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Biodegradation studies on fatty amines used for reverse flotation of iron ore

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ABSTRACT

Bacteria present in effluents from the process of iron ore flotation were isolated and identified in an attempt to identify the microorganisms responsible for fatty amine degradation. Water samples collected at the tailings dam led to the identification of the bacterial species *Serratia marcescens* as the microorganism responsible for degradation, while in laboratory flotation conditions, a strain of *Enterobacter cloacae* was shown to be the biodegrading agent. Both *S. marcescens* and *E. cloacae* are Gram-negative, non-sporulated, mobile and facultative anaerobic bacteria. Monitoring of the effluent had shown that after 5 days, around 34% of amine was already consumed, increasing to 75% after 10 days; these data are important for testing the reuse of the fatty amines contained in effluents. Biodegradation experiments carried out with *S. marcescens* revealed the significant role of temperature and concentration on the biodegradation rate of the etheramine EDA 3B. For the concentration of 10 mg L^{-1}, amine biodegradation rates are very close at all temperatures. However, as amine concentration increases, the influence of temperature can be better observed, mainly for concentrations of 40 and 60 mg L^{-1}. This isolate will be potentially useful in biotreatment of wastewaters and bioremediation of contaminated soils.

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1. Introduction

Fatty amines are compounds bearing one or more nitrogen atoms attached to an alkyl group of 6 or more carbon atoms. Carbon chains constitute the hydrophobic portion of the amines and have different sizes and degrees of unsaturation, depending on the starting material used. Main sources of such carbon chains are fatty acids derived from vegetable oils and animal fats (Combs, 1985; Riggs and Andress, 1996; Krogh and Sipe, 2001). Fatty amines and their respective salts have been used in important industrial sectors as petroleum additives, oil field chemicals, asphalt emulsifiers, germicides, antiseptic agents, and mining agents, among other things (Visek, 2003).

Amine salts are useful as flotation agents (collectors) and at present are the most important cationic collectors used in iron ore flotation. All silicate flotations in the Brazilian state of Minas Gerais are accomplished using fatty amines and basically consist of the selective separation of quartz particles from iron oxides. In this process, the fatty amine is added to the system being adsorbed on the quartz surface, and both are removed from the system as a foam. However, at the pH where this process occurs (close to 10), both quartz and hematite surfaces are positively charged and, therefore, can adsorb the amine despite its preferential attraction for quartz. In order to avoid competitive iron oxide flotation, starch is used as a depressing agent (Houot, 1983; Leja, 1983; Numela and Iwasaki, 1986).

After amines are used in industrial flotation processes, such compounds are released to the tailings dam and remain there. It is estimated that approximately 5500 tons of amine derivatives per year are used in flotation processes only in Brazil. With the ongoing world demand for iron ore, amine consumption tends to constantly increase. Environmental introduction of high levels of fatty amines can be harmful for aquatic organisms (Saube, 1986; Newsome et al., 1991; Schultz et al., 1991). Aquatic toxicity to the bacteria *Photobacterium phosphoreum* and the crustacea *Daphnia magna* has been evaluated by García et al. (2007). The results showed that the bacteria proved to be more sensitive to the toxic effects of the amine oxide-based surfactants (LC_{50} from 0.11 to 11 mg L^{-1}) than crustacea (LC_{50} from 6.8 to 45 mg L^{-1}). Saube (1986), however, reports toxicity levels for surfactant diestearyldimethyl ammonium chloride as an LC_{50} of 1.5–40 mg L^{-1} for fish and 4.0–100 mg L^{-1} for *D. magna*.

