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Studies on bioremoval of copper and zinc by *Desulfovibrio desulfuricans*

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Abstract

This paper reports the study on the feasibility of bioremoval of copper and zinc using sulphate reducing bacteria (*Desulfovibrio desulfuricans*) grown on agro waste carbon sources like rice husk, saw dust and manure. Efficient growth of wild strain was achieved with all the agro waste carbon substrates. The inhibition effect of copper and zinc sulphate on the growth of *D. desulfuricans* cells was established and was found to be concentration dependent. 25 ppm Cu(II) and 30 ppm Zn(II) ions were able to completely inhibit the growth of cells. Strains tolerant to higher metal ion concentrations were obtained by serial subculturing and used for bioremoval studies. Direct bioremoval of copper and zinc during the growth was achieved for all the strains. Strains grown in presence of rice husk had higher bioremoval efficiency with percent removal of nearly 72% and 86% for 500 ppm of initial copper and zinc concentration respectively.

Keywords: Zinc Oxide nanoparticles, biological method, XRD, SEM and agro waste carbon substrate.

Introduction.

Extensive sulphide mining activities has resulted in several abandoned mines that have accumulated large volumes of acid mine drainage (AMD) in open pits and streams. AMD is characterized by high concentrations of heavy metals and sulphates as well as low pH values, which will affect severely the ecosystems and population health.

Several conventional physical and chemical processes are available for treatment of AMD. However, they have generally demonstrated low efficiency and high cost. The use of biological processes with sulphate reducing bacteria (SRB) to reduce sulphate and remove high amounts of heavy metals as insoluble metal sulphides, has great potential due to low installation and operation costs. During the growth of SRB, biologically produced H_2S precipitates metals as metal sulfides, while biogenic bicarbonate alkalinity neutralizes acidic waters.

Cadmium, copper, iron, lead, mercury, nickel, and zinc are some of the metals that will precipitate as metal sulfides. In addition, arsenic, antimony, and molybdenum form more complex sulfide minerals. Metals such as manganese, iron, nickel, copper, zinc, cadmium, mercury, and lead may also be removed to some extent by co-precipitation with other metal sulfides (Figueroa 2005). Furthermore, SRB species have been found that can reduce certain metals to a more insoluble form, such as reduction of uranium (VI) to uranium (IV) (Spear et al., 2000). Sulfate reduction also consumes acidity, raising the pH. Increasing the pH facilitates the above precipitation reactions and creates suitable conditions for precipitation of metal hydroxides (Gadd 2004). The latest developments of using various agro waste substrates as carbon sources for SRB together with new bioprocess designs are increasing the uses and applications of SRB-based bioreactors in AMD control and selective metal recovery. The present investigation examines the feasibility of bioremoval of copper and zinc using sulphate reducing bacteria (*Desulfovibrio desulfuricans*) grown on agro waste carbon sources like rice husk, saw dust and manure.

Materials and Methods

Microorganism: Culturing & Growth studies

Strain of *Desulfovibrio desulfuricans* (NCIM 2539) used in this study were obtained from National Collection of Industrial Microorganisms, National Chemical Laboratory, Pune, India. They were subcultured in the laboratory using modified Barr's medium as given in Table I (Baars 1930; Grossman and Postgate 1953).

The bacteria were cultured in sealed serum bottles under anaerobic conditions by inoculating 10 ml of pure strain of the bacterial cells to the Barr's medium. The serum bottles were maintained air tight using rubber cork. After inoculation, the bottles were purged with

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nitrogen gas to maintain anaerobic conditions. This was incubated at 37 ± 1^0C in a orbital shaker at 200 rpm. Bacterial cell count was done by direct observation under a phase contrast microscope using Petroff-Hausser counter.

Table I: Composition of modified Baars medium

Components	Amount
K_2HPO_4	0.5 g/l
NH_4Cl	1 g/l
$CaSO_4$	1 g/l
$MgSO_4 . 7H_2O$	2 g/l
Sodium lactate	40 ml/l
Yeast extract	1 g/l
$FeSO_4(NH_4)2SO_4 . 6H_2O$	0.5 g/l
Sodium thioglycollate	0.02 g/l
Distilled water	1000 ml

Rice husk, saw dust and manure were used as agro waste carbon substrates to replace tryptone carbon source in the growth medium. The carbon substrate filtrate medium was prepared by interacting 10g of the carbon substrate with 100ml of deionised milli-Q water for 30min at 200rpm and 30^0C followed by filtration through Whatman 41 filter paper.

