate values. Later parameters optimized were treated with synthetic wastewater and are applied. The reactors were kept in an incubator for biological oxygen demand for 7 days under optimized and static conditions. Performed sampling from the reactors was at 0 h and incubation was carried out in 7 days.

### 2.4. Various parameters analysis

Samples of liquid were centrifuged using a 32 centrifuge of Hettich Rotofix at 4000 rpm for 15 min, prior to determination of chemical oxygen demand, hexavalent Chromium and sulfate in the supernatant form. Spectralab chemical oxygen demand digester (2015 M) and chemical oxygen demand COD titrator (CT-15) were used to determine COD of soluble nature in the supernatant form. By atomic absorption spectrophotometer (Shimadzu AA-6300, Japan), total residual chromium was quantified. The diluted sample sulfate was measured using methods of standard nature. According to the methods of standard recommended, pH was also measured [12]. The heavy metal removal (%) was determined using the equation (2.1)

$$\text{Heavy Metal Removal (\%)} = \frac{C_i - C_f}{C_i} \times 100 \qquad (2.1)$$

where $C_i$ is the concentration of initial heavy metal and $C_f$ is the concentration of final heavy metal

## 3. RESULTS AND ANALYSIS

The process outcome was rated through sulfate diagnosis, metals ions and COD bioreduction in wastewater of simulated project. The formation of black precipitate is symptomatic of sulfide as a sulfate reduction (SO42−) to sulfide (S2−)[9]. In the study of batch biosorption, experiments were conducted for various factors optimization such as pH, temperature, concentration of hexavalent chromium, and hydraulic retention time. The results are described in the following sections.

### 3.1. PH consequence

The pH has a main consequence on the solution of ions in biogenic sulfide ions and prompted the reduction in hexavalent chromium. The tests were lead at a constant initial of 60 mg/L hexavalent chromium concentration at a temperature 37 ◦C, by pH varying from 3 to 9. As evident from the results, at pH 7, removal of maximum COD was in day 7 at 55%. As shown in following figure A1, there was a biosorption progressive increase of hexavalent chromium with pH increase up to 7.0 and later there was decrease in Cr (VI) removal trend investigation with more pH increase. Percentage of maximum removal of hexavalent chromium and sulfate was found to be 85.79% and 49.9% respectively at pH 7. Then an efficiency reduction of sulfate removal above pH 7.0 was observed. Found by [11] these results are coincided. They found that 7.5 pH was the optimum value for removal of Cr (VI) water contaminated by (SRB). [10] reported similar findings as sulfate reduction maximum rate is accomplished at 7.25 pH. It is reported that pH of optimum value for SRB growth is between pH 5.6 and 9 [9]. Mostly bacteria has thermophilic growth near pH of neutral value from 5 to 9 but value in pH exceeds the bounds and is responsible for causing SRB activity deactivation [8].

### 3.2. Temperature impact

Temperature of optimal value was obtained for the consortia in isolated form by experiments conducted at temperatures ranging from 20 to 30 ◦C at the hexavalent chromium concentration of constant initial metal ion at 60 mg/L. As shown in figure A2, chemical oxygen demand of maximum reduction and sulfate was observed to be 46.9% and 75.6% respectively. The utmost removal of hexavalent chromium was at 90.4% , 37 ◦C and at pH of 7.9. With temperature increase, the performance of removal was decreased. From the results it can be concluded that at 37 ◦C, sulfate-reducing bacteria maximum growth and parameters of maximum reduction such as chemical oxygen demand, sulfate and concentration of metal ion will be obtained. [7] At 37 ◦C outlined similar findings was found to be for the sulfate reduction at optimum temperature and removal of Cu (II) from wastewater.

### 3.3. Heavy metal dosing consequence

Initially, increasing chromium (VI) doseup to 60 mg/L increased the growth in microbial functions and it indicated that chromium acts as growth essential nutrient. Beyond hexavalent chromium of 60 mg/L, growth in microbial was inhibited due to its toxicity. The activity and bacteria growth were affected strongly by metals as it intervenes with acids in nucleic and active sites enzyme [13]. Many enzymes and co-enzymes depend on some traces of minimal amount of their activation and activity metals. When large amounts present, they cause micro-organisms an inhibitory or toxic consequence [12]. Thus concentration of 60 mg/L was considered as the metal in optimum further experiments loading dose. The hexavalent chromium maximum metal and COD removal efficiency at 60 mg/L were determined to be 81.2% and 86.5% but it decreased with further metal ion dosing increments as shown in figure A3. The efficiency of maximum sulfate removal was observed to be 76.9% due to more preparation for H2S but it decreased with further dosing of metal ion beyond 60 mg/L. This may be due to the increase in the metal ion count competing for availability binding biosorbent sites and also due to binding sites deficit for the complexation as concentration level is higher.

