4
Methodology

4.1.
Microorganism and culture media

A strain of *Rhodococcus opacus* obtained from the Brazilian Collection of Environmental and Industrial Microorganisms (CBMAI-UNICAMP) was used in this study. The maintenance solid medium consisted of 10.0 g/L of glucose, 5.0 g/L of peptone, 3.0 g/L of malt extract, 3.0 g/L of yeast extract, 2.0 g/L of $CaCO_3$, and 12 g/L of agar (Mesquita *et al.,* 2003). In addition, the composition of the liquid medium was the same as the solid media with the absence of agar. The bacteria was incubated by 3 days in a rotatory shaker at 125 rpm, temperature of 28 °C and pH of 7.2.

4.2.
Crude biosurfactant extraction and characterization by FTIR

The fluxogram based on lipid hot ethanol extraction studies (Moreau *et al.*, 2003) is shown in the Fig. 3-1. The crude biosurfactant concentrate was stored at 4°C for a maximum of 5 days. In addition, in order to identify the functional groups presented in the crude biosurfactant and the bacteria, infrared absorption spectra was carried out on a Nicolet FTIR 2000 spectrophotometer; a KBr matrix was used as reference and a deuterated triglycine sulphate (DTGS) as detector. The sample was dried at 50 °C and it was properly mixed with the KBr. Similar procedure was found in literature about the study of *Rhodococcus opacus* functional groups (Botero et al. 2008).

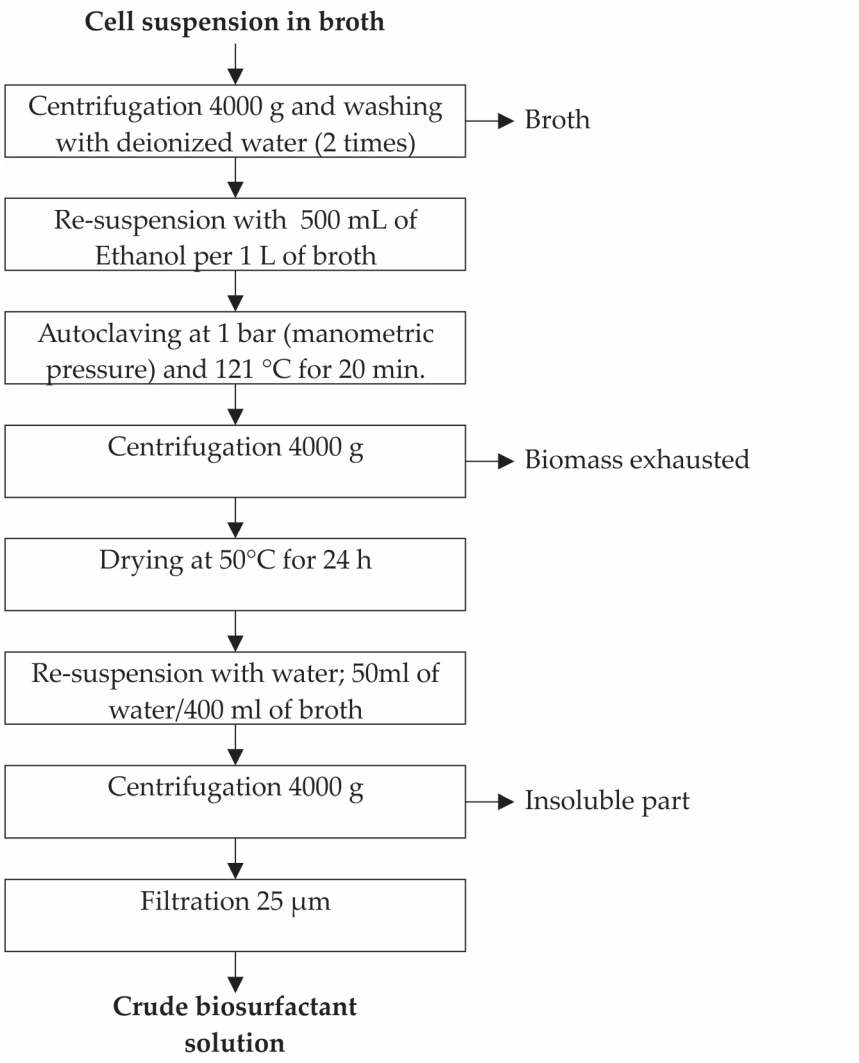

Figure 4-1: Fluxogram of the hot ethanol extraction protocol.