Growth inhibition studies

The inhibition effect of copper or zinc on the growth of bacteria was studied by adding copper sulphate or zinc sulphate at different concentrations to the culture bottles prior to inoculation.

Growth adaptation studies

Adaptation to copper and zinc metal ions were done by serial subculturing of *Desulfovibrio desulfuricans* cells with increasing concentrations of copper and zinc ions respectively. The cells in the exponential phase of growth were harvested by centrifuging at 12,000rpm (8050 ×g) at 4 ºC in a refrigerated highspeed centrifuge for 20 min. The cell pellet was washed with Milli-Q water twice and again centrifuged. The cell pellet so obtained was dispersed in fresh medium of higher metal ion concentration for further adaptation.

Sulphate precipitation studies

Sulphate precipitation studies were carried out using *Desulfovibrio desulfuricans* cells in modified Barr's medium devoid of iron in the presence of copper or zinc metal ions. Stock solutions of $CuSO_4$ and $ZnSO_4$ were added to the serum bottles to give the desired metal concentrations. Aqueous sulphate, copper and zinc concentrations of the growth medium were periodically estimated. Sulphate, copper and Zinc estimation was done using a HACH spectrophotometer according to the methods given in HACH Water Analysis Hand Book [5th edition] which is accepted by USEPA for reporting waste water analyses, and adapted from 'Standard method for the examination of water and waste water'.

Results and Discussion

Growth of Desulfovibrio desulfuricans cells

The strain of *Desulfovibrio desulfuricans* cells used reached maximum growth in 100-110 hrs and the black precipitate was observed after 200 hrs.

The cells were adapted to grow in presence of agro waste carbon substrates by repeated subculturing in presence of these carbon substrates. Adaptation was considered achieved when the growth in presence of these alternate carbon sources matched that of growth in presence of control medium. Figure 1 shows the growth of *Desulfovibrio desulfuricans* cells in presence of rice husk, saw dust and manure as carbon substrates. Highest cell count was obtained when cells were grown with rice husk filtrate as carbon source.

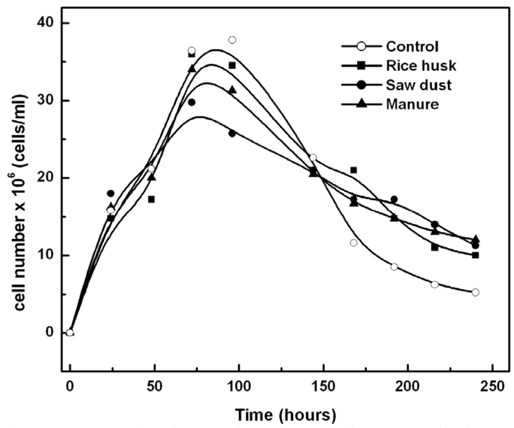

Figure 1: Growth of *Desulfovibrio desulfuricans* cells in presence of rice husk, saw dust and manure as carbon substrates

Effect of copper and zinc inhibition

Growth of *Desulfovibrio desulfuricans* at different concentrations of copper and zinc ions is shown in Figure 2a. The toxicity of Cu(II) to *Desulfovibrio desulfuricans* was dependent on Cu(II) concentration. 5ppm and 10ppm of initial copper concentration had no significant effect on the lag phase observed, However with an initial concentration of 10ppm the rate of cell growth were lower than the control flask, which contained no copper ions. Further increase in copper ion concentration resulted in extended lag phase and decrease in cell yield. The reduction in cell yield observed at 15ppm and 20ppm of copper concentration was 22% and 49% respectively. Copper ions completely inhibited the growth of *Desulfovibrio desulfuricans,* at an initial copper concentration of 25ppm.

