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CHARACATERIZATION OF BACTERIA ACIDOPHILIC IN SAMPELS OF WATERCOMING INTO A REGION THAT SUFFERS INFLUENCE OF URANIUM MINE IN CALDAS (MG)

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ABSTRACT

The fundamental condition for the bioleaching of the uranium ore is the presence of metallic sulfide such as pyrite associated with the ore, which is found in the ore and in the waste at the Unidade de tratamento de Minerio (UTM) of Poços de Caldas, State of Minas Gerais, Brazil. The present study aims to determine the chemical and microbiological characteristics in effluents of uranium mining from the UTM and in Antas dam, which receives treated effluents from the UTM. Water samples were collected Pit Mine (CM), located within the UTM facilities and from site 41 (Antas dam) in July and October 2008. We verified low pH values in water samples from CM (3.7) in comparison to the ones found at site 41 (6.65). There was a higher medium density value of *Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans* and heterotrophic acidophilic bacteria in water samples at site CM compared to the values recorded from samples at site 41. Medium values of Fe^{2+}, uranium and zinc in samples from the site CM were higher than at site 41. The concentration of fluoride (68.5 mL^{-1}l) and manganese (2.34 mL^{-1}) in water samples from site 41 were above the limits fixed for water bodies in Resolution CONAMA 357. The relative seasonal variation of some variables observed at site CM (low pH values, high densities of *Acidithiobacillus* sp. and heterotrophic acidophilic bacteria) shows that this site is one of the main sites of occurrence of acid mine drainage and action of bioleaching bacteria at UTM.

1. INTRODUCTION

The Osamu Utsumi Uranium Mine is located in Poços de Caldas Plateau (Minas Gerais - Brazil), is an open-pit mine. It was the first mine to extract uranium ore in Brazil, and is located at the Unidade de Tratamento de Minerio – Indistrias Nucleares do Brazil (UTM-INB). Among its facilities and utilities, there are an open-pit, areas of the position of mining tailing physical ore treatment plant, chemical treatment plant for extraction of uranium from tailing and waste basin [1]. Activities of Osamu Utsumi Mine began in 1982 and the operations for ore mining and mineral processing, as well as chemical treatment of pulp for production of uranium concentrate were closed permanently in 1995 [2].

One of the environmental problems related to mining is acid drainage from mine (ADM) and the fundamental condition for it to occur is presence of metallic sulfides such as pyrite (FeS_2) associated with ore, which occurs in the UTM-INB. The action of *Acidophillus* bacteria belonging to the genus *Acidithiobacillus* spp. is known to accelerate the generation of acid mine drainage: the oxidative bacterial action on pyrite produces sulfuric acid and ferric ion. Thus, these products provide a direct solubilization of uranium ore [3]. The acidophilic species *A. ferrooxidans* and *A. thiooxidans* belonging to chemoautotrophic organisms group, that fix CO_2, removing the carbon for the synthesis of new cellular material, obtain energy from oxidation of Fe^{2+} or sulphur compounds reduced or partially reduced (including sulfide, elemental sulfur, and thiosulphate) present in medium [4].

The Antas dam is located in Poços de Caldas Plateau, was built in 1982 with the purpose of supplying water to uranium treatment plant. In addition to supplying water to industrial process, the reservoir also receives treated effluent from the UTM-INB, acid drainage from waste generated from the mine tailings (waste rock) of low content uranium ore [5] In this context, the present study was carried out to determine chemical and microbiological characteristics (Acidophilic heterotrophic bacteria, *A. ferrooxidans* and *A. thiooxidans*) in effluents from uranium mine and in one location at the Antas dam that receives treated effluent from this plant.

2. MATERIALS AND METHODS

2.1 Sampling

Water sampling at the UTM-INB were taken em July and October to 2008 during the morning at two points (CM e 41) and immediately underwent chemical and microbiological analyses. The point CM is located in the Osamu Utsumi mine pit and the point 41 is located in Antas dam , which is a location to discharge of acid effluent treated by INB-UTM and it is a local of interface between the nuclear installation and the environment.

