



Technical note

Monitoring of sulfate-reducing bacteria in acid water
from uranium mines

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Abstract

One of the most serious environment problems created by the mining industry is acid mine drainage. In one plant of Nuclear Industries of Brazil—INB, this problem is a matter of concern. The presence of iron sulfites, such as pyrite, generates water with acidity above the levels allowed by the legislation and therefore, inappropriate for releasing straight into the environment. The industry maintain a high cost treatment in acid water from mines and waste disposal which consists in neutralizing and precipitating heavy metals. The treatment of acid water using sulfate-reducing bacteria (SRB) has been used in other countries with quite good technical results as well as economical advantages and thus, the object of this research. A seasonal study was carried out on the sulfate-reducing bacteria present in the liquid effluent discharged from two wastes disposal of the uranium mine, in phase of decommission, in Poços de Caldas. This study shows the presence of SRB in the analyzed environmental, as well as some factors that are related with the amount of SRB presents, such as: dissolved oxygen, pH and organic matter.

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Keywords: Sulfate-reducing bacteria; Acid mine drainage**1. Introduction**

One of the most serious environmental problems created by the mining industry is acid mine drainage. In one plant of Nuclear Industries of Brazil—INB, this problem is a matter of concern. In that unit the uranium ore was processed until 1995 and generated a big amount of barren ore with U_3O_8 lower than 200 ppm. These barren ores were deposited in large neighboring areas from the mining region, in waste rock piles. The sulfite minerals present in these areas, including pyrite, generated high acidity water with radionuclides elements (uranium, thorium and radium) and stable elements (manganese, zinc, fluorite, iron,

etc.). The level of these elements were above the environmental legislation content (*Resolução CONAMA, 2005*). Actually the treatment involves chemical precipitation and any interruption leads to an increasing pollution on the aquatic system (*Cipriani, 2002*). The treatment of acid water using sulfate-reducing bacteria—SRB have been used in other countries with quite good technical as well as economical results. SRB are useful to abate acid mine drainage—AMD due to two fundamental reasons. Firstly because of their capacity to reduce sulfate to sulfite. These sulfites react additionally with certain metals dissolved in the contaminated waters, such as copper, iron and zinc, forming insoluble precipitates. On the other hand, the system acidity is reduced by their own action of sulfate reductions and by the carbon metabolism of the bacteria (*Garcia et al., 2001*). In this context the objective of this study consisted in seasonal diagnostic of the sulfate-reducing bacteria present in the aquifer system of the INB Industry Complex in Caldas, Brazil.

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2. Methodology

2.1. Sampling

Water and sediment samples were collected in liquid effluents from the Pit mine (PM) and from the waste rocks 4 (WR4) and 8 (WR8) in different depth and periodically for the seasonal study between February and December 2004.

2.2. Microbiological analysis

The determination of the occurrence of SRB in water samples and sediment was accomplished with cultivation medium—Postgate B using the method of the multiple tubes, series of five tubes (Postgate, 1979).

For the total bacteria counting aqueous solution of coloring acridine orange was added to the water samples previously conditioned with formaldehyde, used to fix the microorganisms. After a 5 min contact the sample was filtered in polycarbonate membrane placed on sheet dies and analyzed under epi-fluorescence microscope.

2.3. Physical and chemical analyses

The water pH was measured during the sample collection. The determination of dissolved oxygen in water was carried out by titrimetry (Eaton, 1995). The material in sus-

pension (organic and inorganic) was measured according to Teixeira et al. (1965).

The analyses of manganese and zinc were performed by spectrometry of atomic emission with coupled plasma. The phosphate and sulfate were analyzed by spectrophotometry and the fluoride by potentiometry with selective electrode.

3. Results and discussion

Table 1 shows the chemical analysis of the water and sediment samples collected in the sites PM, WR8 and WR4. Garcia et al. (2001) verified that the acid water from the Spanish pyrite showed sulfate values between 1800 and 2000 ppm and zinc grade between 30 and 50 ppm. These levels were higher than the experimental values found in this study.

Table 2 shows the pH and O₂ measures and biological analysis for samples collected from WR8. The highest value of total bacteria counting (13.3×10^5 bacteria mL⁻¹) was observed in February in this site.

