Original Article

https://doi.org/10.4322/2176-1523.20181525

APATITE BIOFLOTATION USING SPENT YEAST (*SACCHAROMYCES CEREVISIAE*) CELLS AS COLLECTOR

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Abstract

The main source of phosphate in Brazil are igneous rocks, which need to be concentrated through froth flotation to separate phosphate ore from the other minerals. Searching for new sources of reactants to be used in mineral processing, spent yeast cells (SYC), from a local brewery was tested. Saccharomyces cerevisiae was choose because its relative easy industrial grow, has no biological risk and can be found worldwide. Microflotation experiments were conducted in a modified Hallimond's tube with high purity apatite mineral samples at pH 10 in order to investigate the influence of the collector dosage in the mineral recovery. The results showed that despite SYC has had lower recovery than collectors industrially adopted, it has a potential future use in mineral flotation as a cheap, biodegradable, eco-friendly, and sustainable reagent with no acquisition cost since the brewery complex is located less than 30 km from Catalão and Ouvidor mine sites in Brazil.
Keywords: Bioflotation; Spent yeast cells; Flotation reagent.

BIOFLOTAÇÃO DE APATITA UTILIZANDO LEVEDURA CERVEJEIRA (*SACCHAROMYCES CEREVISIAE*) SAPONIFICADA COMO COLETOR

Resumo

A principal fonte de fosfato no Brasil é a rocha ígnea, que precisa ser concentrada através da flotação no intuito de separar o minério de fosfato dos outros minerais. À procura de novas fontes de reagentes a serem utilizadas no processamento de minerais, as células de levedura de cerveja gastas (SYC) provenientes de uma cervejaria local foram testadas. A espécie Saccharomyces cerevisiae foi escolhida devido ao seu fácil crescimento industrial, não possuir risco biológico e poder ser encontrada em todo o mundo. Experimentos de microflotação foram conduzidos em um tubo de Hallimond modificado em pH 10 com amostras de apatita de alta pureza a fim de investigar a influência da concentração do coletor na recuperação do mineral. Os resultados mostraram que apesar das SYC terem uma recuperação menor do que coletores adotados industrialmente, estas possuem potencial para uso futuro na flotação mineral, se apresentando como um reagente barato, biodegradável, ecológico e sustentável, sem custo de aquisição, uma vez que o complexo da cervejaria fica a menos de 30 km das minas nas cidades de Catalão e Ouvidor, Brasil.
Palavras-chave: Bioflotação; Levedura de cerveja gasta; Reagente de flotação.

1 INTRODUCTION

The Brazil's economy is strongly based on agricultural commodities [1]. Its arable territory and tropical climate put us as one of the world's largest producer of vegetables and meat, setting us among the world six largest agricultural producer and exporter [2]. However, most of Brazilian soils present low fertility and fit the nutritional requirements of the plants need chemical fertilizers must be used [3,4]. This places Brazil both as one of the biggest exporters of

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agricultural products and as one the biggest importer of fertilizers [5].

The three essential elements of plant nutrition are Phosphorus, Nitrogen and Potassium. The first one is absorbed mainly in the form of phosphate ions and may present problems of fixation in the soil, becoming unavailable to the plants. It is an essential component in the energetic metabolism of cells, participates in the formation of RNA and DNA, and others biomolecules [6]. The Brazilian main source of phosphorous is phosphate rock, which requires mineral processing to reduce the content of accessory minerals including quartz, chert, clay, feldspar, mica, calcite, and dolomite [7]. The principal separation method mineral processing for separation and concentration of phosphates is the froth flotation [8] and fatty acids soaps derived from different vegetable species are utilized as collectors [9].

According to Behera and Mulaba-Bafubiandi [10], conventional chemical reagents used on flotation process such as petroleum derivate, xanthates, cyanides, amines etc., are toxic, non-degradable and exorbitant in nature. The use of alternative reagents has become a new trend in mineral processing in the last years, receiving attention from industries and government alike. These reagents can improve froth flotation recovery and be more eco-friendly. The use of other industries sub products has the potential to solve environmental problems, such as discharge of organic matter in rivers and soils, and can be profitable by aggregating economic value to industrial residues.