2.2 Physical and chemical measurenments

The following physical and chemical measurements were done: redox potential, pH, conductivity, fluoride, manganese and zinc (atomic absorption spectrophotometry - AAS); uranium and thorium (spectrophotometry with arsenazo III); sulfate (spectrophotometry with barium chloride); $Fe^{2+}$and Fe^{3+} (spectrophotometry with orthophenanthroline). Values for water samples' physical and chemical variables from point 41 (Antas dam) were compared to the limits established in Resolution CONAMA 357 [6] for Class II water. This resolution provides for the classification of water bodies in Brazil, giving reference limits, and establishing conditions and patterns of effluent discharge.

2.3 Microbiological measurements

2.3.1Acidophilic heterotrophic bacteria

To determine the density of heterotrophic acidophilic bacteria the technique of the Most Probable Number (MPN) was used, in a series of five test tubes. Two liquid PSC culture media were prepared; with one value of pH adjusted to 3.0 and the other to 5.0 (modified from [7]) and these means were used for bacteria cultivation. Each sample of water from UTM-INB was subjected to serial dilution in sterile distilled water. Then, 1 mL aliquots from each dilution were transferred to test tubes containing CPS culture medium, in accordance with the above. They were further incubated at 25 °C for 10 days.

2.3.2 *A. ferrooxidans* and *A. thiooxidans*

The three-tube series, most probable number technique, was used to determine the population density of *A. ferrooxidans* and *A. thiooxidans*. 2,5 ml aliquots from each dilution were then transferred to erlenmeyer containing 50 ml T & K medium with ferrous ion (Fe^3+) as a source of energy and erlenmeyer containing the same basal medium with elemental sulphur as an energy source, for the detection of *A. ferrooxidans* and *A.*

thiooxidans, respectively. The prepared samples were incubated at room temperature in shaker (150 rpm) for 21 days[8].

3. RESULTS

Results showed that the average concentrations of manganese (1.06 mg L^{-1}) and fluoride (2.34 mgL^{-1}) at the point of effluent discharge, ie point 41, were above the limits established in Resolution CONAMA 357 for class II water

Water samples from CM point were had highest average concentrations for ferrous ion, ferric iron, uranium, zinc, sulfate, as well as the highest average values of electrical conductivity and oxy-reduction potential, when compared to average values found in water samples collected in point 41 (table 1). Water samples collected at point CM had lower average values of pH (3.7) when compared to the results recorded in the samples of point 41 (6.7).

Table 1 shows that in points CM and P41, high average values were observed for populations of *A. ferrooxidans* (CM = 5.000 $NMPmL^{-1}$ and P41 = 351.5 $NMPmL^{-1}$) when compared with the populations of *A. thiooxidans* (CM = 950 $NMPmL^{-1}$ and P41 = 3 $NMPmL^{-1}$). In experiments for determination of acidophilic heterotrophic bacteria, it was observed that in test with pH 3.0 the presence of bacteria was only detected in water samples from CM point (16.840 $NMPmL^{-1}$). For test results obtained at pH 5.0, the occurrence of acidophilic heterotrophic bacteria was recorded in samples from CM point (9.450 $NMPmL^{-1}$) and P41 (1.000 $NMPmL^{-1}$).

Table 1: Average Values for chemicals variables analyzed in water samples from UTM/INB and environment interface samples. SD = standart deviation.

