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Cellulase as a new phosphate depressant in dolomite-phosphate flotation

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Abstract: Recently, many research efforts have been made to reduce the magnesium content in the phosphate concentrate to meet the requirement for phosphoric acid production and other applications. A bioprocessing technique is among these efforts. However, this paper was devoted to study the use of cellulase enzyme as a new phosphate depressant during fatty acid flotation of a calcareous phosphate rock. The flotation behavior of collophane and dolomite as single minerals using oleic acid as a collector and cellulase enzyme as the phosphate depressant was investigated in details. The results from single mineral flotation tests were discussed based on FTIR and zeta potential measurements to find out the mechanism of cellulase depression of phosphate. The findings from the single minerals tests were used to develop a selective flotation process for recovery of phosphate minerals from the natural phosphate ore. The flotation experiments were carried out to apply this new process using the calcareous phosphate ore. Under the optimum flotation conditions, 0.04% cellulase and 0.5 mM oleic acid, a phosphate concentrate containing 0.89% MgO with a P_2O_5 recovery of 75% was obtained from the phosphate ore containing 2.2% MgO.

Keywords: flotation, cellulose, cellophane, dolomite, oleic acid

Introduction

The phosphate/oleic acid flotation system has been studied extensively since the beginning of the flotation reached. Oleic acid in either salt or acid form is the most widely used collector for phosphate flotation, and its solution chemistry features are well documented in literature (Dubel, 1990). On the other hand, using oleate as a collector provides limited selectivity, and no clear phosphate concentrate is produced unless depressants are used. In the recent decade, several separation techniques have been proposed by researchers for separation of dolomite from phosphate ores (El-Shall et al., 2004, Abouzeid et al., 2009, Ahmed et al., 2013). The most significant difficulty that faces separation of carbonate gangue minerals from phosphate ores arises from

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the fact that surface physicochemical properties of phosphate and carbonate minerals are very similar (Houot et al., 1982). The surface properties of phosphate are affected not only by phosphate's own solution chemistry but also by dissolved species from other salt-type minerals present such as calcite and dolomite (Somasundaran, 1999). From theoretical considerations, depending on solution conditions, the apatite surface can be converted to calcite and vice versa through either surface reactions or bulk precipitation of the more stable phase (Amankonah and Somasundaran, 1985). Meanwhile, apatite is depressed by depressants such as different acids, various organic and inorganic salts (Sis and Chander, 2003). Reverse flotation of carbonate gangue using fatty acid with depression of the phosphate mineral is one of the more promising selective separation techniques that have been tried by several researchers (Zheng and Smith, 1997, Guimaráes, 2005, Antony, 2006). The application of phosphate depressants, particularly phosphoric acid while floating carbonates in acidic media (pH below 5.5) has been successfully conducted by many workers (Anzia and Hanna, 1987, El Shall et al., 1996, Abdel-Khalek, 2000, Boulos et al., 2000). It was predicted from thermodynamic considerations that selective flotation of carbonates from phosphates in acidic media can be enhanced by increasing HPO_4^{-2} ions in the system (Abdel-Khalek, 2000). Besides, at such low pH, calcite is expected to dissolve and generates carbon dioxide bubbles that nucleate on its surface. The presence of CO_2 enhanced the selectivity of calcite flotation with respect to apatite (Predile, 1969). However, reverse flotation for calcareous phosphate confronts some difficulties because the optimal pH range is acidic (Al-Fariss et al., 2014, Boulos et al., 2014). In this case, a portion of P_2O_5 is lost due to dissolution of phosphate in the acidic medium. Moreover, a large amount of calcium and magnesium ions in the pulp precipitates with a collector molecule which results in the increase of collector consumption (Dos Santos et al., 2012).