### 3.4. Hydraulic retention time consequence (HRT)

Hydraulic retention time (HRT) is the main factor that affects bioreactors proficiency and has been reported in [11]. A narrow hydraulic retention time may not allow enough time for sulfate-reducing bacteria activity to neutralize acidity and metals of precipitated size. A longer hydraulic retention time may imply depletion of either the available source in organic matter or the sulfate source for sulfate-reducing bacteria. The 7 days reactors were set up at a temperature in optimum conditions of 37 ˚C with lactate of sodium as carbon source. As shown in figure A4, it could be concluded that the reactor pH fluctuates between 8.0 and 8.49, which was optimum for the SRB activity. The reduction of continuous COD was observed and 86.8% was achieved after incubation period of 168 h. Removal of Cr (VI) and sulfate were noticed to be 83.6% and 92.4%, respectively on incubation period day 7.

### 3.5. Simulated water treatment under optimum conditions

The simulated wastewater treatment was performed under the optimized Cr (VI) 50 ppm condition, 7 days, temperature of 37 ◦C and pH 7. In this study the two reactors of 1000 mL capacity were used for removal of Cr (VI) from wastewater of simulated environment. In these two reactors one was kept as a control and was run parallel to check the efficiency of removal without growth in microbial (SRB). SRB innoculum of 100 mL was added into the mixture of 900 mL in Postgate growth media in 1:1 ratio (v/v) and wastewater in the reactors. The study for wastewater treatment was carried out in duplicate and the average value was informed. First reactor was inoculated with SRB inoculum of 100 mL, while the second reactor was set as control having media for postgate only. The conditions in anaerobic environment were created by maintaining all air-tight reactors.

The reactors were coupled with bottles for aspirated level of labeled water. Decreased level of water in bottles for the aspirated one indicated the gas production which was due to growth of SRB. The water level was continuously monitored while control reactor water level remained the same. The reactors were kept in an incubator for BOD at conditions which are optimized i.e. temperature 37 ˚C with seven days, hexavalent chromium 60 mg/L for under static conditions. In this method sampling from reactors was performed at the incubation time (0 hr) and after days specified. The effluent pH increase from 6.45 to 7.93 indicated sulfate-reducing bacteria growth due to formation of hydroxyl ions. At this pH, sulfate-reducing bacteria were capable of removing 94.5% sulfate, 82.8% COD and 88.3% Cr (VI) from an anaerobic bioreactor simulated wastewater operated under condition of alkalinity with lactate as a source in organic carbon. The wastewater in microorganisms present convert this sulfate into sulfides of biogenic form which helps in precipitation of heavy metal.

## 4. CONCLUSION

The study concludes that the chromium maximum removal was found to be in simulated wastewater of 88.4% with Cr (VI) loading initially at 60 mg/L and sodium lactate as source in carbon for hexavalent chromium for 7 days. Synthetic wastewater treatment under conditions of optimized parameters resulted in efficiency of high removal rate for Cr (VI), sulfate and COD because sulfate-reducing bacteria utilized substrate in organic form from wastewater as a source of carbon.

The obtained results confirmed that SRB consortium was found suitable for an efficient development and biosorbent in economic form from wastewater for sulfate removal and heavy metals.

## REFERENCES


[1] Q.Zhou, Y.Chen, M.Yang, W.Li and L.Deng, Enhanced Bioremediation of Heavy Metal From Effluent by Sulfate-Reducing Bacteria with Copper-Iron Bimetallic Particles Support, Bioresource technology, Vol. 136, 2013, pp: 413-417.

[2] S.Chelliapan, P.J.Sallis, Anaerobic Treatment of High Sulphate Containing Pharmaceutical Wastewater, Journal of Scientific and Industrial research, Vol. 74, No .09, 2015, pp. 526-530.

[3] F.Omil, P.Lens, A.Visser and L.W.Hulshoff Pol, Long-term Competition Between Sulfate Reducing and Methanogenic Bacteria in UASB Reactors Treating Volatile Fatty Acids, Biotechnology and Bioengineering, Vol. 57, No. 6, 1998, pp 678-685, http://dx.doi.org/10.1002/(SICI)1097-0290(19980320)57:6<666::AID-BIT4>3.0.CO;2-J.

[4] Mohita Sharma, Jhansi L.Varanasi and Pratiksha Jain, Influence of Headspace Composition on Product Diversity by Sulphate Reducing Bacteria Biocathode, Bioresource Technology, Vol. 165, 2014, pp. 365-361, http://dx.doi.org/10.1016/j.biortech.2014.03.075.

