# Carbon drought and its effects on the biochemical events in a chemo-bioreactor treating Acid Mine Drainage

Bidus Kanti Das, Jayanta Bhattacharya

*Department of Mining Engineering, Indian Institute of Technology Kharagpur, 721302, India.*

**Abstract** A pilot scale chemobioreactor was tested for acid mine drainage (AMD) treatment. During continuous run, parameters like $Fe_{influent}$ : $Fe_{effluent}$ and Sulfate $_{influent}$ : Sulfate $_{effluent}$ was over 47 and 69 respectively, when SRB population was over $10^5$ cells/ ml and DOC concentration over 100 mg $L^{-1}$. After $14^{th}$ week, temporary process failure was seen to take place when DOC value and SRB population fell below 50 mg $L^{-1}$ and $10^3$ cells /ml. pH was below 6 at this stage. Sulfur was one of the major elements in blackish green effluent precipitate. Metal binding, nutritional contribution and $CO_2$ formation were major reactions on reactor bed.

**Key Words** Chemobioreactor; metal sulfate reduction; bacterial abundance; carbon drought

## Introduction

Different technologies have been applied to mitigate the acid mine drainage (AMD) problem in situ or ex-situ, in which biological strategies also find favour (Johnson and Hallberg 2005). Biogenic alkalinity generations not only raise the pH, but also help to precipitate the metals in AMD (Das et al 2009). Modern biological AMD treatment technologies are based on sulfate reducing bacteria (SRB) mediated system because of simultaneous removal of soluble metals and sulfate, production of less sludge and products of lower solubility (Kaksonen et al 2004).

Common requirements in these technologies are suitable carbon source, pH (> 5.0), anaerobic environment and stable microstructure for SRB growth (Hao 2003). Uses of complex but common available organic waste materials are seen to result in sufficient sulfate reduction and make the process more economical (Neculita et al 2007). Spent mushroom compost (SMC) is a good carbon source as it shows higher relative performance than other waste materials (Chang et al 2000). It can provide sufficient dissolved organic carbon (DOC), nitrogen (DON) and nutrient additives. Polysaccharide content of SMC is generally degraded by hydrolytic fermentative anaerobes to alcohols and fatty acids that support the growth of SRB (Chang et al 2000). Because of its slow degradation rate and bulk physical property, it can be chosen as a suitable substrate in bioreactor for long term operation (Bhattacharya et al 2008). A higher pH (>5.0) is preferable to avoid the decline of solubility and sulfate reduction efficiency of metal sulfides (Neculita et al 2007). A successive alkalinity producing system (SAPS) with limestone is widely used to increase the pH in presence of SMC in bioreactor (Bhattacharya et al 2008).

The present study focuses on the performance of a down-flow chemo-bioreactor using SMC as growth substrate, and limestone as neutralizing material in continuous experiment to treat synthetic AMD. SMC has long been applied for treating metals in AMD and coal mine drainage. Its organic carbon liberating capacity and bacterial load in AMD during treatment have been studied here. In addition, the fate and the composition of metal precipitates along with metal adsorption by SMC have been analyzed. The objective of this study was to evaluate how long SMC could liberate available organic carbon, bacterial abundance (especially sulfate reducing bacteria) in the environment, utilization of organic matrix and also the evaluation of limestone in terms of exhaustion for better performance of chemo-bioreactor in continuous treatment.

## Experimental

***Chemo-Bioreactor and its operation:*** The down-flow gravity fed pilot-scale chemo-bioreactor system contained six transparent cylindrical chambers with an openable headspace (Fig.1). A is the reservoir; B and C are settlement tanks to prevent the entry of excessive total suspended solids (TSS) into tank D where substrate materials were present. Tanks E and F are for settlement of the precipitate coming from tank D. Height of the reactive chamber (D) is 145 cm with inner diameter of 30 cm. Rest of the chambers are identical in size (48 cm × 30 cm). D tank was packed with limestone (4.8 kg), SMC (5.12 kg) and gravels (5.3 kg) from bottom that made bed height of 20.5 cm. Empty column above the bed was used in calculations of HRT (Hydraulic Retention Time) and loading rates.

Synthetic acid mine drainage was prepared following Gray and O'Neill (1995) procedure, containing metal ions (mg $L^{-1}$) Fe (460), Mn (220), Mg (267) Cu (264), and sulfate (2611) with pH 2.8 and acidity 1038 mg/L as $CaCO_3$. 80 liters of this mixture solution was fed to the chemo-bioreactor (D) initially with filling other three (A, B and C) tanks; and was left to settle. Pond sediment (80 ml) was poured into the reactor for inoculation of SRBs. After 15 days, continuous flow was started with a HRT of 11.1 days and was fixed in this state until end (18 weeks). During this period the water volume was kept constant by controlling flow rate.