Currently, the research orientations to solve this problem can be classified into two basic types. One is to exploit the efficient depressants that can be applied in a neutral medium such as microorganism, which was utilized for enhancing the separation selectivity of minerals by modifying minerals surface characteristic (Ahmed et al., 2001, Smith and Miettinen, 2006, El-Mahdy et al., 2013). Recently, there has been a growing interest in using a biotechnological route for mineral processing due to its flexibility and lower operating costs. It also represents a process that is environmentally mild. For the past two decades, several studies have been carried out on the use of microorganisms and their secretions such as proteins and polysaccharides as environmentally friendly flotation reagents (Smith and Miettinen, 2006). Bioflotation, in which biological compounds or microorganisms act as reagents, frothers, collectors or modifiers, making possible for the selective separation of minerals (Smith and Misra, 1993), has become very attractive for presenting a great technological potential and flexibility in the choice of microorganisms (Abdel Khalek et al., 2015). Microorganisms can modify the mineral surfaces, either directly or indirectly. The direct mechanism involves adhesion of cells to mineral particles, while the indirect mechanism refers to the biological reagents such as excreted metabolites act as surface-active reagents (Sharma and Rao, 2003). These processes could not be carried out under commercial-scale conditions where it is impossible to prevent contamination by undesirable microorganisms. The other one is to exploit high selectivity collectors such as chelating agents (Wang et al., 2006). In addition, many polymers were used as phosphate depressants (Reis et al., 1988, Viana and Souza, 1988). It was indicated that a small amount of carboxymethyl cellulose (CMC) acted as a phosphate depressant, while calcite was floated, increasing the phosphate recovery by about 10% (Lin and Burdick, 1988). Adsorption of CMC on the mineral surface depended on the OH-OH distances in the cellulose molecule and Ca-Ca distances along the crystallographic orientation exhibited by the mineral particles. It was found that there was a better size fit for phosphate/CMC than calcite/ CMC. From the foregoing, it can be realized that the flotation processes to recover phosphate from the calcareous rock need to be improved. In this concept, the major objective of this study was to understand the flotation behavior of collophane and dolomite as single minerals using oleic acid as a collector and cellulase enzyme, which was not affected by other bacteria in the surrounding atmosphere, as a new phosphate depressant, as well as to use this information as a basis to develop a selective flotation process for the recovery of phosphate minerals from the phosphate ore.

Materials and methods

Materials

Cellulase from *Aspergillus Niger*, 22U/mg, was purchased from Fluka. Oleic acid (cis-9- octadecanoic acid; purity 99.4%) was supplied by Alfa Products. All other chemicals such as sodium hydroxide and hydrochloric acid were purchased from El-Nasr Co., for Chemical Industries (Egypt), and were reagent grade. A natural pure collophane, $Ca_5(PO_4,CO_3)_3OH$, and dolomite, CaMg (CO_3), minerals were supplied by Ward's science company. A sample from the Abu-Tartur phosphate deposit (Western Desert of Egypt) was used in this study. Table 1 presents the complete chemical analysis of the Abu- Tartur phosphate sample.

Table 1. Chemical analysis of Abu Tartur phosphate sample

Constituent	P_2O_5	Fe_2O_3	CaO	MgO	SiO_2	F	Al_2O_3	K_2O
%	27.15	3.65	43.50	2.20	4.45	2.90	1.20	0.12

Experimental techniques

The sized feed samples (-150+106 μm) of either single minerals or natural ore were first conditioned with the oleic acid as a collector for 20 min at a pulp density of 1% solid. In the case of using enzyme and after conditioning with oleic acid at pH 9, the slurry was treated with enzyme and conditioned at pH 6. Flotation pH was adjusted to

9. After conditioning, the pulp was transferred to a carefully cleaned froth column. The flotation experiments were performed using a micro-flotation column (2.5 cm in diameter and 26 cm in height). The total volume of oleate solution was 100 cm^3. A flotation time of two minutes was used at a nitrogen flow rate of 70 cm^3/min. The experiments were repeated three times, and the reported values were the average of three experiments.