[5] Jiemin Zhou, Effect of Electron Donors on the Performance of Haloalkaliphilic Sulfate-Reducing Bioreactors for Flue Gas Treatment and Microbial Degradation Patterns Related to Sulfate Reduction of Different Electron Donors, Biochemical Engineering Journal, Vol. 96, 2015, pp. 14-22, http://dx.doi.org/10.1016/j.bej.2014.12.015.

[6] Yiwen Liu, Yaobin Zhang, and Bing-Jie Ni, Evaluating Enhanced Sulfate Reduction and Optimized Volatile Fatty Acids (VFA) Composition in Anaerobic Reactor by Fe (III) Addition, Environmental Science and Technology, Vol. 49, No.4, 2015, pp. 2123-2131, http://dx.doi.org/10.1021/es504200j.

[7] Chunxi Kang, Pingxiao Wu , Yuewu Li, Bo Ruan, Liping Li, Lytuong Tran , Nengwu Zhu and Zhi Dang, Understanding the Role Of Clay Minerals in the Chromium(VI) Bioremoval Bypseudomonas Aeruginosa CCTCC AB93066 under Growth Condition: Microscopic, Spectroscopic and Kinetic Analysis, World Journal of Microbiology and Biotechnology, Vol. 31, No. 11 , 2015, pp 1765-1779, http://dx.doi.org/10.1007/s11274-015-1928-9.

[8] Cetin Kantar, Cihan Ari and Selda Keskin, Comparison of Different Chelating Agents to Enhance Reductive Cr(VI) Removal by Pyrite Treatment Procedure, Water Research, Vol. 76, 2015, Pages 66–75, http://dx.doi.org/10.1016/j.watres.2015.02.058.

[9] Gulumser Acar Doganlı and Nazime Mercan Dogan, Reduction of Cr(VI) to Cr(III) by Thermal Bacillus Licheniformis B22 Under Different Temperatures using Binary and Ternary Combinations of Organic Acids, Desalination and Water Treatment, Vol. 52, No. 37-39, 2014, pp.7163-7171, http://dx.doi.org/10.1080/19443994.2013.823117.

[10] V. Janaki, Mi-Na Shin, Song-Hee Kim and Kui-Jae Lee, Application of Polyaniline/Bacterial Extracellular Polysaccharide Nanocomposite for Removal and Detoxification of Cr(VI), Cellulose, Vol. 21, No. 1, 2014, pp 463-472, http://dx.doi.org/10.1007/s10570-013-0100-7.

[11] Xi Mo, Zhao-hui Yang and Hai-yin Xu , Combination of Cathodic Reduction with Adsorption for Accelerated Removal of Cr(VI) Through Reticulated Vitreous Carbon Electrodes Modified with Sulfuric Acid–Glycine Co-Doped Polyaniline, Journal of Hazardous Materials, Vol. 286, 2015, Pages 493–502,

http://dx.doi.org/10.1016/j.jhazmat.2015.01.002.

[12] Thi Quynh Hoa Kieu, Thi Yen Nguyen and Thi Yen Dang, Optimization of Sulfide Production by an Indigenous Consortium of Sulfate-Reducing Bacteria for the Treatment of Lead-Contaminated Wastewater, Bioprocess and Biosystems Engineering , Vol. 38, No. 10 , 2015, pp 2003-2011, http://dx.doi.org/10.1007/s00449-015-1441-4 .

[13] Subhabrata Dev , Aditya Kumar Patra, Abhijit Mukherjee and Jayanta Bhattacharya, Suitability of Different Growth Substrates as Source of Nitrogen for Sulfate Reducing Bacteria, Biodegradation, pp 1-16, http://dx.doi.org/10.1007/s10532-015-9745-2.