Currently, the behavior of amines in tailings—particularly their degradation and the products formed—is not completely understood, although some studies on amine biodegradation have been reported. Yoshimura et al. (1980) studied the metabolism of several fatty amines by *Pseudomonas* sp, measuring their biochemical

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oxygen demand as an indication of susceptibility to biodegradation. van Ginkel et al. (1995), studying the activity of *Pseudomonas* sp on dodecylamine, reported a bacterial degradation yield of 80% in one week's time. García et al. (2007) studied the aerobic and anaerobic biodegradability of two fatty amine oxides and one fatty amide amine oxide. The three amine oxide-based surfactants tested were readily biodegradable under aerobic conditions, but only the alkyl amide amine oxide was found to be easily biodegradable under anaerobic conditions. Work on *Bacillus polymyxa* (Deo & Natarajan, 1998; Chockalingam et al., 2003) showed this organism could degrade dodecylamine by taking out carbon and nitrogen—required for growth—from the amine and yielding polysaccharides, proteins and fatty acids as metabolic products. In this case, the utilization of microorganisms is highly beneficial since the products formed are much less toxic than their precursors.

Fatty amine degradation constitutes, therefore, an important way to remove these compounds from effluents in order to keep them at low levels in the environment. In the present work, microorganisms present in iron ore flotation effluents were isolated, aiming at identifying the microorganism(s) responsible for amine degradation. Initially, the effluent was characterized in this way. After the identification of key biodegradation microorganisms, further studies were carried out to verify the ideal biodegradation conditions for fatty amines.

2. Materials and methods

2.1. Reagents

All reagents were of p.A. grade. Amines used were Flotigam EDA 3B and Flotigam 2835, both produced by Clariant (Brazil), with the molecular formulae R–O–$(CH_2)_3$–NH_3 (Flotigam EDA 3B), where R = alkyl group with 10–14 carbon atoms, and R–O–$(CH_2)_3$–NH–$(CH_2)_3$–NH_3 (Flotigam 2835), where R = alkyl group with 10 or more carbon atoms.

2.2. Tailings dam

For physico-chemical characterization of tailing water, pH, temperature, dissolved oxygen and conductivity were measured at the same spots where the samples were collected. Samples of water were manually collected by using an aluminum sampler and then transferred to plastic bottles. Containers for sample storage were previously sterilized in the laboratory by using acids, and the containers for sample storage of microorganisms were previously sterilized using an autoclave.

Water samples were filtered with 0.45 μm membrane filters to remove possible interfering materials such as particles in suspension that could interfere in nitrate, nitrite, ammonium and amine analysis. Amine quantification was carried out using bromocresol green methodology and nitrate, nitrite and ammonium by using methodology described by ALPHA (1998).

The dissolved oxygen was measured by use of selective electrode to O_2. Portable oxygen meter model CG867, manufactured by Schott Geräte, was used for measurements in the sampling place, before transferring the water to the sampling bottles. The conductivity was also measured in the sampling place, using a portable device model LF37, manufactured by Leittähigkeit.

Water from five spots was sampled for analysis. Spot 1 (S1) is located on a concentration unit outlet and spot 2 (S2) refers to the place where all of the plant's reject material is discarded. The plant has two tailings dams (effluent from barrage 1 goes to barrage 2), whose inlets were chosen as spots 3 and 4 (S3 and S4), and finally, spot 5 (S5) was the treated water outlet. From spot 5, treated water is sent straight to the natural watercourse.

2.3. Culture medium

The culture medium used for isolation and experimental growth was Bromfield, prepared in distilled water at pH8.0, which contained the following composition: 0.5 g L^{-1} of KH_2PO_4, 0.2 g L^{-1} of $MgSO_4.7H_2O$, 1.0 g L^{-1} of $(NH_4)_2SO_4$, 0.15 g L^{-1} of yeast extract and 20.0 g L^{-1} of glucose (Anand et al., 1996).

The medium was sterilized in an autoclave for 15 min. Due to their volatility, amines and substrates were always added after sterilization.

2.4. Growth and isolation of microorganisms

For microorganism isolation, samples from the tailings dam and also water samples obtained from flotations carried out in the laboratory were used. Aliquots of 10 mL of Bromfield medium and 1 mL of water sample were placed in 250 mL Erlenmeyers and incubated at 30 °C. After two days, 0.5 mL of the broth was transferred to Petri dishes containing 39 g L^{-1} nutrient agar. Petri dishes were incubated under the same conditions: at 30 °C, for 3 days. Upon growth, microorganisms presenting different shapes and colors were individually transferred with the help of a transferring needle to new Petri dishes for the isolation of individual strains.