A laser zeta meter Malvern Zetasizer 2000 was used for zeta potential measurements of different samples such as enzyme, oleic and minerals in an aqueous medium. A 0.01 mol/dm^3 aqueous KCl solution was used to get the required ionic strength. The Fourier transform infrared spectroscopy (FTIR) study was carried out on Nicolet IS-10 FTIR, USA to analyze functional groups using a potassium bromide (KBr) disk method.

Results and discussion

Flotation of dolomite and collophane

The major objective of the research described in this section is to understand some of the physico-chemical factors affecting the flotation behavior of dolomite and collophane single minerals using oleic acid as the collector in the presence of cellulase enzyme as a surface modifier. This new finding will be used in flotation of real phosphate ore to lower its magnesium content.

In order to find the optimum collector dosage for dolomite and collophane floatability, flotation tests were conducted as a function of oleic acid concentration at pH 9. The results plotted in Fig. 1 indicate that dolomite has a higher floatability than collophane. The recovery of dolomite increases gradually to 100% at a collector concentration of about $2\cdot10^{-4}$ mol/dm^3. It is seen that collophane, at 10^{-4} mol/dm^3 of oleic acid, does not float, while under the same conditions about 70% of dolomite is floated. The oleic acid concentration of $2\cdot10^{-4}$ mol/dm^3 is chosen for the flotation experiments in the presence of enzyme. At this concentration of oleic acid it is assumed that both minerals have not multilayers of oleic acid adsorbed on their surfaces where enzyme can interact with mineral surface.

Figure 2 presents floatability of collophane and dolomite minerals as a function of cellulase concentrations using $2\cdot10^{-4}$ mol/dm^3 oleic acid. It shows that there is a complete flotation of dolomite at any enzyme concentration. The dolomite recovery under similar experimental conditions is unchanged from that without enzyme addition (Fig. 1). This indicates that the addition of enzyme has no major effect on the dolomite recovery. On the other hand, collophane does not float at the low dose of enzyme and starts to float at higher enzyme dose. However, Figure 2 shows that separation of dolomite from collophane is feasible at the cellulase concentration of about 0.01%. At this concentration, the dolomite recovery remains at 100% where collophane reaches only 23%.

The pH of the system has a pronounced effect on enzyme activity. Each enzyme has a characteristic pH at which its activity is the highest. The effect of conditioning pH with enzyme on single mineral flotation of collophane and dolomite using $2\cdot10^{-4}$ mol/dm^3 oleic acid and 0.03% of cellulase is illustrated in Fig. 3. It is shown that there is also a complete flotation of dolomite over the entire pH range. On the other hand, the flotation recovery of collophane, in general, is low in comparison to the recovery of dolomite. In the acidic region, the floated collophane reaches 36% and there is a gradual increase at pH 8 reaching about 52% and decreases again at pH 10 reaching 31%. The collophane recovery, at this level of oleic acid addition and pH 6 without enzyme, is in the range of 56%. There is a clear separation zone, for dolomite and collophane that is achieved at pH 6 which is the natural pH of the suspension in the presence of cellulase.

Flotation tests of the pure collophane and dolomite single minerals, using oleic acid as a collector, are followed by electrokinetic measurements and FTIR to elucidate the mechanism of the observed selectivity in the presence of enzyme.

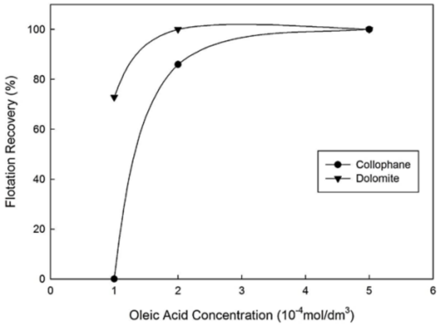

Fig. 1. Effect of oleate concentration on floatability of collophane and dolomite single minerals