Spent yeast cells are a sub product from alcohol beverage industries, such as beer and wine. The alcoholic fermentation process is accompanied by yeast cells growth and in some cases is possible to reuse the yeast cells from previous fermentation on the next fermentation process. However, at some point, the generated biomass exceeds the inoculum rate and the yeast cells become a residue. SYC are used nowadays as animal feed supplement, but with a low aggregate value, mainly because the yeast biomass production exceeds the demand [11]. The yeast cell main composition is lipids, proteins and two types of polysaccharides, a mannan and a glucan [12]. The lipids react with sodium hydroxide forming soluble lipids [13], named as alkaline saponification.

According to Dwyer et al. [14], the microorganisms already identified for their potential application in microbial induced flotation and flocculation of mineral samples are considerably diverse and include hydrophobic and surfactant producing species of bacteria, fungi and some species of yeast. Microorganisms such as these interact with minerals to effect surface changes by three different mechanisms: first, via attachment of the cell to the mineral surface; second, microorganisms may catalyse oxidation or reduction reactions that modify the mineral for the generation of energy for growth; or third, extracellular proteins and polysaccharides produced by the microorganism may interact with the mineral surface. According to Rao and Subramanian [15], the microorganisms adhere to mineral surfaces for various reasons and the feasibility of adherence of a microorganism to a mineral surface will depend on the charge characteristics as well as the hydrophobicity of both the mineral surface and the microorganism.

The purpose of this work was a preliminary demonstration of the potential use of SYC after saponification as collector in apatite froth flotation, replacing the commonly used fatty acids. This approach seems to be the first related in the specialized literature, opening a new way possible use yeast cells from brewery.

2 MATERIALS AND METHODOLOGY

2.1 Spent Yeast Cells (*Saccharomyces cerevisiae*)

Cervejaria Catalão, a brewing industry located in the city of Catalão, Goiás, Brazil, provided SYC. Brewer's wort samples containing *Saccharomyces cerevisiae* cells were collected from the bottom of the conical fermenter and packed in 18 L polypropylene buckets. The samples was centrifuged at 3400 rpm at room temperature for 10 minutes in order to obtain a mass of cells separated from the wort. The obtained pellets were suspended in distilled water. The centrifugation loop was repeated three times, under the same conditions described. The final pellet was dried in an oven at 70 °C for 48 hours. The obtained mass was disaggregated using a stainless steel mortar and pestle.

2.2 Mineral Characterization

Apatite samples were comminuted in a jaw crusher followed by a ball mill, and granulometric separated through wet sieving using a Tyler sieving series during 15 minutes. The samples were then dried in an oven at 60 °C during 24 hours. A ferrite magnet with field of 2 kG was used to remove and possible contamination from the previous stages. The mineralogical phase characterization was performed at UNIFESSPA using an X-ray Diffractometer Empyrean from PANalytical with graphite monochromator, operating at 30 kV/15 mA, angular step of 0.02° and acquisition time of 2 seconds. The sample chemical composition was determined at Copebras S/A laboratory using an X-ray Fluorescence spectrometer AXIOX MAX series DY 5001 from PANalytical. Samples images were acquired with a SEM JSM-6610 from Jeol coupled with EDS probe from Thermo Scientific NSS Spectral Imaging at Labmic /UFG. The particle size was performed wet with addition of Na_2P2O_7 (1 g/L) as dispersant agent and tap water at IFAC/TUC using a HELOS laser diffraction particle size analyser from Sympatec. The apatite zeta potential was measured at LPI/EM/UFOP from pH 3.5 to 12.5 with distillate water and having KCl at

10^{-3} mol/L as indifferent electrolyte using a ZS90 Zetasizer Nano from Malvern.

