Original Article

Apatite flotation using saponified baker’s yeast cells (Saccharomyces cerevisiae) as a bioreagent

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ABSTRACT

Brazil has many mineral resources, one of them being phosphate rocks. Established in 1978, the current main mineral-processing route for the Brazilian phosphate rock igneous deposits consists in a direct flotation using alkaline-saponified fatty acids as collector. Looking for new sources of reactants to be used in mineral processing, commercial baker’s yeast cells (BYC) from Fleischmann were tested. Saccharomyces cerevisiae was chosen because it is relatively easy to grow industrially, has no biological risk, and can be found worldwide. Microflotation experiments were conducted in a modified Hallimond’s tube with high-purity apatite samples in order to investigate the influence of the pH and the biocollector dosage on the apatite recovery. High saponification levels for the BYC occurred many hours after its saponification and an optimal 96 h aging was established. The industrially adopted collector (Flotigam 5806 from Clariant) was used as collector benchmark. Apatite recoveries with the 96h-aged saponified BYC were higher than with Flotigam 5806 (above 95% in all tested pH for dosages of saponified BYC ≥200 mg/L). Good results were found also at pH 7, which could lead to a reduction in the phosphate rock flotation pH in the future. No major differences were visually found between the 96h-aged saponified BYC and the Flotigam 5806 regarding the froth aspect and stability, suggesting that the biocollector possibly acted as a frother.

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1. Introduction

Nowadays, Brazil’s economy is strongly based on agricultural commodities [1]. Its arable territory and tropical climate put Brazil as one of the world’s largest producer of vegetables and meat, and among the world’s six largest agricultural producer and exporter [2]. However, most of Brazilian soils present low fertility and does not fit the crop’s nutritional requirements. Therefore, chemical fertilizers must be used [3,4]. This places Brazil both as one of the biggest exporters of agricultural products and as one of the biggest importers of fertilizers [5].

The three essential elements for plant nutrition are nitrogen, phosphorus, and potassium. The Brazilian main source of phosphorous is phosphate rock from igneous deposits, which requires mineral processing to reduce the content of gangue minerals, such as quartz, chert, clay, feldspar, mica, calcite, and dolomite [6]. Flotation is the major concentration method [7] using alkaline-saponified fatty acids as collectors [8]. The fatty acids-based collectors can be synthetic or extracted from different vegetable species. Conventional chemicals used in other flotation systems, such as petroleum derivate, xanthates, cyanides, and amines, are toxic, non-degradable, and exorbitantly expensive in nature [9].

The application of microorganisms in mineral processing is not relatively new, but is still in the early stages [10]. It started as a solution to actual problems in mineral flotation, such as the depletion of high-grade ores, the necessity of concentrate fine and ultrafine particles, and to the constant quest of environmental friendly reagents [11]. For the authors, the term biofloation turned out attractive not only as the solution of the problems mentioned before, but also to its technological potential and mineral selectivity. Biofloation exploits the differences in surface characteristics of solids suspended in an aqueous medium, adjusting and controlling their surface energies and interfacial tensions using microorganisms with hydrophobic properties [10]. In their opinion, a significant increase in the researches related to the electrophoretic behavior of minerals/bacteria systems, thermodynamic adhesion of different bioreagents onto mineral surface, and biofloation kinetics, to name a few, are required in order to have a better understanding of the fundamental aspects of mineral biofloation. Mineral biofloation encompasses the principles and methods used in mineral flotation using microorganisms as flotation reagents.

The attachment of bacteria on the mineral surface occurs by a variety of methods such as the secretion of a slime layer, hydrophobic interactions, extracellular polymeric substances and other surface appendages, protein-binding receptors, chemical attachment, physical adsorption, and with the use of glycolax [12]. The feasibility of adherence of a microorganism to a mineral surface will depend on the charge characteristics as well as the hydrophobicity of both mineral surface and microorganism [13]. Microorganisms already identified for their potential application in microbial-induced flotation and flocculation are considerably diverse and include hydrophobic and surfactant producing species of bacteria, fungi, and some species of yeast [14].