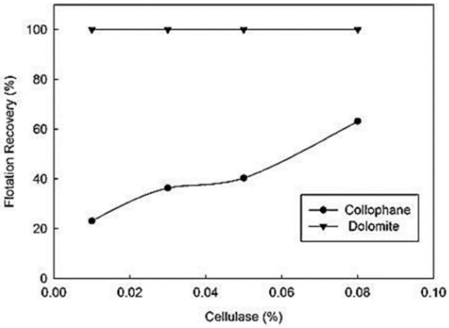

Fig. 2. Effect of cellulase concentration on floatability of collophane and dolomite single minerals using $2\cdot10^{-4}$ mol/dm^3 oleic acid

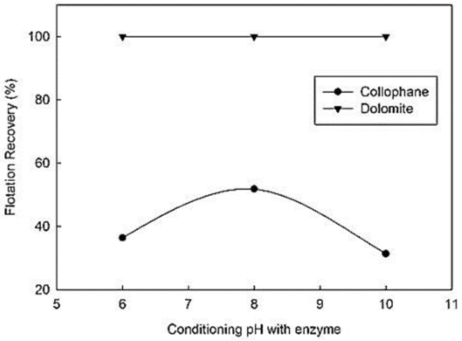

Fig. 3. Effect of conditioning pH, with enzyme, on floatability of collophane and dolomite single minerals in presence of cellulase using $2\cdot10^{-4}$ mol/dm^3 oleic acid

Zeta potential of minerals

Flotation is affected by the surface charge of mineral particles suspended in the pulp. The surface charge of mineral particles can either enhance or hinder bubble-particles interactions and supply the driving force for adsorption of surfactant on mineral surfaces. The objective of these measurements is to determine the surface charge behavior of collophane and dolomite minerals in the presence of oleic acid in the absence and presence of enzyme. However, a number of tests is conducted to determine the effect of cellulase enzyme on the zeta potential of collophane and dolomite with the adsorbed oleate as shown in Fig. 4. It is clear from the results in Fig. 4 that the zeta potential of both minerals decreases in magnitude with the increasing oleic acid concentration. This lowering of zeta potential magnitude is due to adsorption of the ionized oleic acid species $RCOO^-$ on the mineral surfaces. It is also noticed that the presence of enzyme decreases the negativity of the zeta potential of both minerals with the adsorbed oleic acid. For example, the zeta potential of dolomite is about -60 mV using 10^{-4} mol/dm^3 oleic acid and is increased to about -20 mV after the treatment with cellulase enzyme. This indicates that enzyme is adsorbed on the mineral surface, and there is a hydrophobic bond between the hydrocarbon chain of oleic acid (adsorbed on mineral surface), and the hydrophobic pocket is found at the enzyme surface (Ahmed and Reheem, 2012). The created new surface becomes hydrophilic due to the presence of hydrophilic groups at the enzyme surface (Michelle and Robert, 2001). The hydrophilic groups of enzyme contained a proteinaceous amino group which carried a positive charge. This is the reason for decreasing the negativity of the zeta potential of the mineral particles treated with enzyme as shown in Fig. 4. However, the electrokinetic behavior of both minerals is remarkably influenced by addition of enzyme and results in reduction of the negative charge on the mineral surfaces. However, the electrokinetic behavior of both minerals are remarkably influenced by the addition of enzyme resulted in a reduction of the negative charge on the mineral surfaces.

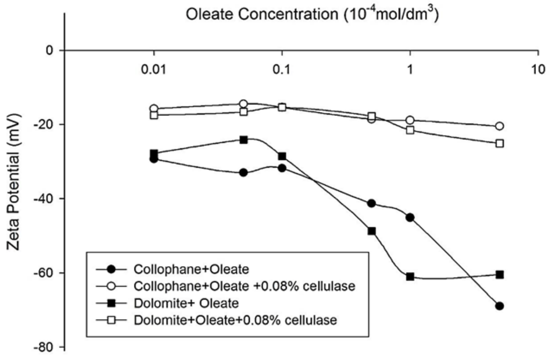

Fig. 4. Effect of different concentrations of oleate and oleate/0.08% cellulase on zeta potential of collophane and dolomite at pH 6