The flotation experiments were performed in a Denver bench-scale flotation machine with a 2-L cell. The conditions of the experiment were: 1200 rpm, 45% solids, conditioning starch (300 g t^{-1}) for 5 min, conditioning the collector for 1 min and removal of froth. The tests were done at pH10.5.

2.5. Test for amine degradation

In order to verify which colonies were able to degrade ether-amine, the following test was carried out in modified Bromfield medium (only 1% of original described glucose concentration was used): 200 mL of modified Bromfield medium was placed in a 500 mL Erlenmeyer and autoclaved. The experiment was carried out as follows:

$$\text{Flask 1} \rightarrow \text{medium} \rightarrow \text{bacteria inoculation} \rightarrow 24h \rightarrow \text{addition of EDA 3}B(10 mgL^{-1})$$

$$\text{Flask 2(substrate control)} \rightarrow \text{medium} \rightarrow \text{bacteria inoculation} \rightarrow 24h \rightarrow \text{no addition of EDA 3B}$$

$$\text{Flask 3(bacteria control)} \rightarrow \text{medium} \rightarrow \text{no bacteria inoculation} \rightarrow 24h \rightarrow \text{addition of EDA 3B}(10\ mg\ L^{-1})$$

After 7 days, remaining amine was quantified in the samples. The buffer solution of bromocresol green at pH 5 was used for sample quantification; 25 mL of chloroform, 10 mL of bromocresol green buffer solution and 10 mL of sample were placed in a separatory funnel. After agitation, the liquid in the funnel turned yellow. After decanting the chloroform fraction, absorbance reading was taken in the spectrophotometer (Araujo et al., 2009).

2.6. Identification of microorganisms

Microorganisms were identified using classical taxonomy tools. The analyses were performed in the Fundação André Tosello, São Paulo, Brazil. The samples were in contact with agar in glass tubes that were preliminary autoclaved. Identification was based on comparative morphology, physiology and biochemical metabolism

of the strains in comparison with reference data for the strains found in the literature.

2.7. Determination of conditions necessary for bacterial growth

After isolation and identification of bacteria present in the samples from laboratory flotation, two further flotation tests were carried out. In the first experiment, iron ore samples were autoclaved prior to the flotation experiments to assure that no microorganisms would remain in the sample. Flotation was accomplished, and the remaining water solution was kept for 7 days at room temperature. After this period, bacteria isolation, growth and biodegradation experiments were carried out as described previously. In the second experiment, all methodology was identical, except that the iron ore was not added to the sample.

2.8. Biodegradation screening using Serratia marcescens

For evaluation of *S. marcescens* behavior towards etheramine, this strain was fed to different concentrations of amine EDA 3B (10, 40 e 60 mg L^{-1}) in Bromfield medium (200 mL) prepared without glucose. Tests were carried out at several temperatures: 25, 30, 35 and 38 °C. Aliquots (10 mL) were taken after 8, 16, 24, 32, and 36 h of incubation to quantify the amount of amine remaining in the system. The quantification of the amine was performed using a Hewlett Packard UV/Vis spectrophotometer.

2.9. Infrared characterization of degradation product

For characterization of degradation product Infrared Perkin Elmer Spectrum GTX – FTIR equipment was used. The samples were analyzed in KBr cells.

The samples used in the analyses were extracted from the solution used in the degradation tests, with ethylacetate. The organic phase was dried with sodium sulphate, filtered and taken to a rotavapor. The products were purified by chromatography over silica column gel, using, as eluent, hexane/ethylacetate. Column fractions were monitored by CCD (elution system: hexane/ethylacetate 9:1, using vanillin/H_2SO_4 15% under heating as a revealing agent).

CCD was conducted in order to verify products purity. The products obtained presented oily aspect and were analyzed by infrared spectroscopy.