Saccharomyces cerevisiae (SC) commonly refereed as baker’s yeast cells (BYC) is widely used to produce bread. Lipids, proteins, and two types of polysaccharides (α-mannan and α-glucan) mainly compose it [15]. SC had been used in biofloation before, but only after submitted to adaptation for the targeted minerals and without being saponified. For example, calcite was successfully separated from quartz using mineral-adapted cells of SC [16]. BYC had potential to be used as a biocollector in mineral flotation mainly not only because of its performance as biocollector, but also because of its price, relatively easy to grow industrially, and high biological safety and availability. In this paper, the effect of the pH, dosage, and aging of the saponified BYC on the apatite recovery was studied. The results show a possible use of saponified BYC as apatite collector.

2. Materials and methods

2.1. Mineral samples

Blue crystals of igneous apatite from Ipira-BA, Brazil, were comminuted in a jaw crusher followed by a ball mill and granulometrically separated through wet sieving using a Tyler sieving series for 15 min. The samples were then dried in an oven at 60°C for 24 h. A ferrite magnet with a field of 2 kG was used to remove any possible contamination from the previous stages. The mineralogical phase characterization was performed using an X-ray diffraction Rigaku Miniflex II with Cu tube and Cu Kα radiation, graphite monochromator, operating at 30 kV/15 mA, angular step of 0.02°, and acquisition time of 2 s. The sample chemical composition was determined using an X-ray fluorescence spectrometer PANalytical AXIOS MAX series DY 5001. The sample images were acquired with an SEM JEOL JSM-6610 coupled with EDS probe Thermo Scientific NSS Spectral Imaging. The particle size analyzed was performed wet with the addition of Na2P2O7 (1 g/L) as dispersant agent and tap water using a laser diffraction particle size analyzer Sympatec HELOS. The apatite zeta potential was measured at the pH range from 3.5 to 12.5 using distilled water and KCl (10−3 mol/L) as indifferent electrolyte using a Zetasizer Malvern Nano ZS90.

2.2. BYC preparation and saponification

Dry BYC manufactured by Fleishmann were locally purchased in 100 g packages, containing the cells and emulsifying agents. The BYC was solubilized in distilled water to produce a solution of 20 mL/g of BYC. The solution was centrifuged at room temperature for 5 min at 3400 rpm in order to remove the emulsifying agents. The obtained pellets were suspended in distilled water. The centrifugation loop was repeated three times, under the same conditions described. The final pellets were dried in oven at 70°C for 48 h. The processed BYC were disaggregated using a stainless steel mortar and pestle.

A solution of 100 g of processed BYC and 600 mL of distilled water (pH 7) was added into a 1 L glass beaker under magnetic stirring at 130 rpm at room temperature until there was complete solubilization of the solids. The BYC saponification was performed by the addition of 400 g of NaOH solution (10%, w/v) to the solution.
The saponification index (SI), which is the mass of potassium hydroxide (in mg) required to neutralize the fatty acids resulting from the complete hydrolysis of 1 g of fat, was used to study the BYC saponification reaction kinetics. Previous results indicated that BYC saponification kinetics was slow, requiring the aging of the solution before its use as a collector. To test the hypothesis that BYC requires some aging to achieve high apatite recoveries, saponified BYC solutions were prepared and their SI was measured. The methodology adopted for the SI measurement was adapted from [17], where 100 mL of saponified BYC was stirred with 25 mL of methanolic KOH (1 N) and the excess of the mixture was then titrated with HCl (0.5 N) using phenolphthalein as indicator.