Fourier transform infrared spectroscopy studies

The flotation experiments provide some indication about the nature of the phosphate depression reaction using cellulase enzyme but they are insufficient to completely identify the chemical nature of the reaction products. Fourier transform infrared spectroscopy (FTIR) is used for this purpose. The surface compounds, formed on the minerals surface (collophane and dolomite) after collector adsorption and subsequent a bio-treatment process, are examined through the FTIR spectroscopy technique. The assignments of the bands are made based on the reported values for the various functional groups (Herman, 1963; Socrates, 1980). Figure 5 represents FTIR spectra of collophane interacted with oleic acid and cellulase. It is clear from Fig. 5 that the peak at 1044 cm^{-1} due to P-O stretching of collophane interacted with oleic acid and cellulase enzyme, is broader, and its intensity is lower than that of oleate interacted with collophane. The peaks at 1634 cm^{-1} due to oleic acid, and at 1637 cm^{-1} due to cellulase are disappeared, and are included in the peak at 1044 cm^{-1}.

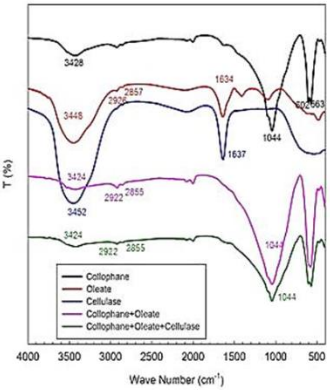

Fig. 5. FTIR spectra of collophane, oleate, cellulase and treated collophane

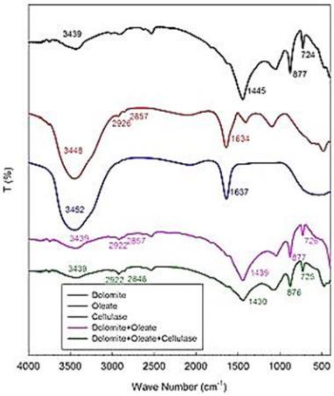

Fig. 6. FTIR spectra of dolomite, oleate, cellulase and treated dolomite

Figure 6 represents the FTIR spectra of dolomite, dolomite interacted with oleic acid and cellulase enzyme. The FTIR spectrum of oleate interacted dolomite after treatment with cellulose (Fig. 6) indicates that the peak at 1439 cm^{-1}, in oleate interacted dolomite, shifts to 1430 cm^{-1}, and becomes broader and lower in intensity. Additionally, the peaks at 1634 cm^{-1} due to oleic acid, and at 1637 cm^{-1} due to cellulase are disappeared, and are included in the peak at 1430 cm^{-1}. However, FTIR spectroscopy indicates that there is an interaction between collophane and dolomite with adsorbed oleic acid and enzyme. In this case, collophane is depressed in the presence of enzyme, where floatability of dolomite is not affected.