***Water sampling and analysis:*** Influent and effluent water were collected at 7day intervals regularly. Samples for analytical purpose were filtered through a 0.45 µm nylon membrane filter to remove precipitates and other solid materials. Metal concentrations were analyzed by ICP-MS (Varian 820-MS). Sulfate was measured by titration method in electrometric auto-titrator (Orion 950 Ross FASTQC, USA) in the fixed millivolt (mv) increment mode for first derivative endpoint detection. pH was measured by pH meter. Total organic carbon (TOC) was measured by TOC analyzer using the filtered sample and reported as DOC (Khan et al 1996). Microbial abundance was evaluated on reactor bed for total and sulfate reducing bacteria. Total microbial count was performed by epifluorescent microscope applying propidium iodide and acridine orange. Most Probable number method described by Fortin et al (2000) was applied for SRB count. SMC before and after loading and the precipitate formed in the E tank was analyzed for precipitate comparison. Samples were examined by a field emission scanning electron microscope (FESEM) with attached EDAX analyzer. Infrared spectra of SMC and precipitates were recorded on a Nexus™ 870 FT-IR (Thermo Nicolet, USA) spectrophotometer.

## Results and Discussion

***Chemobioreactor performance:*** Two performance parameters in terms of $Metal_{influent}$: $Metal_{effluent}$ and $Sulfate_{influent}$: $Sulfate_{effluent}$ are used that directly point out the state of performance of the chemo-bioreactor. The quotient of influent to effluent is taken as a parameter because it would be expectedly more than one, thus showing the number of times the effluent is less than the influent, after treatment. Values ≤ 1 would mean non-performance or even addition. All tested metals (Fe, Mn, Mg and Cu) and sulfate showed a ratio more than 1, in most cases, during the experiment (Fig. 2). However, after first week it was near 0.5 for Mg indicates its addition into the effluent. The peak intensity of Mg in SMC after experiment was much less than it was before, as shown in EDAX data (Fig.5b) indicating Mg leaching. Mg shows the least of reduction, while Cu was reduced the most in the same time. Fe, Mn, Cu and sulfate ratios rose steadily till the second week but then fluctuated. Highest acheived reduction was seen for Mg ($Mg_{inf}$ : $Mg_{ef}$ at 14.35) at 2nd week, Mn ($Mn_{inf}$ : $Mn_{ef}$ at 27.59) at 3rd week, Cu ($Cu_{inf}$ : $Cu_{ef}$ at 32.71) at 9th week, Fe ($Fe_{inf}$ : $Fe_{ef}$ at 47.56) and sulfate ($Sulfate_{inf}$: $Sulfate_{ef}$ at 69.68) at 12th week. However, ratios for metals and sulfate were reduced after 14th week and continued to be steady value - indicating some kind of process failure at this stage. DOC fell from a moderate value of over 400 mg $L^{-1}$ to below 50 mg $L^{-1}$ after 14th week and became constant (Fig. 3). The pH could be maintained over 6 up to 13 weeks period, after which it fell below 6. Increase of the pH above 5 in the chemobioreactor is the combined action of microbial alkalinity generation and limestone dissolution. Long water column could make the envi-

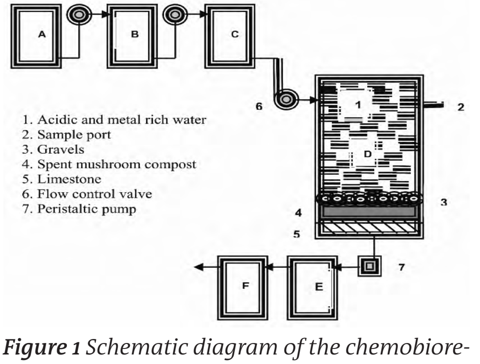


*Figure 1 Schematic diagram of the chemobioreactor configuration*

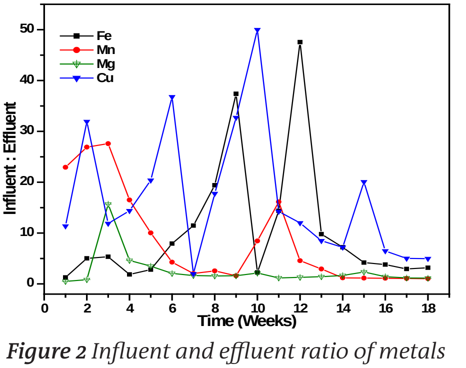


*Figure 2 Influent and effluent ratio of metals*

ronment anoxic. Figure 2 and 3 clearly state that the chemobioreactor was always able to reduce the metals and sulfate concentration and to provide required alkalinity generation during the experiment.