2.3. **Microflotation tests**

Microflotation tests were carried out at room temperature (around 25 °C) in a modified Hallimond tube (addition of an extender between the bottom and the upper part of the tube, in order to reduce the effects of hydraulic entrainment) with 320 mL of internal volume. The airflow was kept at 40 cm³/min and the air pressure at 10 psi in order to minimize the hydraulic entrainment. The mineral mass used in each test was 1.0 g with particle size between +106–150 μm (+150–100 #). The conditioning time was 7 min and the flotation time was 1 min.

A first experimental campaign was carried out in order to study the influence of the saponified BYC aging on the apatite recovery, at pH 9 and biocollector dosage of 400 mg/L. Both flotation test and SI measurement were carried out at the same time. With the results of the first experimental campaign, it was established an optimal aging period for the BYC.

Five different dosages of aged saponified BYC (50, 200, 400, 600, and 800 mg/L) and four different pH values (7, 8, 9, and 10) were tested. The alkaline pH was chosen to mask the pH industrially adopted in Brazil for phosphate rock flotation (normally around 10–10.5). The apatite flotation in neutral pH (7) was also tested, which could lead to a cost reduction with pH regulators. BYC has a theoretical lipid mass composition of 12.6% (db) [15]. Therefore, the tested dosage range is similar to dosages of fatty acids collectors tested by other authors in apatite flotation [7,18,19]. Flotigam 5806 from Clariant, a synthetic mix of fatty acids highly used in phosphate rock flotation worldwide, was used as a collector benchmark and it was prepared following the manufacturer recommendations. Three different pHs (8, 9, and 10) and four dosages (2.5, 5.0, 7.5, and 10.0 mg/L) were tested. The neutral pH was not tested because Clariant suggests that Flotigam 5806 must be used at a pH range from 8 to 10, with dosages between 50 and 300 g/ton. The slurry pH was adjusted with HCl and NaOH, both at 1%. Distilled water was used throughout the experiments. All tests were performed in triplicate.

### Table 1 - XRF results for the apatite samples (in %).

<table>
<thead>
<tr>
<th>CaO</th>
<th>P₂O₅</th>
<th>K₂O</th>
<th>SiO₂</th>
<th>SO₃</th>
<th>I</th>
<th>Cl</th>
<th>Na₂O</th>
<th>ThO₂</th>
<th>Fe₂O₃</th>
<th>MnO</th>
<th>BaO</th>
<th>SrO</th>
</tr>
</thead>
<tbody>
<tr>
<td>54.02</td>
<td>38.49</td>
<td>4.20</td>
<td>1.10</td>
<td>0.63</td>
<td>0.39</td>
<td>0.34</td>
<td>0.27</td>
<td>0.18</td>
<td>0.11</td>
<td>0.11</td>
<td>0.10</td>
<td>0.06</td>
</tr>
</tbody>
</table>

3. **Results and discussion**

Table 1 shows the XRF results of the apatite samples. Regarding the chemical composition, it is possible to assume that apatite samples had around 95.5% of purity, with presence of traces of Cl, indicating a possible presence of fluorapatite.

Fig. 1 shows the SEM images (BEC and SEI) of the apatite sample. It is possible to notice the association of apatite with a secondary acicular mineral phase. EDS results are shown for two points, one on the acicular phase (Fig. 1c) and the other on the apatite phase (Fig. 1d). Fig. 1d shows that the main mineral phase is composed by Ca, P, and O, as expected for apatite (Ca₅(PO₄)₃). Since Cl and F were also observed, this could be an indication of the existence of fluorapatite and chlorine-apatite in the sample (Ca₅(PO₄)₃(F,Cl)). The existence of the fluorine–chlorine–apatite phase agrees with the XRF results and does not affect its flotation in anyway. The acicular phase (Fig. 1c) could not be correctly identified based only on this result. The presence of Ag in the EDS spectra of the secondary mineral phase is rather unusual considering its association with igneous apatite.