## APPENDIX A

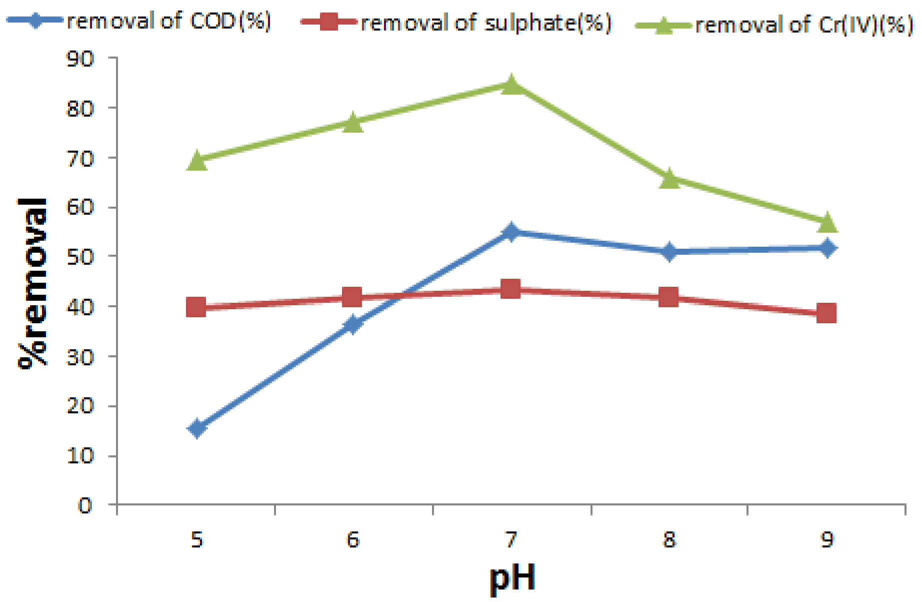



Figure A1.pH consequence on various parameters reduction at dose of constant metal 60 mg/L, temperature 37 ° C and time 7 days.

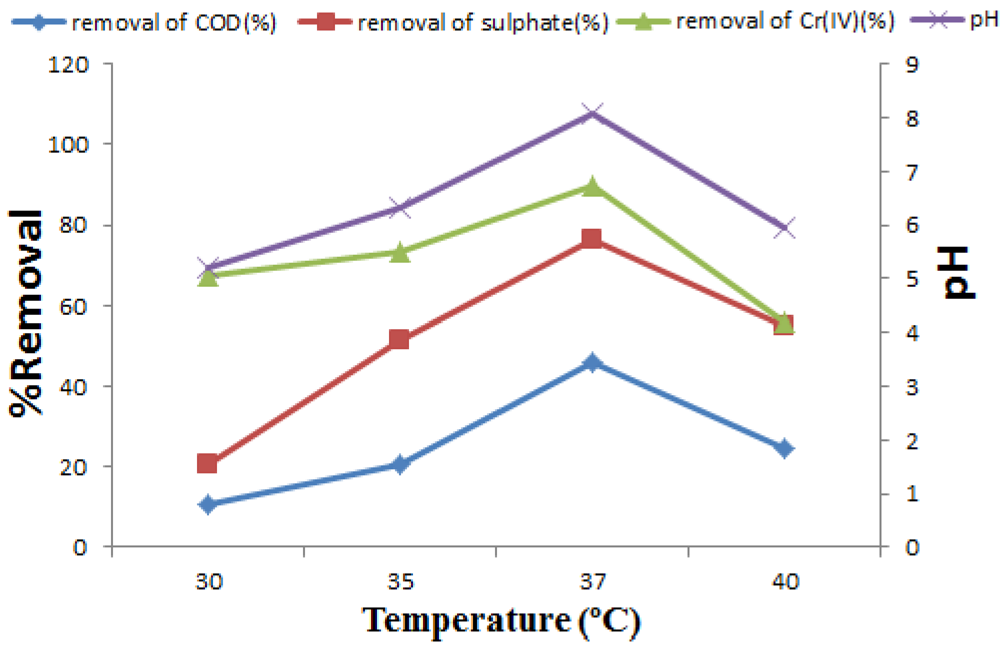



Figure A2. Consequence of temperature in incubation on various parameters reduction at pH 7 in optimized, 60 mg/L in constant metal dose and time 7 days of incubation.