***Bacterial Abundance:*** Double fluorescence stained samples showed both live and dead cells were present in the effluent samples. Percentage of live cells was over 65% throughout the continuous experiment. Total bacterial population was over $10^5$ cells /ml whereas SRB population was over $10^4$ cells/ ml at DOC higher than 50 mg $L^{-1}$ (Fig. 3). Results also indicate that initially there was a predominance of other bacteria over SRBs in the chemobioreactor. With time, growth of SRBs predominated and from $5^{th}$ week onwards SRBs began to comprise most of the bacterial population. After this point, SRB curve follows the same pattern like total bacterial population. At $11^{th}$ week SRB population reached at maximum, and after $13^{th}$ week both began to fall exponentially. SRB population dropped down to $10^3$ cells /ml when DOC was less than 35 mg $L^{-1}$. From $10^{th}$ to $15^{th}$ week total bacteria was mainly comprised of sulfate reducing bacteria. Noticeably, after $13^{th}$ week SRB population decreased more sharply correlating significantly with reducing DOC release. There is a positive correlation between SRB abundance and sulfate reduction ratio (Fig. 3 and 4). After $12^{th}$ week, both the values got reduced and after $15^{th}$ week they were nearly constant. Therefore, whatever sulfate concentration was reduced was primarily due to SRB. And the process failure was mainly due to organic carbon drought that affects both SRB biomass production as well as SRB mediated sulfate reduction.

***Microscopic and FTIR analysis of SMC and precipitates:*** SEM images of exhausted SMC reveal Biofilm formation on the SMC bed (Fig. 5). Attached EDAX analysis demonstrate that the major elements in exhausted SMC were Si, Fe, Ca and O. Blackish green precipitates were formed in the first effluent tank (E) from the beginning of the experiment and continued to form till the completion of the experiment. Major elements of this precipitates were Fe, Mn, Ca, C, S, clearly indicate presence of sulphur containing metal complex.

FTIR spectrum (Fig. 6) of raw and exhausted SMC, and effluent tank precipitate showed broad peaks at 3418 $cm^{-1}$, 3415 $cm^{-1}$, 3405 $cm^{-1}$ respectively corresponding to the OH stretching of polymers or phenolic group and N-H stretching of primary amides that might be associated with cellulosic cell wall of mushroom and plant debris (Belamy 1975). Peaks near 1100 $cm^{-1}$ [C-O; stretching and O-H deformation vibration of secondary alcohol (Belamy 1975)] are present in both SMC sample but absent in precipitates. That indicates the microorganisms in the chemo-bioreactor could have utilized the alcoholic group in SMC for their growth. Decrease in intensity of peak near 3400 $cm^{-1}$ in exhausted SMC can be attributed to the involvement of the H-bonded OH and N-H group in metal biosorption. Shifting of peak in the region of 1650 $cm^{-1}$ [C = O stretching of primary amide (Pagnanelli et al 2000)] of SMC to lower frequencies at 1620 $cm^{-1}$ in effluent precipitate can be due to interaction of this group with metal ions. A new peak with small intensity at 2360 $cm^{-1}$ region in exhausted SMC and precipitate was found [$\upsilon_3$ band of $CO_2$ (Nakamoto 1986)]. That points toward either microbial metabolism-evolved-$CO_2$ in the chemo-bioreactor or residual limestone-evolved-$CO_2$. New peaks in precipitate at 1252 $cm^{-1}$ and at 1345 $cm^{-1}$ can be assigned to $SO_3$ stretching, and inorganic $NO_2$ stretching, (Nakamoto 1986) respectively. It seemed the deposition of sulfate ions also occurred with metal ions.