Fig. 2 shows the XRD diffraction pattern for the apatite sample. A peak match correlation of 95.75% was obtained between the sample and fluorapatite reference diffraction pattern 96-901-3556. A semi-quantitative analysis using the Reference Intensity Ratio method showed that the sample was composed by 100% of fluorapatite. When the apatite sample XRD pattern was compared with chlorine-apatite (references 96-901-3555 and 96-901-3557), the peak match correlation was only 23.06%, confirming that the sample was mainly composed by fluorapatite.

Regarding the Zeta potential measurements (Fig. 3a), the apatite isoelectric point (IEP) was not detected in the tested pH range (3.5–12.5) and the apatite surface charge remained negative in all measurements. According to the literature, apatite has negative superficial charge in a solution with alkaline pH, which agrees with the results found, and the adsorption of anionic collectors on its surface is due to chemisorption [20]. Fig. 3b presents the granulometric analyses of the apatite sample. It is possible to notice the presence of particles below 103 μm (68.3%) and above 175 μm (20%), even though the samples had been size classified through wet sieved (+106–150 μm). This fact can be explained by the imperfection of the sieving process and by possible mistakes in the laser diffraction analysis related to the particle morphology.

Fig. 4 shows the influence of the saponified BYC aging on both collector’s SI and average apatite recovery. During the aging process, no visual or aromatic changes in the collector was detected. The BYC SI increased abruptly in the first 24 h after the saponification and did not stabilize during the testes, even after 144 h. On the other hand, the apatite recovery stabilized around 94% when using saponified BYC with
Figure 1 – SEM images for apatite sample: (a) BEC and (B) SEI image at \( \times 750 \) magnification. EDS results for points (c) 1 and (d) 2 marked in (a).

Figure 2 – Apatite sample X-ray diffraction.

Figure 3 – (a) Zeta potential and (b) particle size distribution for the apatite sample.
aging ≥96 h (selected as the optimal aging). The results indicate that even though saponification reactions still occur after 96 h, apparently they do not have major influence on apatite recovery. For the 96 h-aged solution, the average apatite recovery was 94.9 ± 0.4% and the SI was 76.7 ± 1.4 mg KOH/g. The found SI was lower than the observed for fruit’s oil used as fatty acids sources in apatite flotation, such as 192 ± 3.5 mg KOH/g for pequi [18] and values ranging from 196 to 212 mg KOH/g for Amazon fruits [19], but the apatite recovery was as high as found by the same authors. It is possible to notice that apatite recoveries were considerably low in the first for 24 h aged saponified BYC (around 35%), indicating that freshly prepared saponified BYC should not be used in flotation. A major increase in the apatite recovery was noticed after 48 h of aging when compared with the results for 24 h (2.19 times higher). A linear correlation between BYC SI and the apatite recovery was obtained with a Pearson correlation coefficient of 93% (which is inside the 95% confidence interval).

Apatite flotation test results using the 96 h-aged saponified BYC and the Flotigam 5806 are presented in the Fig. 5. Since the error bars for the biocollector presented in the figure are too small, the complete data set (average apatite recovery and standard deviation) for the 96 h-aged saponified BYC test is presented in Table 2. A non-linear fitting (exponential decay) was used in order to fit the experimental data and to model the recovery stabilization plateau. The occurrence of such plateau is a regular feature in the flotation of many minerals. In sphalerite flotation using Bacillus circulans as collector, for example, the plateau was found for biocollector dosages above 2 mg/g [12].

Average apatite recoveries above 95% were obtained for the 96 h-aged saponified BYC at dosages ≥200 mg/L in all tested pHs, including pH 7. This is a remarkable result, since fatty acids, the main industrially adopted collector for apatite in Brazil, usually do not show high recoveries at this pH. An industrial flotation of phosphate rock performed at pH 7 could lead to cost reduction with pH regulators and to the reduction of the electrolyte load in the slurry (especially regarding Na+). The pH influence on the apatite recovery with the 96 h-aged saponified BYC was evident for the dosage of 50 mg/L. The pH increases from 7 to 10 promoted a reduction in the average apatite recovery with the biocollector from 48.4 ± 0.2 to 8.4 ± 0.2, respectively.