3. Results

3.1. Physico-chemical characterization of the tailings dam water

Table 1 presents the results for physico-chemical analysis of tailings dam water. Values found for dissolved oxygen (DO) in the different sampling locations were low, possibly affecting aquatic life in this environment. Usually the tailings dams collect a huge amount of organic biodegradable material. Aerobic microorganisms present in the barrage water will degrade all this material by using all available oxygen. In this way, the high levels of etheramine, among other biodegradable compounds present, increase oxygen consumption.

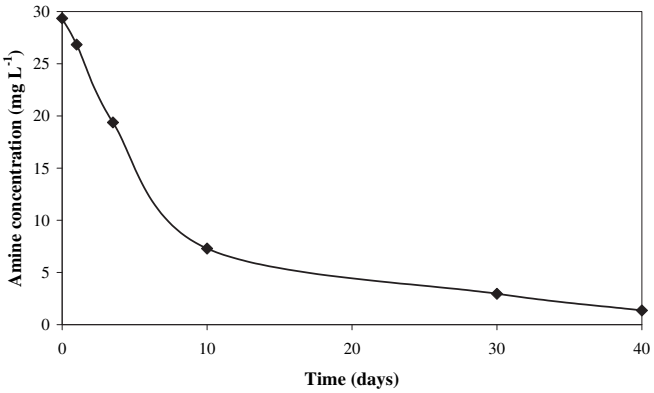

Fig. 1. Degradation of amine in tailings dam water.

High conductivity values were also found and can be explained by the high levels of dissolved ionic compounds coming from the ore itself, and also by the presence of acidic and basic compounds used for water neutralization. The temperature in the studied region remained at the average temperature of 25 °C during the time of sample collection.

Inorganic dissolved nitrogen was measured as nitrite, nitrate and ammonium, to evaluate the conversion of nitrogen present in the etheramines (EDA 3B and F2835) in some of these chemical species. Table 1 shows the results found with detection of low levels of nitrite, nitrate and ammonium. Amine concentration is expected to decrease as the sampling location becomes further from the concentration plant. Amine concentration also decreased with time as shown in Table 1.

Samples of water from a plant unit at a point with the highest amine level were taken to screen amine concentration in the medium. As shown in Fig. 1, amine concentration dropped off as a function of time. It can be observed that initial amine concentration was 29.3 mg L^{-1} and, after 40 days, the concentration dropped to 1.37 mg L^{-1}. It was also verified that, after 5 days, around 34% of amine was already consumed, increasing to 75% after 10 days. The sample contained both etheramines EDA 3B and F2835.

3.2. Isolation of bacterial biodegradation strains

After effluent water plating and careful transfer of single strains to fresh Petri dishes, four different bacterial colonies were isolated. Each of the strains were individually tested in the amine degradation screening, and only one was able to decrease amine concentration. This strain was identified as the Gram-negative *S. marcescens* Bizio, a non-sporulated, mobile and facultative anaerobic species. This species has been used as a reducing agent for metals such as

Table 1
Physico-chemical characteristics of the water in the tailings dam.

Sampled spot	DO (mg L^{-1})	pH	Conductivity (μS cm^{-1})	Nitrite (μg L^{-1})	Nitrate (mg L^{-1})	Ammonium (mg L^{-1})	Amine (mg L^{-1})
S1	NM	NM	NM	38.3	0.31	0.009	13.48
S2	NM	NM	NM	48.1	0.50	0.051	23.88
S3	5.0	8.81	514	12.2	0.21	0.007	7.98
S4	2.5	7.90	349	13.8	0.19	0.029	3.70
S5	4.9	8.16	352	13.3	0.29	0.037	5.86

DO = Dissolved oxygen NM = Not measured S1 = Concentration plant S2 = Plant outlet to tailings dam 1 S3 = Outlet of tailings dam 1 to tailings dam 2 S4 = Tailings dam 2 S5 = Outlet of tailings dam 2 to the water stream.

molybdenum (Shukor et al., 2008) and also for chlorophenol and nitrophenol degradation (Pakala et al., 2006; Singh et al., 2007).