Similar behaviour was observed with zinc ions (Figure 2b). 5ppm, 10ppm and 15ppm of initial zinc concentration had no significant effect on the lag phase observed, however the rate of cell growth were lower than the control flask with 15ppm initial zinc concentration. Further increase in zinc concentration resulted in extended lag phase and decrease in cell yield. The reduction in cell yield observed at 15ppm, 20ppm and 25ppm of copper concentration was 16%, 28% and 53% respectively. Zinc ions completely inhibited the growth of *Desulfovibrio desulfuricans,* only at an initial copper concentration of 30ppm.

The obtained results suggest that copper ions are more toxic to *Desulfovibrio desulfuricans* compared to zinc ions.

Adaptation of the strains grown in presence of different carbon substrates to copper and zinc ions

Adaptation to copper and zinc metal ions were done by serial subculturing of *Desulfovibrio desulfuricans* cells with increasing concentrations of copper and zinc ions respectively. The strains were considered adapted when the growth rate of cells in presence of metal ions were similar to control strains grown in the absence of metal ions. Strains adapted to 500 ppm copper and 500 ppm zinc ions were obtained by serial subculture and these strains were used for sulphate reduction studies.

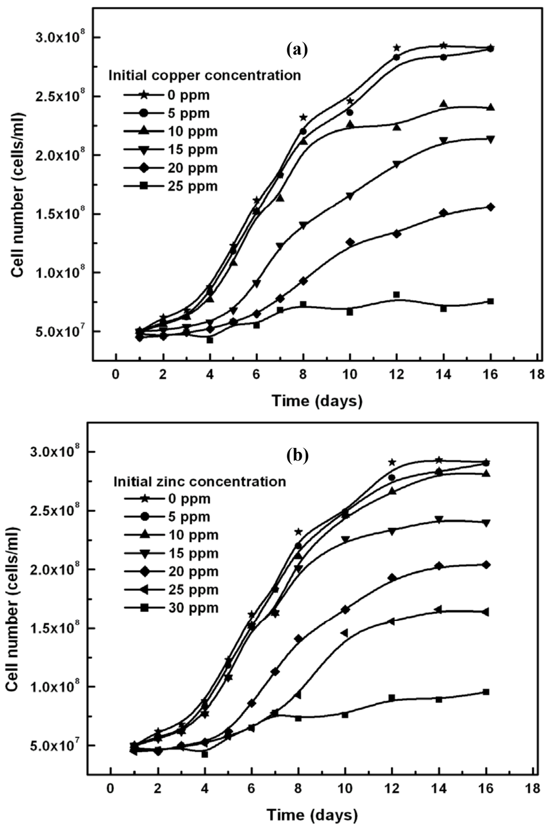

Figure 2: Effect of (a) copper (Cu(II)) and (b) Zinc ions on the growth of *Desulfovibrio desulfuricans* cells.

Sulphate reduction studies

Aqueous metal ion concentration was monitored during the growth of cells in presence of different concentrations of copper and zinc ions, and the results obtained are shown in Figure 3 and Figure 4 respectively. $CuSO_4$ and $ZnSO_4$ were added to the medium to obtain the desired metal ion concentrations. Strains grown in presence of agro waste carbon substrates and previously adapted to 500 ppm copper or 500 ppm zinc ions were used for these studies.

When grown in presence of copper sulphate or zinc sulphate, the aqueous metal ion concentration decreased indicating removal of metal sulphate from the solution. The percent removal was found to be nearly 98% and 96% for 50 ppm and 100 ppm of initial copper concentration respectively for all the strains. The bioremoval efficiency with 500 ppm of initial copper concentration was maximum for strains grown in presence of rice husk (72%) followed by saw-dust grown strain (61%) and manure grown strain (52%).

When grown in presence of 100 ppm Zn ions, the percent removal was found to be 11%, 44% and 73% for manure-grown, saw dust-grown and rice husk-grown strains respectively. When the strains grown in presence of 100 ppm Zn was used as inoculum for media containing 200 ppm Zn, the percent removal increased to 67%, 48% and 97% for the manure-grown saw dust-grown and rice husk-grown strains respectively. The bioremoval efficiency with 500 ppm of initial Zn concentration was maximum for strains grown in presence of rice husk (86%) followed by saw-dust grown strain (54%) and manure grown strain (28%).