2.3 SYC Saponification

The saponification was performed according to the described by Pacheco et al. [16] for fatty acids saponification, with minor modifications. A solution of 5.0 g of SYC and 30 mL of distilled water (pH 7) was added to a 250 mL glass becker under magnetic stirring. In order to facilitate the solubilization and the reaction between NaOH solution (10% w/v) and SYC the temperature during the gelatinization was kept at 40°C. The initial pH of the solution dropped since the SYC tends to turn the medium acid. After the pH stabilization, a titration with 1.0 mL of NaOH solution (10%) was started until a total of 30 mL of NaOH solution was added. Every addition of NaOH was followed by a brief pause (between two and five minutes) to allow the solution pH stabilization. Distilled water was added to the solution to reach 100 mL in order to obtain a final solution with dosage of 50 g/L of saponified YSC.

2.4 Microflotation Tests

Microflotation tests were performed on a modified Hallimond tube (addition of an extender between the bottom and upperparts of the tube in order to reduce the effects of hydraulic entrainment) with 320 mL of internal volume, at room temperature (around 25 °C). To minimize the hydraulic entrainment air flow was kept at 40 cm^3/min, pressure at 10 psi, apatite sample at the size range of +106-150 μm (+150-100 #), as suggested by Guimarães et al. [17]. The apatite mass used in each test was 1.0 g. Twelve different dosages (from 100 to 800 mg/L) of SYC were tested. The conditioning time was 10 minutes and the flotation time 2 minutes. Distillate water was used throughout the experiments. All tests were performed in triplicate. Previous results showed that high apatite recovery in microflotation tests using saponified vegetable oils were obtained at pH 10 [8,18,19]. The flotation pH was adjusted to 10 with hydrochloric acid and sodium hydroxide, both at 1%.

3 RESULTS AND DISCUSSION

Table 1 shows the results of X-ray fluorescence of apatite sample. The follow oxides were analysed but not found in the apatite sample: Co_3O_4, Ta_2O_5, Cr_2O_3, CuO, HfO_2, MgO, Nb_2O_5, TiO_2, WO_3, Y_2O_3, Yb_2O_3, ZnO, and ZrO_2. Since pure apatite contains amounts CaO and P_2O_5 of 55.07 and 41.82%, respectively, is possible to estimate that the apatite sample has 95.5% of purity, regarding its chemical composition.

Figure 1 shows scanning electron microscope images from apatite samples using backscattered and secondary electron imaging. It is possible to notice the association of apatite with an acicular mineral phase. Energy-dispersive X-ray spectroscopy (EDS) results are show for two points, one on the acicular phase (Figure 1c) and the other on the apatite phase (Figure 1d). Figure 1f shows that the main mineral phase is composed by Ca, P and O, as expected for apatite ($Ca_5(PO_4)_3$). Since Cl and F was also observed this could be an indication of the existence of fluorapatite and chlorine apatite in the sample ($Ca_5(PO_4)_3(F,Cl)$). The existence of the phase Fluorine-Chlorine-Apatite does not affect its flotation in anyway. The acicular phase (1e) could not be correct identified based only on this result.

Figure 2 shows the X-ray diffraction results for the apatite sample. Since the objective of the XRD was to confirm the mineral phases present in the sample, a simple search in the Bragg peaks allowed the confirmation of the apatite phase. Nevertheless, a refinement step of the results was performed to check the sample composition. The found results did not indicate the presence of other mineral phase in the sample.

Regarding the Zeta potential measurements (Figure 3), the apatite zero charge point was not detected in the tested pH range (3.5 to 12.5) and superficial charge was negative. According to Oliveira [20] apatite has negative superficial charge at alkaline pH and the adsorption of anionic collectors on its surface is due chemisorption.

Figure 4 presents the granulometric analyse of the apatite sample using HELOS analyser. Even though the samples had being wet sieved (+ 106-150 μm), it is possible to notice the presence of particles bellow 103 μm (68.3%) and above 175 μm (20%). This fact can be explained by the imperfection of the sieving process.

Figure 5 shows the apatite recovery as a collector dosage in the microflotation tests at pH 10. The higher recovery (approximately 40%) was obtained with 600 mg/L. Silva et al. [8] performed apatite microflotation tests using saponified oil from *Jatropha curcas* L. and the industrially adopted collector Flotigam 5806 from Clariant®. An average apatite recovery around 90% was obtained for both reagents at pH 10 and collector dosage of 7.5 mg/L. The discrepancy in the dosages employed (7.5 to 600 mg/L) can be explained due the fact that no separation of the SYC cellular constituents was performed, besides the membrane lipids.