Possible mechanism

Figure 7 represents a schematic diagram for the probable mechanism of oleic acid and enzyme interaction on the mineral surface. In spite of the fact that enzyme molecules are coated mostly with hydrophilic functional groups, it is understood that they have hydrophobic pockets on their surfaces (Shaltiel, 1975). These pockets are available for interaction with appropriately sized hydrocarbon chains, implanted on an inert matrix forming hydrophobic bonds (Champe and Harvey, 1994). However, it is assumed that there is a hydrophobic bond between the hydrocarbon chain of oleic acid (adsorbed on the mineral surface), and the hydrophobic pocket found on the enzyme surface (Michelle and Robert, 2001, Ahmed Yehia, 2012). The created new surface becomes hydrophilic due to the presence of hydrophilic groups on the enzyme surface. Apparently, this occurs on the phosphate mineral surfaces and not on the dolomite surfaces. This affinity of enzymes to P atoms is related to the structural integrity of the enzyme molecules and strong adsorption on the phosphate surface (Yasutaka et al., 2011). Besides, the heats of adsorption show that phosphate mineral surfaces are polarized and forms strong bonds with either polar or polarizable molecules (Beebe et al., 1975). In general, the mode of adsorption of either oleate or any polar collector at a mineral surface is affected by the surface polarizability (Read et al., 1972). However, the presence of P atoms on the mineral surface can provide a surface environment suitable for formation of hydrophobic bond between the hydrocarbon chain of oleic acid (adsorbed on the mineral surface), and the hydrophobic pocket found on the enzyme surface. If so then, it seems that the orientation of the oleate hydrocarbon chains at the phosphate surface favored its accommodation by enzyme and differed in conformation from the oleate hydrocarbon chains at the dolomite surface. It is noteworthy that the mechanism of cellulase enzyme and cellulose, used as depressants for phosphate, are sufficiently different. The cellulase mechanism depends on formation of the hydrophobic bond between the hydrocarbon chain of oleic acid (adsorbed on mineral surface) and hydrophobic pocket found on the enzyme surface. On the other hand, the cellulose mechanism depends on the OH-OH distances in the cellulose molecule and Ca-Ca distances along the crystallographic orientation exhibited by the mineral particles.

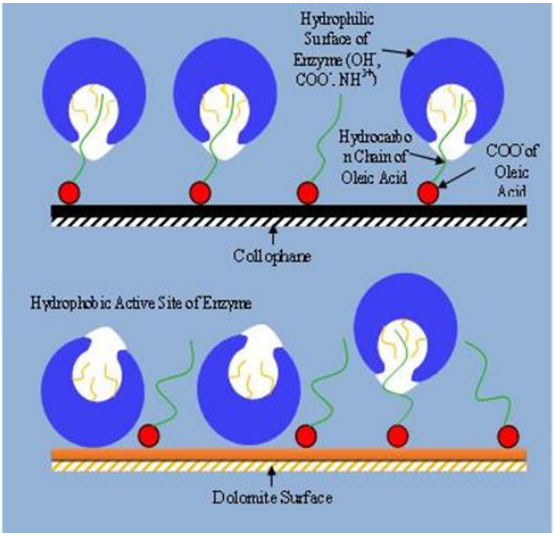

Fig. 7. Schematic diagram for probable mechanism of interaction of oleic acid and enzyme on the mineral surface

Flotation of phosphate ore

In the previous section, it was demonstrated that it was possible to separate dolomite from collophane in the froth column flotation cell. This was accomplished by floating dolomite, using oleic acid as the collector, in the presence of cellulose, which acted as the depressant for collophane. These data suggested a possible process for reverse flotation operation, where dolomite would be concentrated in the froth product to leave a non-float enriched in collophane. However, to evaluate the selectivity achieved from the single minerals tests, flotation of phosphate ore, from Abu-Tartur, is subjected to flotation employing the method of the present new investigation. The separation performance is presented in terms of P_2O_5 recovery and MgO% of the final concentrate.

In order to find the suitable dosage of oleic acid, to be used in phosphate flotation in the presence of cellulase enzyme, flotation tests are conducted as a function of oleic acid concentration at pH 9. Figure 8 shows floatability of the Abu-Tartur sample as a function of oleic acid concentrations at pH 9 without enzyme. It is seen that no flotation is observed up to $4\cdot10^{-4}$ mol/dm^3 of oleic acid and the complete flotation is obtained at 10^{-3} mol/dm^3 oleic acid. However, it is decided to use the concentration of $5\cdot10^{-4}$ mol/dm^3 oleic acid, where about 80% of the ore is floated, for the subsequent flotation experiments in the presence of cellulase enzyme. This concentration of oleic acid is used to avoid the multilayer formation of oleic acid on the minerals surface.