Frömmichen et al. (2004) carried out studies of microcosm simulating of small pond role mine with acid water (pH 2.6). The bacteria total counting in the water column obtained by these authors changed from 10^5 to 10^6 bacteria mL⁻¹, according to the medium values of total bacteria obtained in that work in the WR8 (1.1×10^6 bacteria mL⁻¹), PM (3.5×10^5 bacteria mL⁻¹) and WR4 (3.4×10^5 bacteria mL⁻¹) points.

Table 1
Chemical analysis of samples from the points PM, WR8 and WR4

Site	F ²⁻	PO ₄ ²⁻	Mn	Zn	SO ₄ ²⁻
<i>Water</i>					
PM	70.95	4.60	97.66	16.83	1385.50
WR8	116.81	<0.03	137.61	22.59	1548.75
WR4	121.44	<0.03	107.38	17.95	1247.14
<i>Sediment</i>					
PM	4.16	0.137 + 0.006	4.94 + 0.03	0.935 + 0.001	12.10
WR8	0.06	0.217 + 0.006	0.24	0.06	2.91
WR4	0.04	0.254 + 0.006	1.14	0.04	0.43

Concentrations of the species in water—mg L⁻¹ and (%) in sediment.

Table 2
Biological analyses, pH and O₂ measures in samples from WR8 site

Month	Water					Sediment	
	pH	SRB MPN (mL ⁻¹)	Total bacteria (bacteria mL ⁻¹)	O ₂ (mg L ⁻¹)	Organic matter (mg L ⁻¹)	SRB MPN (mL ⁻¹)	Organic matter (mg L ⁻¹)
February	5.15	2.8	13.29×10^5	5.69	0.005	1.4	16.60
March	3.38	1.1	11.58×10^5	1.58	0.006	1.2	15.90
April	3.50	0	12.16×10^5	0.65	0.004	1.4	19.30
August	3.35	0	8.84×10^5	1.60	0.004	15	22.70
September	3.51	0	10.74×10^5	1.62	0.004	0.7	14.90
October	3.45	0	11.59×10^5	1.62	0.005	2	24.70
November	3.52	0	13.00×10^5	13.34	0.004	12	20.50
December	3.87	0	—	11.24	0.005	110	11.90

MPN/mL—most probable number/mL.

The medium values of SRB in water samples were low as we can see in Table 2. Some studies showed that high concentrations of heavy metals and low pH values (3.0–3.5) are limiting factors that influence the SRB increasing acid water from mine (Frömmichen et al., 2004; Garcia et al., 2001). The lowest pH value (below 3.5) was measured during the months of March and April and higher number of SRB (2.8 MPN mL^{-1}) was detected in February in WR8 site when the water pH was higher (5.2). Anaerobic reactors studies for the metals and sulfites removing from mine acid water have shown high SRB increasing, from 4.0×10^7 to 8.0×10^8 bacteria mL^{-1} on acid medium mainly due to organic matter enrichment in these systems (Jong and Parry, 2003).

According to expected results, the highest values of SRB were detected in the period between February and August in water samples collected 12.5 m of depth in the PM, where it was also detected the lowest oxygen concentrations along the period. The dissolved oxygen values measured in bottom samples of the mine lake (UTM) changed from 4.0 to $10.1 \text{ mg O}_2 \text{ L}^{-1}$ and were according to the values obtained by Meier et al. (2004) using sediment/water interface samples of acid water from Lusatia (Germany).

The values of SRB as well as most of its occurrences in the sediment samples in the three sampling sites were higher if compared with water samples along the year. This was expected as lower concentrations of dissolved oxygen were found in the sediment and higher concentrations of available organic matter for the populations of SRB were found in the sediment samples. These results are on a par with different studies conducted with acid water from mine.

4. Conclusion

The results obtained from the microbiological variables presented seasonal variation in the three points evaluated.

The results showed that SRB was present in water samples presenting low pH values and high concentrations of heavy metals, as well as in samples with high oxygen levels. The sediment was the preferential place for SRB occurrence and WR8 site presented the highest values of SRB detected in that experimental study.

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