By doing this, the preparation cost of the SYC was very low, but proteins, carbohydrates, and genetic material

Table 1. XRF results in percentage for apatite sample

CaO	P_2O_5	K_2O	SiO_2	SO_3	I	Cl	Na_2O	ThO_2	Fe_2O_3	MnO	BaO	SrO
54.02	38.49	4.20	1.10	0.63	0.39	0.34	0.27	0.18	0.11	0.11	0.10	0.06

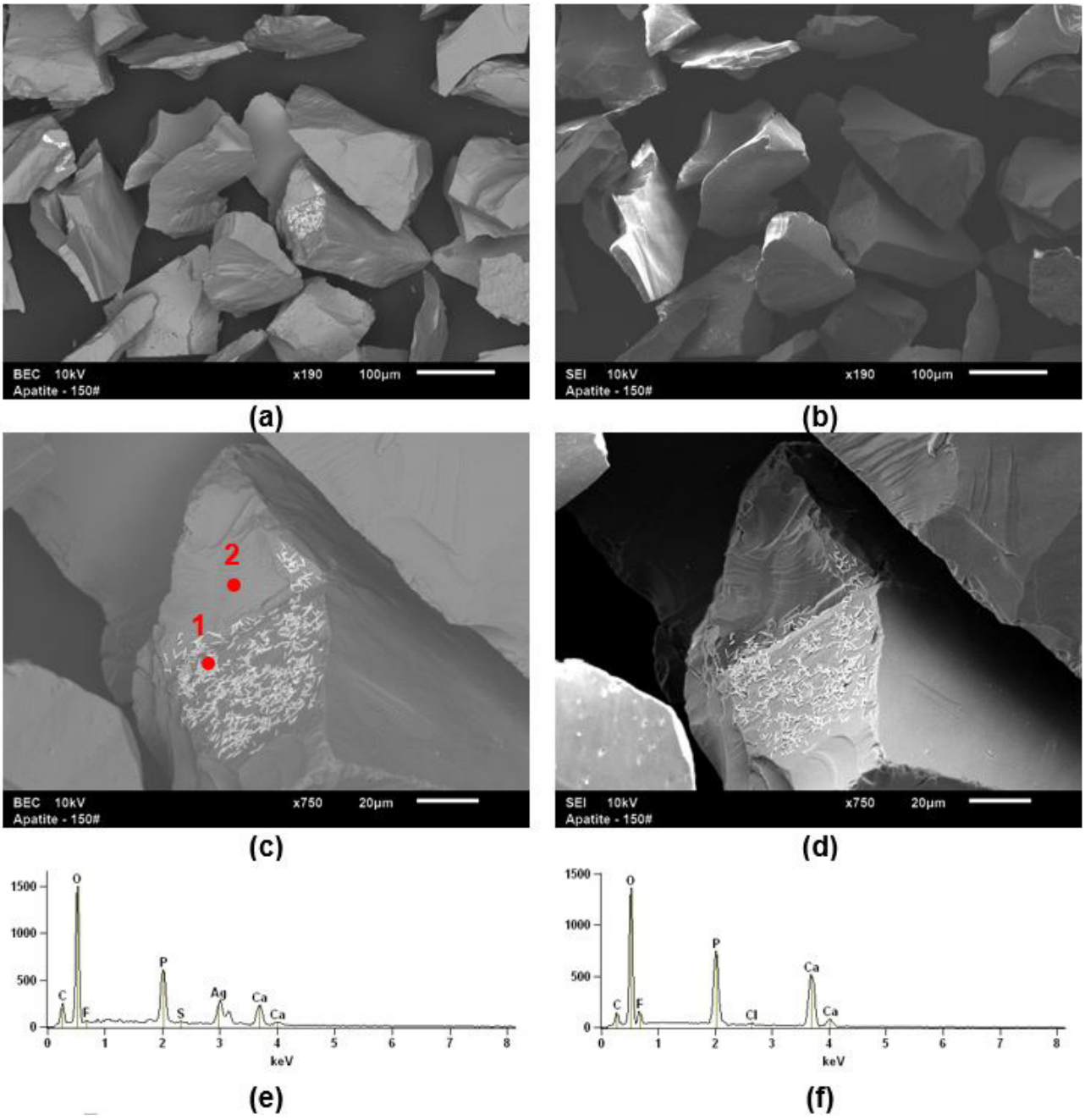

Figure 1. SEM images for apatite samples (a) BEC and (b) SEI image at x190 and (c) BEC and (d) SEI image at x750 magnification. EDS results for points 1 (e) and 2 (f) marked on figure 2c.

(DNA and RNA) were also present. Therefore, the SYC mass that has been effectively saponified may be less than 5% of the total saponified mass.

Padukone and Natarajan [21], worked with *Saccharomyces cerevisiae* yeast and their metabolites as collectors in order to selective separate calcite from quartz through flotation. The authors adapted the cells to a specific mineral (calcite or quartz), proceeding with the