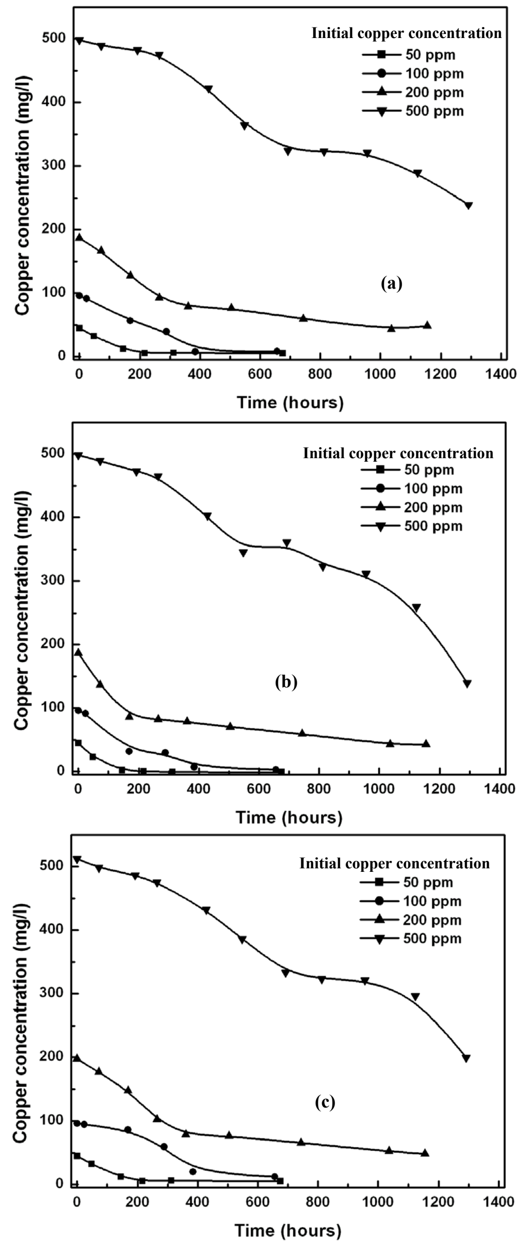

Figure 3: Aqueous copper ion concentration during growth of (a) manure (b) rice husk and (c) saw dust grown strains of *Desulfovibrio desulfuricans* in presence of copper sulphate.

This removal of metal ions could be attributed to the following processes: (i) biosorption onto cell surfaces (Chen et al. 2000), (ii) release of extracellular polymeric substances that can complex and detoxify (Beech and Cheung 1995), (iii) complexation and precipitation of Cu(II) as CuS (Bhattacharya et al. 1981; Poulson et al. 1987), and (iv) intracellular penetration and accumulation (Erardi et al. 1987).

Conclusions

Efficient growth of *Desulfovibrio desulfuricans* cells was achieved on agro waste carbon sources like rice husk, saw dust and manure. The inhibition effect of copper and zinc sulphate on the growth of cells was found to be concentration dependent. 25 ppm Cu(II) and 30 ppm Zn(II) ions were able to completely inhibit the growth of cells. However, strains tolerant to higher metal ion concentrations were obtained by serial subculturing and used for bioremoval