Flotation was also carried out on a laboratorial scale, and remaining solutions containing residual amine were plated. Some strains were isolated from these solutions and tested for amine degradation. The only one able to accomplish this process was identified as *Enterobacter cloacae*, also a Gram-negative, non-sporulated, mobile and facultative anaerobic bacteria. *E. cloacae* has been used in the biodegradation of chlorobenzenes, explosives such as trinitrotoluene (TNT) and pentaerythritol tetranitrate (PETN) (Binks et al., 1996; French et al., 1998; Robertson and Jemba, 2005; Adebusoye et al., 2007), and in the biological control of plant diseases (Nelson and Craft, 1991; Abdel-Salam et al., 2007).

3.3. Biodegradation screening using S. marcescens

Biodegradation results for concentrations of 10, 40 and 60 mg L^{-1} can be seen in Fig. 2 in response to the temperatures tested (25, 30, 35 and 38 °C). Biodegradation rates are initially slow, especially at higher amine concentrations. At 10 mg L^{-1} (Fig. 2), biodegradation rates were very close to each other at all tested temperatures, showing low interference of the temperature parameter at this stage of the process. At 38 °C, the reaction sped up slightly. After 16 h, 70.6% of the amine was already degraded, while at 25 °C, only 45% of amine was consumed. After 24 h, amine concentrations were about the same in all tested temperatures.

At concentrations of 40 and 60 mg L^{-1}, temperature had a significant influence on biodegradation rates, the greatest influence occurring at 38 °C. After 36 h, 93.8% of all amine was already degraded at 40 mg L^{-1}, while at the concentration of 60 mg L^{-1}, degradation reached 91.3%, after the same period of time.

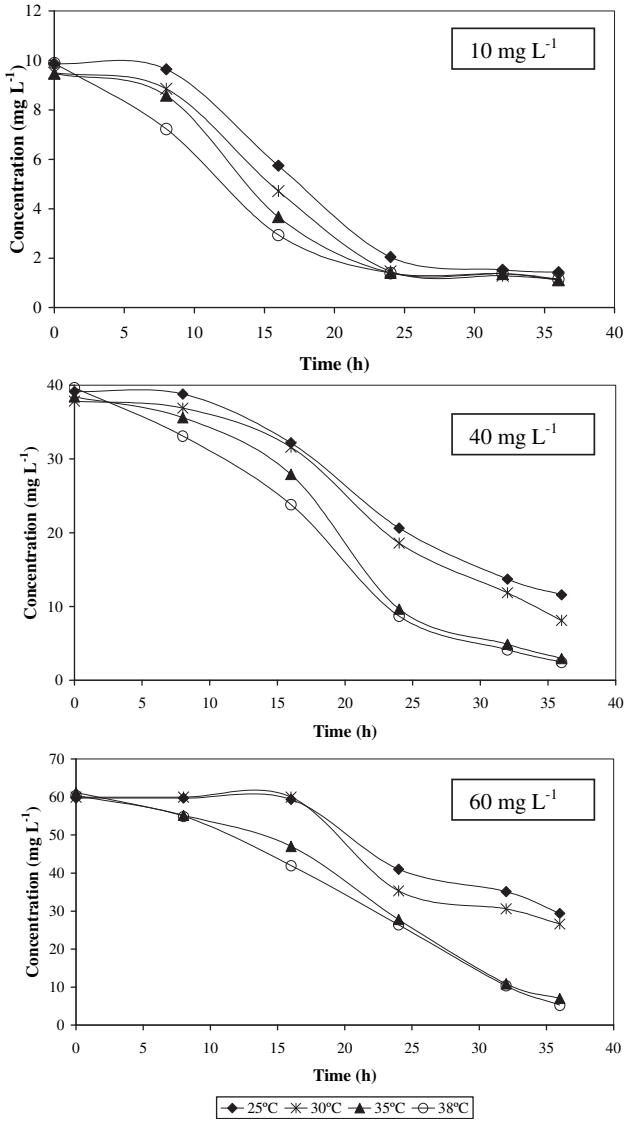

Fig. 2. Biodegradation of EDA 3B as a function of time and temperature at a concentration of 10, 40 and 60 mg L^{-1}.