growth of subcultures in growth medium with a gradually increase of the mineral concentration. The presence of the mineral and the growth medium shifted some superficial physicochemical properties of cells, such as hydrophobicity. The cells adaption to a specific mineral demand an apparatus to produce biomass for the mineral industries, adding cost for the process. Despite this procedure be more specific for some mineral, the methodology adopted in this work is cheaper and less time consuming once the cells had been already produced outside the mine company.

Merma et al. [22] working with *Rhodococcus opacus* bacteria obtained apatite recovery of 90% at pH around 5, in

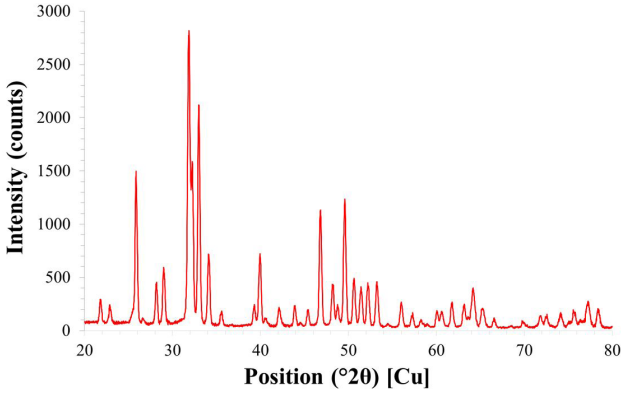

Figure 2. Apatite sample X-ray diffraction.

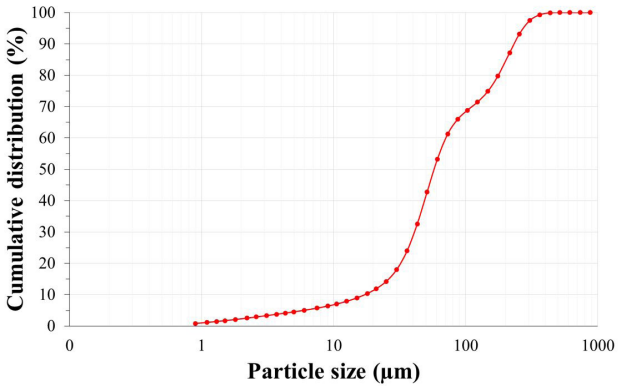

Figure 4. Particle size distribution for apatite sample.

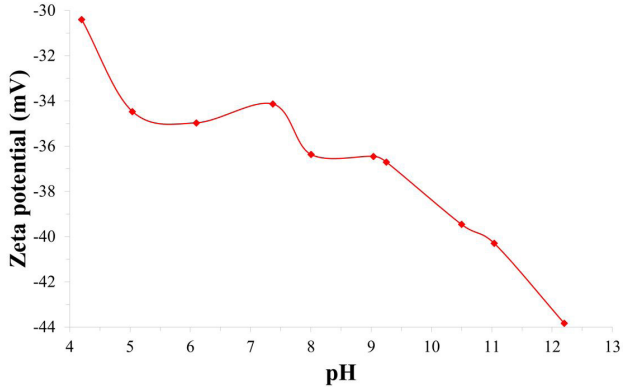

Figure 3. Apatite sample Zeta potential.