	CM	**41**	**Res. 357 CONAMA (water class II)**
pH	3.7± 0.4	6.65 ± 0.7	6.0 a 9.0
Eh	524.7 ±524.7	218.7 ± 49.9	
Fe^{2+} (mL^{-1})	0.34 ± 0.02	0.15 ± 0	
Fe^{3+} (mL^{-1})	2.58 ± 0.82	0.19 ± 0.02	
F^- (mL^{-1})	68.5 ± 6.5	2.34 ± 2.3	1.4 (mL^{-1})
Mn (mL^{-1})	88.3 ± 7.4	1.06 ± 0.25	0.1 (mL^{-1})
U mL^{-1})	5.1 ± 0.87	0.07 ± 0.03	0.02 (mL^{-1})
SO_4^{2-} (mL^{-1})	1,848.5 ± 16.5	244.8 ± 200	250 (mL^{-1})
Zn (mL^{-1})	15.2 ± 3	0.02 ± 0	0.18 (mL^{-1})
Af (NMP/ mL^{-1})	5,000 ± 4,300	351.5 ± 492.8	
At (NMP mL^{-1})	950 ± 550	3 ± 0	
Acid-3 (NMP mL^{-1})	16,84 ± 16,160	1.8 ± 0	
Acid-5 ($NMPmL^{-1}$)	9,450 ± 4,550	1,000 ± 1,412.9	

4. DISCUSSION

Values of variables manganese and fluoride in samples of treated effluent collected in Point 41 are above the limits for class II water mentioned in the CONAMA resolution 357 [12]. Such results showed the need to revise the chemical treatment employed. According to Possa & Santos [9] manganese generally precipitates in pH values ranging from 9 to 9.5. Sometimes however, it is necessary to raise the value to 10.5 to promote its complete precipitation.

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Brandenberger et al. [10] found in a sub-tropical reservoir (Corpus Christi - USA), located in a uranium extraction region, uranium concentrations in water, ranging from 0.001 to 0.011 $mg.L^{-1}$. In this study, results showed higher average value of uranium found in water samples from the Antas dam, ie point 41 (0.07 $mg.L^{-1}$), in relation to that found by Brandenberger et al. [10].

Water samples collected at point CM had high average values for oxy-reduction potential and low average values of pH in relation to the results obtained in point 41. In addition, water samples collected at this point showed the highest average values of ferric ion, ferrous ion, sulfate, and fluoride that contributed to the higher electrical conductivity.

High average values of *A. ferrooxidans* were reported in points CM and 41. Colmar & Hinkle, 1947; Temple & Colmar [11] observed that in leaching of sulfide minerals the bacteria *A ferrooxidans* shows to be the most promising in dissolution of these sulfides, being most commonly found in acid water from mine.

In this study, the higher density of *A. ferrooxxidans* was found in point CM, at the same time that it registered the highest average value of ferrous ion and ferric ion. Fowler et al [12] found that the species of *A. ferrooxidans* increased the concentration of ferric ions released into solution during pyrite leaching, when compared to experiments performed in the absence of bacteria.

Preliminary tests showed that in water samples from the point CM was more density of acidophilic heterotrophic bacteria in cultivation medium with pH 3.0 when compared to results obtained at pH 5.0. In water samples collected in point 41, the highest occurrence of acidophilic heterotrophic bacteria was found in pH 5.0. Such results showed that the higher density for acidophilic heterotrophic bacteria were recorded in culture medium that had pH near the pH recorded in the environment, and may indicate that these populations are adapted to environmental conditions of where they occur naturally. Few studies have addressed occurrence of acidophilic heterotrophic bacteria in samples from acid mine drainage. Wichlacz and Uns [13] studied a mine deep in Hollywood with mine acid drainage and reported values of density of acidophilic heterotrophic bacteria from 20 to 250 cells / ml. The densities values of acidophilic heterotrophic bacteria found in samples from point P41 were always lower than those recorded in sample point CM. This result may be related to effects of effluent treated and discharged by UTM-INM, as well as other parameters not evaluated in this study

5. CONCLUSIONS

The relative seasonal variation of some variables observed at site CM (low pH values, high densities of *Acidithiobacillus* sp. and heterotrophic acidophilic bacteria) shows that this site is one of the main sites of occurrence of acid mine drainage and action of bioleaching bacteria at the UTM, and it should be considered a critical spot, facing a possible decommissioning action. The sampling site located at the interface UTM-environment (site 41) was considered susceptible to acid mine drainage and to the activity of bacteria involved in bioleaching of metals.

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