3.4. Determination of conditions necessary for bacterial growth

Three flotation tests were carried out in the laboratory at distinct conditions to observe the growth of *E. cloacae* in the resulting liquid. Flotations were accomplished using reagents and usual conditions, and bacterial growth was observed. When previously autoclaved iron ore was added to the medium, bacterial growth was observed, but when iron ore was not added to the medium, *E. cloacae* did not grow, showing that minerals present in iron ore are essential for normal development of this strain. Chemical analysis of the iron ore used in the experiments has shown that species like Fe (52.540%) and SiO_2 (32.500%) are present in higher concentrations than elements like P (0.026%), Ca (0.012%), Al (0.210%), Mn (0.017%) and Mg (0.012%).

3.5. Infrared characterization of degradation product

The infrared spectrum of etheramine EDA 3B is shown in Fig. 3 (Transmission, in arbitrary unity a. u.), where a peak can be observed at 3361 cm^{-1}, very typical for an ammonium salt due to the axial deformation of N–H bond. An intense peak can also be seen at 1113 cm^{-1}, characteristic of a C–O–C axial asymmetric deformation on an ether group.

The infrared spectrum of the product recovered after etheramine degradation, shown in Fig. 3, has a different profile. A novel peak is seen at 1731 cm^{-1}, typical of a C=O axial deformation with an absorption frequency very close to the one typical of carbonyl in ester groups or carboxylic acid. In accordance with this, an absorption observed at 1272 cm^{-1} would correspond to an axial deformation of a C–O bond on an ester group. Peaks representing C–H groups were seen in both starting material and products, suggesting an oxidation of the product with a carbon chain remaining. At 1463 cm^{-1}, an angular deformation of CH and at 1378 cm^{-1}, angular deformation of a CH_3 group can be seen.

An oxidative degradation of the initial alkylamine to alkanal, and then to an acid group, has been proposed in the literature (Deo and Natarajan, 1998; van Ginkel et al., 2008).

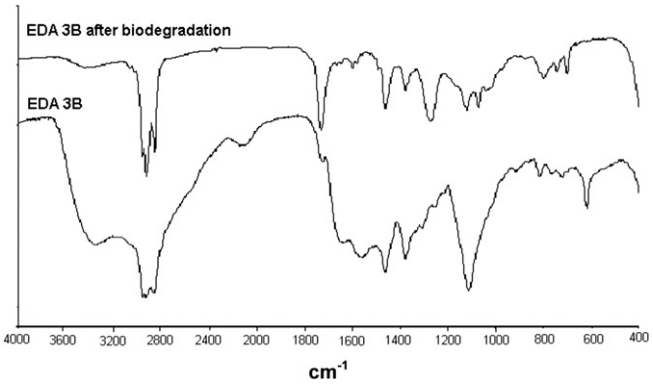

Fig. 3. Infrared spectrum of etheramine EDA 3B before and after biodegradation.

4. Conclusions

The screening for the identification of microorganisms responsible for fatty amine degradation showed that, in field samples, the metabolism of this compound is accomplished by *S. marcescens*, but when flotation is carried out in the laboratory, *E. cloacae* is the organism responsible for amine biodegradation. Studies on bacterial growth showed that the iron ore is a key substrate in the medium, providing the minerals essential for bacterial development. In biodegradation tests using *S. marcescens*, the significant influence of temperature and substrate concentration on the rate of etheramine EDA 3B degradation was observed. For concentrations of 10 mg L^{-1}, rates of etheramine EDA 3B degradation are very similar. However, by increasing amine concentration, the temperature influence is more apparent, mainly at concentrations of 40 and 60 mg L^{-1}.

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