The flotation tests of the ore are also performed at different concentrations of enzyme. Figure 9 shows the effect of cellulase concentration on the flotation behavior of the phosphate ore using $5\cdot10^{-4}$ mol/dm^3 oleic acid. It can be seen that by increasing the concentration of enzyme the floated product increases. On the other hand, the wt. % of the sink product decreases with cellulase dosage and MgO% increases. It is observed from Fig. 9 that using 0.04% cellulase, there is an effective separation of dolomite mineral (conentrate contains 4.79% MgO, but only 0.89% MgO in the sink fraction) with 74.60% P_2O_5 recovery in the sink fraction (tailing) and 28.07% P_2O_5 in grade from phosphate ore containing 2.20% MgO.

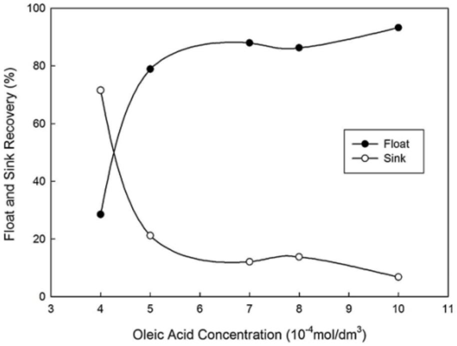

Fig. 8. Effect of oleic acid concentration
on the flotation of Abu-Tartur phosphate ore sample

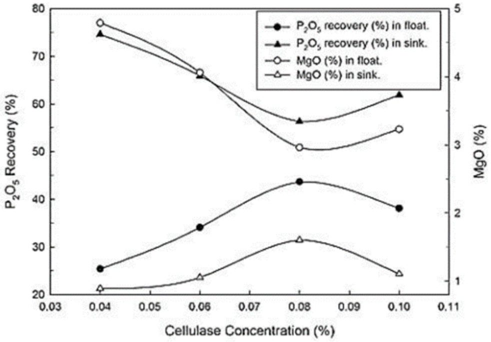

Fig. 9. Effect of cellulase concentration
on the flotation of Abu Tartur phosphate ore

Conclusions

The aim of this work was to study a possible use of cellulase enzyme as a new phosphate depressant in fatty acid flotation of single minerals of collophane and dolomite to find the best separation conditions. It was further used to develop the selective flotation process for the recovery of phosphate minerals from the natural phosphate ore.

Separation of dolomite from collophane in the single mineral tests was possible at a cellulase concentration of about 0.03% since the dolomite recovery remained at 100%, whereas the recovery of collophane reached 36.34%. There was a complete flotation of dolomite over the entire pH range. On the other hand, the recovery of collophane particles reached 36.34% in the acidic region, and there was a gradual increase at pH 8 reaching about 52%, and then decreased again at pH 10 reaching 31.32%. FTIR spectroscopy and zeta potential measurements indicated that there was interaction between collophane and dolomite with adsorbed oleic acid and enzyme but in different ways. Collophane was depressed in the presence of enzyme where floatability of dolomite was not affected.

The optimum flotation conditions for flotation of phosphate ore from Abu-Tartur were: 0.04% cellulase and $5\cdot10^{-4}$ mol/dm^3 oleic acid. It was possible to obtain the phosphate concentrate containing 0.89% MgO with a P_2O_5 recovery of 74.60% from phosphate ore containing 2.20% MgO. These results indicated that the used enzyme, under the appropriate flotation conditions, was a good depressant for flotation of phosphate (phosphorous-containing mineral), and showed no effect on dolomite flotation (a magnesium-containing mineral) when oleic acid was used as the collector.

A probable mechanism of oleic acid and enzyme interaction on the mineral surface was suggested. This mechanism explained this new finding according to the hydrophobic bond between the hydrocarbon chain of oleic acid (adsorbed on mineral surface) and the hydrophobic pocket found on the enzyme surface. The created new surface became hydrophilic due to the presence of hydrophilic groups on the enzyme surface. Apparently, this occurred on the phosphate mineral surfaces and not on the dolomite surfaces. In such case, the phosphate mineral was reported to the sink fraction since the enzyme tended to be the depressant for phosphate, while carbonate minerals floated.

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