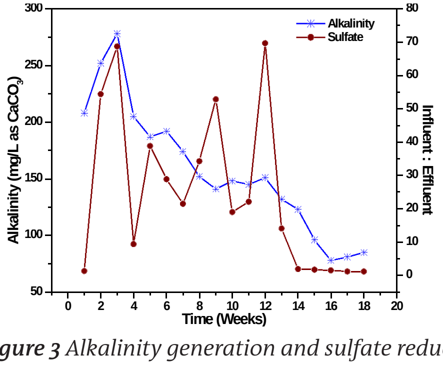


***Figure 3*** *Alkalinity generation and sulfate reduction*

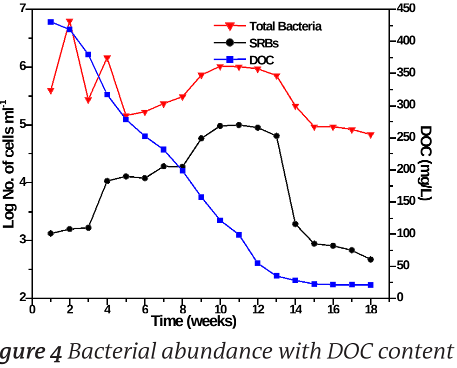


***Figure 4*** *Bacterial abundance with DOC content*

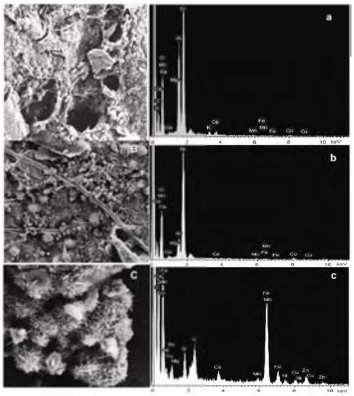

***Figure 5*** *SEM and EDAX micrograph of SMC before (A and a) and after (B and b) experiment and precipitates in effluent tank (C and c)*

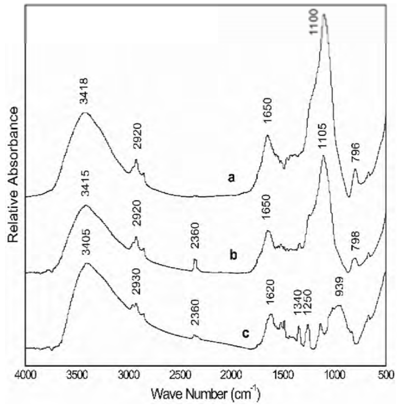


***Figure 6*** *FTIR spectra of (a) SMC before experiment (b) SMC after experiment and (c) precipitate formed in the effluent tank*

## Conclusions

This research produced the following findings: a) The experimental chemobioreactor to neutralize synthetic AMD is suitable for application in laboratory scale, to reduce metals and sulfate concentration. b) SRB population decreases with DOC release, as DOC gets spent. c) The microbial process of sulfate reduction ceased, due to insufficient electron donor and enhanced alkalinity, after $14^{th}$ week of running.

## References

Bhattacharya J, Ji SW, Lee HS, Cheong YW, Yim GJ, Min JS, Choi YS (2008) Treatment of acidic coal mine drainage: design and operational challenges of successive alkalinity producing systems. Mine Water Environ 27: 12—19

Bellamy LJ (1975) The infrared spectra of complex molecules. Chapman and Hall, London.

Chang IS, Shin PK, Kim BH (2000) Biological treatment of acid mine drainage under sulphate-reducing conditions with solid waste materials as substrate. Water Res 34: 1269—1277

Das BK, Roy A, Koschorreck M, Mandal SM, Wendt-Potthoff K and Bhattacharya J (2009) Occurrence and Role of Algae and Fungi in Acid Mine Drainage Environment with Special Reference to Metals and Sulfate Immobilization. Water Res 43: 883—894

Fortin D, Roy M, Rioux JP, Thibault PJ (2000) Occurrence of sulfate-reducing bacteria under a wide range of physico-chemical conditions in Au and Cu-Zn mine tailings. FEMS Microbiol Ecol 33: 197—208

Gray N F, O’Neill C (1995) Artificial acid mine drainage for use in laboratory simulation studies. Fresenius Environ Bull 4: 481—484

Hao OJ (2003) Sulfate reducing bacteria, in Handbook of water and wastewater microbiology, ed Mara D and Horan NJ. Academic press

Johnson DB and Hallberg KB (2005) Acid mine drainage remediation options: a review. Sci Total Environ 338: 3—14

Kaksonen AH, Franzmann PD, Puhakka JA (2004) Effects of hydraulic retention time and sulfide toxicity on ethanol and acetate oxidation in sulfate-reducing metal-precipitating fluidized-bed reactor. Biotechnol Bioeng 86: 332—343

Khan E, Babcock Jr. RW, Stenstrom MK, Suffet IH (1996) Biodegradable organic carbon for characterizing reclaimed and treated wastewaters. Water Environment Federation, $69^{th}$ annual conference & exposition. Dallas, Texas. October 5—9.

Nakamoto K (1986) Infrared and Raman spectra of inorganic and coordination compounds. John Wiley & Sons, Inc.

Neculita CM, Zagury GJ, Bussiere B (2007) Passive treatment of acid mine drainage in bioreactors using sulfate-reducing bacteria: critical review and research needs. J Environ Qual 36: 1—16.

Pagnanelli F, Papini MP, Trifoni M, Vegli F (2000) Biosorption of metal ions on Arthrobactor sp.: biomass characterization and biosorption modeling. Environ Sci Technol 34: 2773—2778