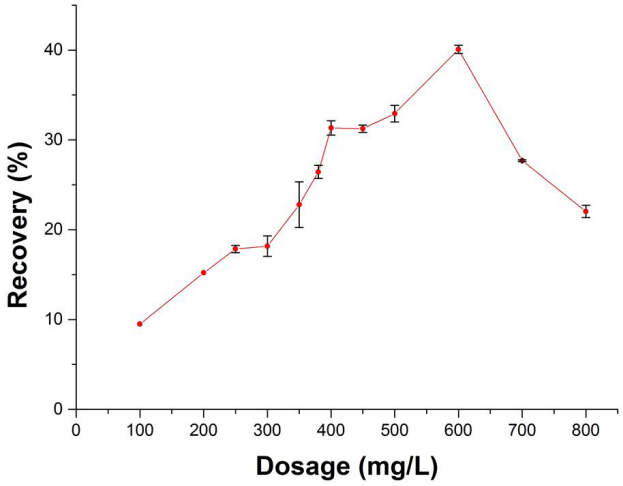

Figure 5. Apatite recovery as function of the collector dosage at pH 10.

the presence of 150 mg/L of bacteria after 5 min of flotation. On the other hand, quartz achieved a recovery of 14% under identical experimental conditions. The fundamental flotation studies performed by the authors revealed the prospect that *R. opacus* presents as a biocollector and biofrother and indicate its promising application in phosphate flotation industry.

4 CONCLUSIONS

Microflotation tests with apatite samples with 95% of purity were performed using saponified SYC as collector. No reference to such use had been found on the literature. No separation (chemical or physical) of the SYC cellular constituents was performed, besides the membrane lipids, in order to maintain the preparation cost of the SYC as low as possible. Therefore, the SYC mass that has been effectively saponified was less than 5% of the total saponified mass (proteins, carbohydrates, and cell genetic material were kept in the mass). This approach forced the use of high concentration of saponified SYC.

No apatite recovery above 40% was obtained for the tested pH and dosages, indicating that the SYC cannot supply the required amount of lipids required for the flotation or the remaining cellular material could be interacting with the flotation process. More work is required in order to test the isolation of the cellular material from the lipids and to understand the adsorption mechanism of the saponified SYC on apatite surface. Despite the low apatite recovery, the results demonstrate a potential future use for SYC in mineral froth flotation as a cheap, biodegradable, eco-friendly, and sustainable reagent.

Acknowledgements

The authors gratefully acknowledge the financial support of the Brazilian agencies CNPq, CAPES, FAPEG e FUNAPE. In addition, we would like to thank the brew master Noêmio Gontijo, Copebras, Labmic, UNIFESSPA, UFOP, UFG, and TUC.

REFERENCES

1 Murphy S, Burch D, Clapp J. Cereal secrets: the world's largest grain traders and global agriculture. Oxford: Oxfam Research Reports; 2012.

2 Rudorff BFT, Aguiar DA, Silva WF, Sugawara LM, Adami M, Moreira MA. Studies on the rapid expansion of sugarcane for ethanol production in São Paulo state (Brazil) using Landsat data. Remote Sensing. 2010;2(4):1057-1076. http://dx.doi.org/10.3390/rs2041057.

3 Prochnow LI. Optimizing nutrient use in low fertility soils of the tropics. Better Crops with Plant Food. 2008;92(3):19-21.

4 Juo ASR, Franzluebbers K. Tropical soils: properties and management for sustainable agriculture. 1st ed. New York: Oxford University Press; 2003.

5 Current world fertilizer trends and outlook to 2016. Rome: Food and Agriculture Organization of the United Nations; 2012.

6 Taiz L, Zeiger E. Plant physiology. 5th ed. Sunderland: Sinauer Associates; 2010.

7 Sis H, Chander S. Reagents used in the flotation of phosphate ores: a critical review. Minerals Engineering. 2003;16(7):577-585. http://dx.doi.org/10.1016/S0892-6875(03)00131-6.