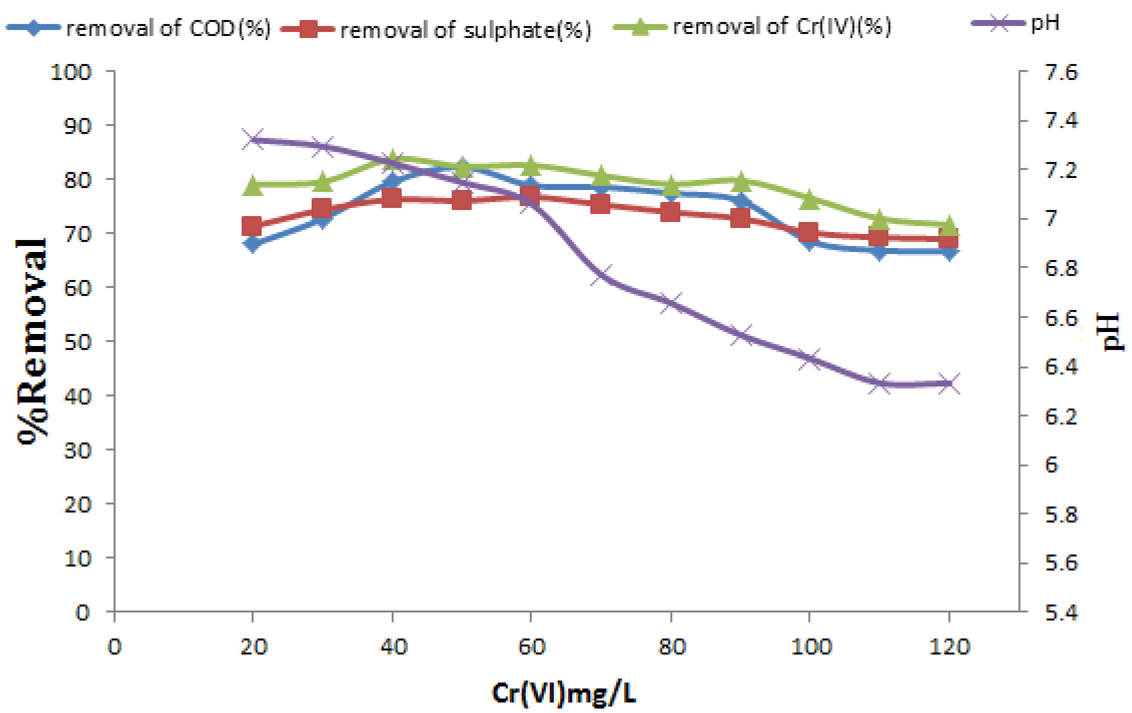



Figure A3.Initial metal dose consequence on various parameters reduction at pH 7 in optimized and temperature 37 ˚ C and time 7 days of incubation.

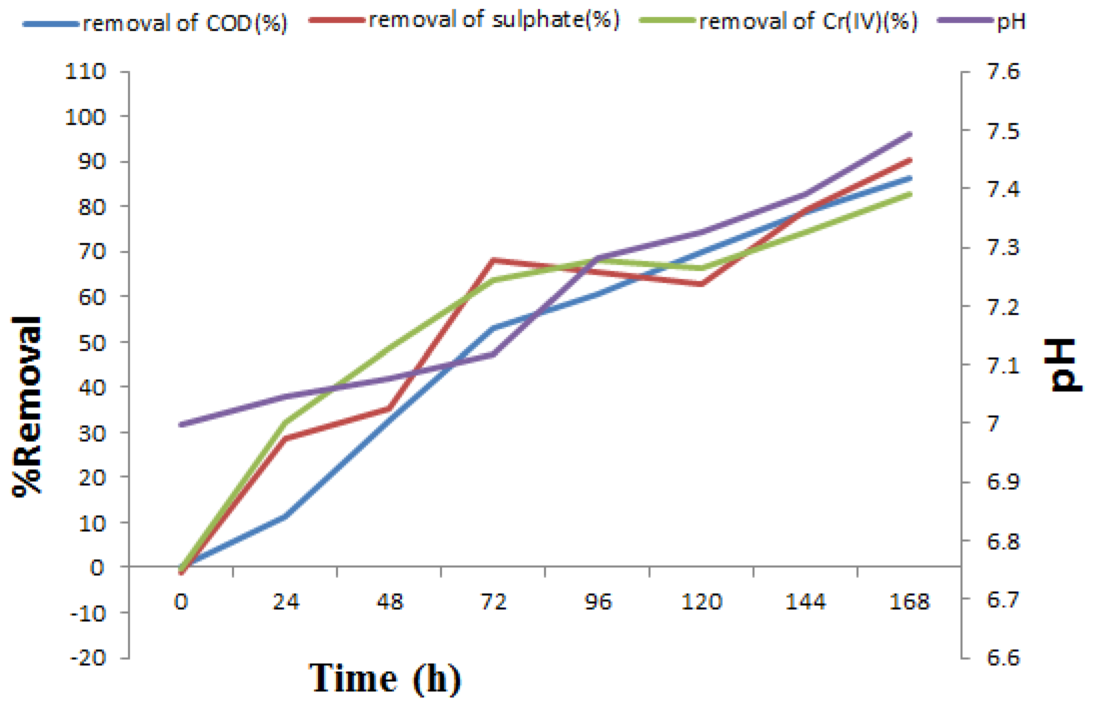



Figure A4.Hydraulic retention time consequence on pH of various parameters (secondary *X* axis), COD, sulfate and Cr (VI) removal by confederation of SRB at pH 7, 60 mg/L in metal dose and temperature 37 ˚ C.