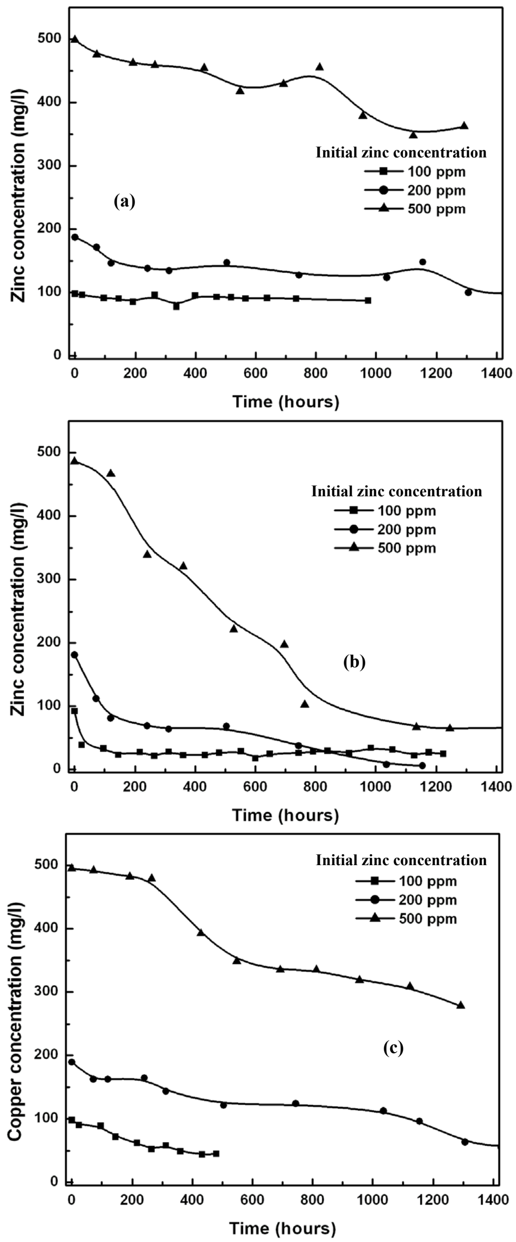

Figure 4 : Aqueous zinc ion concentration during growth of (a) manure (b) rice husk and (c) saw dust grown strains of *Desulfovibrio desulfuricans* in presence of zinc sulphate.

studies. Direct bioremoval of copper and zinc during the growth was achieved with all strains and strains grown in presence of rice husk had higher bioremoval efficiency compared to other strains.

When grown in presence of copper and zinc metal ions, a decrease in the aqueous sulphate concentration was observed indicating that the cells were able to remove the metal sulphates from the solution. The efficiency of removal was higher in case of zinc sulphate compared to copper sulphate and strains adapted to copper or zinc ions showed higher efficiency. The percent removal with rice husk-grown adapted strains was found to be nearly 72% and 86% for 500 ppm of initial copper and zinc concentration respectively.

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References

Baars JK (1930) Over Sulfate Reductive Door Bacterin. Dissertation, University of Delft, Holland.

Beech IB, Cheung CWS (1995) Interactions of exopolymers produced by sulfate-reducing bacteria with metal ions. Int. Biodeterior. Biodegrad. 35:59–72

Bhattacharya D, Jumawan AB, Sun G, Sund-Hegelburg C, Schwitzgebel K (1981) Precipitation of heavy metals with sodium sulfide: benchscale and full-scale experimental results. AICHE (Am. Inst. Chem. Eng.) Symp. Ser. 209:31–38

Chen B, Utgikar VP, Harmon SM, Tabak HH, Bishop DF, Govind, R (2000) Studies on biosorption of zinc(II) and copper(II) on *Desulfovibrio desulfuricans*. Int. Biodeterior. Biodegrad. 46:11–18

Erardi FX, Failla ML, Falkinham JO (1987) Plasmid-encoded copper resistance and precipitation by *Mycobacterium scrofulaceum*. Appl. Environ. Microbiol. 53:1951–1954

Figueroa L (2005) Microbial ecology of anaerobic biosystems treating mining influenced waters. Presented at the Mine Water Treatment Technology Conference, Pittsburgh, PA, August 15-18, 2005

Gadd GM (1992) Metals and microorganisms: a problem of definition. FEMS Microbiol. Lett. 100:197–204

Grossman, J.P., Postgate, J.R., 1953. Cultivation of sulfate reducing bacteria. Nature 171, 600–602

Poulson SR, Colberg PJS, Drever JI (1997) Toxicity of heavy metals (Ni, Zn) to *Desulfovibrio desulfuricans*. Geomicrobiol. J. 14:41–49

Spear JR, Figueroa LA, Honeyman BD (2000) Modeling the removal of uranium U(VI) from aqueous solution in the presence of sulfate reducing bacteria. Environmental Science and Technology 66(9):3711-3721