8 Silva AC, Moraes ILA, Silva EMS, Silva CM Fo. *Jatropha curcas* L. oil selectivity in froth flotation. International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering. 2016;10(10):1-7.

9 Guiamarães RC, Araújo AC, Peres AEC. Reagents in igneous phosphate ores flotation. Minerals Engineering. 2005;18(2):199-204. http://dx.doi.org/10.1016/j.mineng.2004.08.022.

10 Behera SK, Mulaba-Bafubiandi AF. Microbes assisted mineral flotation a future prospective for mineral processing industries: a review. Mineral Processing and Extractive Metallurgy. 2016;38(2):1-10.

11 Yang X, Jin G, Gong Z, Shen H, Song Y, Bai F, et al. Simultaneous utilization of glucose and mannose from spent yeast cell mass for lipid production by Lipomyces starkeyi. Bioresource Technology. 2014;158:383-387. http://dx.doi.org/10.1016/j.biortech.2014.02.121.

12 Northcote DH, Horne RW. The chemical composition and structure of the yeast cell wall. The Biochemical Journal. 1952;51(2):232-236, 236.2. http://dx.doi.org/10.1042/bj0510232.

13 Brandão PRG, Caires LG, Queiroz DSB. Vegetable lipid oil-based collectors in the flotation of apatite ores. Minerals Engineering. 1994;7(7):917-925. http://dx.doi.org/10.1016/0892-6875(94)90133-3.

14 Dwyer R, Bruckard WJ, Rea S, Holmes RJ. Bioflotation and bioflocculation review: microorganisms relevant for mineral beneficiation. Mineral Processing and Extractive Metallurgy. 2012;121(2):65-71. http://dx.doi.org/10.1179/174328512Y.0000000005.

15 Rao KH, Subramanian S. Bioflotation and bioflocculation of relevance to minerals bioprocessing. In: Donati ER, Sand W. Microbial processing of metal sulfides. Dordrecht: Springer; 2007. p. 267-286.

16 Pacheco CAT, Silva AC, Silva EMS, Rocha TWP. Microflotação de apatita com óleos da polpa da castanha de Macaúba. In: Neves AF, Paula MH, Anjos PHR, Silva AG. Estudos Interdisciplinares em Ciências Biológicas, Saúde, Engenharias e Gestão. São Paulo: Blucher; 2016. p. 249-260. http://dx.doi.org/10.5151/9788580391619-16.

17 Guimarães P Jr, Silva AC, Silva EMS. Modelling the hydraulic entrainment phenomenon in microflotation. In: Flogen. Proceedings of the 2nd International Symposium on Sustainable Mineral Processing; 2015 Oct 4-8; Antalya, Turkey. Vancouver: Flogen; 2015. p. 186-192.

18 Rocha TWP. Estudo sobre a utilização do óleo da castanha de Macaúba como coletor na microflotação de apatite [thesis]. Catalão: Universidade Federal de Goiás; 2014.

19 Silva TC. Utilização do óleo de pequi como coletor na microflotação de apatita [thesis]. Catalão: Universidade Federal de Goiás; 2014.

20 Oliveira MS. Minério fosfático sílico-carbonatado: estudo fundamental [thesis]. Belo Horizonte: Universidade Federal de Minas Gerais; 2007.

21 Padukone SU, Natarajan KA. Microbially induced separation of quartz from calcite using *Saccharomyces cerevisiae*. Colloids and Surfaces. B, Biointerfaces. 2011;88(1):45-50. http://dx.doi.org/10.1016/j.colsurfb.2011.05.054.

22 Merma AG, Torem ML, Morán JJV, Monte MBM. On the fundamental aspects of apatite and quartz flotation using a Gram positive strain as a bioreagent. Minerals Engineering. 2013;48:61-67. http://dx.doi.org/10.1016/j.mineng.2012.10.018.

Received: 21 Sept. 2017
Accepted: 06 Feb. 2018