For the Flotigam 5806, the best results were obtained for dosages ≥5.0 mg/L. The exception was at pH 10, in which a high average apatite recovery was also obtained for the dosage of 2.5 mg/L. Lower dosages of Flotigam 5806 were required to produce similar apatite recovery when compared to the 96 h-aged saponified BYC. However, high apatite recoveries were obtained with the 96 h-aged saponified BYC in all tested pHs, except on pH 10, where the results for the two collectors were similar.

The pH influence on Flotigam 5806 was also verified. An increase in the pH produced an increase in the apatite recovery when using Flotigam 5806, which agrees with industrial data and explains the adoption of pH around 10 in the phosphate rock flotation. On the other hand, for the 96 h-aged saponified BYC an increase in the pH produced a decrease in the apatite recovery.

The high apatite recovery could also be related with other constituents of the BYC. S. cerevisiae potential for carboxylic acid production had been suggested in the literature [21] and the interaction between some of the carboxylic acid’s functional groups present in the SC and the apatite surface cannot be neglected. Proteins and nucleic acids were present in the saponified BYC solution since no cellular constituent separation was performed, and the adopted preparation and saponification methodology possibly exposed the cell’s DNA, which has reported biocollector properties [12].

Saponified fatty acids are well known as frother and ionized species can generate excessively stable froths [8]. No major differences were visually found between the 96 h-aged saponified BYC and the Flotigam 5806 regarding the froth aspect and stability, suggesting that the biocollector also acted as a frother.

4. Conclusions

A first attempt to use saponified BYC (S. cerevisiae) as a biocollector in apatite flotation was carried out. High-purity apatite crystals were prepared and characterized. The influence of the biocollector aging in the apatite recovery was investigated and a linear correlation, with statistical significance, was found. The optimal aging of 96 h was established. Average apatite recoveries above 95% were obtained with dosages ≥200 mg/L of 96 h-aged saponified BYC. The average apatite recovery was not affected by changes in the pH for the biocollector at this dosage (no statistically significant difference was found). As expected, the average recoveries with the industrially adopted collector (Flotigam 5806) were around 93%. However, higher apatite recoveries were obtained with 96 h-aged saponified BYC in almost all tests carried out. Regarding the froth, no major differences were visually found between the two tested collectors.

An inverse behavior regarding the pH change was observed between the Flotigam 5806 and the biocollector. An increase in the pH produced and the increase in the apatite recovery when using the Flotigam 5806 and a decrease when using the 96 h-aged saponified BYC. The pH reduction in the industrial flotation of apatite (from 10 to 7) could be an important step...
forward for the mineral industry. The reduction in the use of pH modifiers directly reduces operational costs, the electrolyte load in the slurry, and could also have beneficial effects on the recirculating water quality.

More tests with phosphate rock are required to ensure the potential use of saponified BYC as an industrial bio-collector. Proteins and nucleic acids were present in the saponified BYC solution and the interaction of these constituents with the apatite surface is not clear so far. Therefore, more studies are required to understand the adsorption mechanisms.

5. Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

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Figure 5 – Apatite flotation results at pH (a) 7, (b) 8, (c) 9, and (d) 10 using 96 h-aged saponified BYC and Flotigam 5806.

Table 2 – Apatite flotation data set using 96 h-aged saponified BYC.

<table>
<thead>
<tr>
<th>Dosage (mg/L)</th>
<th>pH 7</th>
<th>pH 8</th>
<th>pH 9</th>
<th>pH 10</th>
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<tbody>
<tr>
<td></td>
<td>Avg. Re. (%)</td>
<td>Std. Dev. (%)</td>
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<td>Std. Dev. (%)</td>
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<td>47.26</td>
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REFERENCES