4.3. Mineral characterization

The hematite samples were obtained from the Mineral Technology Center, CETEM, their chemical composition was determined throughout fluorescence spectroscopy. In addition, an elemental composition analysis was carried out by energy-dispersive X-ray spectroscopy. Figure 4-2 shows the hematite sample preparation fluxogram.

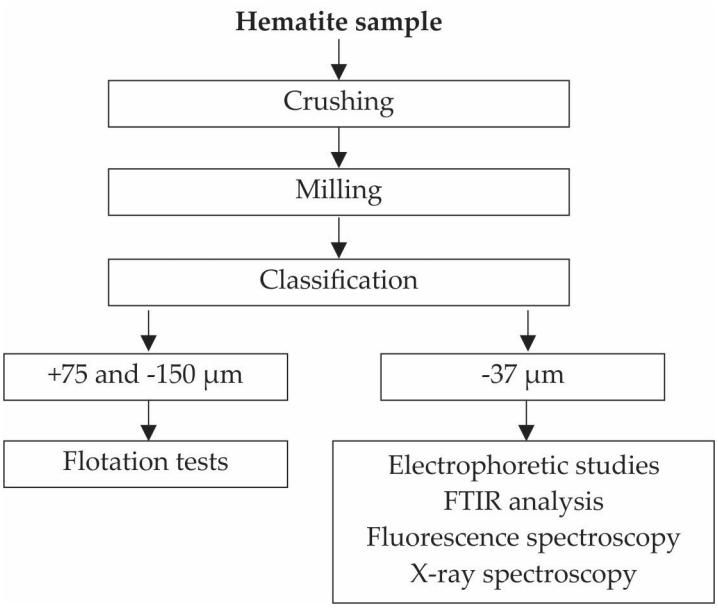

Figure 4-2: Hematite sample preparation.

4.4. FTIR analysis of modified hematite

In addition, hematite samples were analyzed before and after interaction with the biosurfactant, the size fraction was -37 ⇑m. The mineral particles were submerged in a crude biosurfactant solution of 100 ppm for 5 min, after filtration, they were dried out at 40°C for 12 hours, before the FTIR analysis..

4.5. Zeta potential measurements

A Zeta-Meter system 4.0 was used to measure the zeta potential of hematite particles of -37 ⇑m before and after interaction with the crude biosurfactant at a concentration of 120 ppm. In addition, electrophoretic measurements were carried out varying the background electrolyte concentration, similar to the work of Fuerstenau et al. (2005). Details of the experiment can be seen in the table 4-1.

Table 4-1: Experimental plan for the electrophoretic studies.

Mineral	Hematite
Particel size	-35 µm
Mineral suspension	0.1 g dm^{-3}
BS concentration	120 mg dm^{-3}
Background electrolyte	NaCl
Background electrolyte concentrations	10-4 M; 10-3 M; 10-2 M
Voltage	30 V
pH	3; 4; 5; 6; 7; 8.5; 9.5; 11

4.6. Surface tension measurements

The effect of crude biosurfactant concentration on the surface tension of deionized water was studied at neutral pH; varying the biosurfactant concentration from 0 to 250 ppm, with intervals of 50 ppm. The surface tension measurements were performed using the ring method in a Kruss K10 digital tensiometer. The protocol was based on the work of Merma et al. (2013). In addition, to estimate the critical micelle concentration of the crude biosurfactant two tangents at the minimum and maximum surface tension points were drew, corresponding the intersection to the CMC (França *et al.*, 2015).

4.7. Microflotation tests

Microflotation tests were carries out in a Hallimond tube with the bacteria and the biosurfactant. In order to minimize the number of tests and have a relievable correlation between the hematite recovery as a function of the pH and the bioreagent concentration, a multistage factorial design was proposed. A hematite size fraction between +75 and -150 µm, which is a typical size in the mineral flotation process, and an air rate of 35 mL/min were used. Table 4-2 shows the experimental design.

Table 4-2: Multistage factorial design for the floatability tests.

Variable	Units	Type	Stages	Minimum value	Maximum value
Bioreagent concentration	ppm	Independent	7	0	150 (175 for the bacteria)
pH		Independent	5	3	11
Recovery	%	Dependent	-	-	-