

Selective depression of *Escherichia coli* on flotation of collophanite and dolomite

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Abstract: The phosphate rock flotation test was carried out using *Escherichia coli* (*E. coli*) as a depressant of dolomite. The results showed that *E. coli* had a great selective depression on dolomite during flotation. With *E. coli* as a depressant of dolomite, a useful beneficiation index of phosphorus concentrate with P₂O₅ grade of more than 30% and MgO content less than 1.2% can be obtained by closed-circuit of one-stage roughing and one-stage cleaning. Furthermore, the depression mechanism of *E. coli* was studied by adsorption experiments, infrared spectrum, and zeta potential. This study shows that the adsorption ability of *E. coli* onto dolomite is stronger than that of collophanite. When the pH is greater than 6, *E. coli* are chemically adsorbed on the surface of the collophanite and dolomite, which also increases the negative charge on the surface of the two minerals. The selective adsorption of *E. coli* cells to dolomite was best when the pH value is about 7.8.

Keywords: *Escherichia coli*, collophanite, dolomite, flotation, phosphate rock

1. Introduction

China's phosphate resources are plentiful, and marine sedimentary phosphate rock accounts for about 85% of its total reserves. Collophanite in the phosphate rock is the primary ore containing phosphorus (Dos Santos et al., 2010; Wu et al., 2021). Depending on the main gangue dolomite, quartz, and silicate mineral content, phosphate rock can be divided into calcium (magnesium) phosphate rock, siliceous phosphorus rock, and silicate-calcareous phosphate rock (Zhen et al., 2018). The reverse flotation process is the most effective beneficiation method for calcium (Magnesium) phosphate rock. In the current production, pH is often adjusted by H₂SO₄, and gangue mineral, dolomite are collected by anionic collectors of fatty acids. Through multistage separation, the removal rate of dolomite can reach 70% ~ 80%. The process is difficult to be applied to the treatment of phosphate rock containing more silicon minerals, and it is more challenging to process ores containing sesquioxide (Kuang et al., 2018; Mohammadkani et al., 2011). The treatment of low-grade siliceous rock phosphate by single reverse flotation is not effective (Nie, 2016; Li et al., 2019). Theoretically, the best way to treat this kind of phosphate rock is to float phosphate minerals whilst discarding calcareous and siliceous minerals. However, during flotation of phosphate rocks, dolomite and collophanite present similar surface properties which makes difficult the selectivity of the process.

Because bacteria are fast-growing, environmentally-friendly, have economic and other advantages (S. Ghosh et al., 2016; Wang et al., 2014; VÍctorm Luque-Almagro et al., 2018), the use of bacteria in the mineral processing is receiving more and more attention. Bacteria and some of their metabolites can be adsorbed onto mineral surfaces, modifying their surface properties, which affect the mineral inhibition, activation, dispersion and flocculation and as a consequence their floatability (Sarvamangala et al., 2011; Xiao et al., 2021; Wang et al., 2016). The bacteria research applications in mineral processing is mainly focused on flotation of nonferrous metals and coal (Patra et al., 2003; Santhiya et al., 2001). In recent years, the application of bacteria in mineral flotation has increased. In 1999, American scholars (Zheng et al., 1999) studied the flotation separation of apatite and dolomite with anionic collector in the presence of *Mycobacterium phlei* (*M. phlei*), and found that *M. phlei* strains

can be used as inhibitors of these two minerals, and the inhibitory effect on dolomite is better than that on apatite. Bleeze et al. (2018) studied the extracellular polymeric substance (EPS) extracted from *Leptospirillum ferrooxidans*, grown on chalcopyrite for 48 hours, and polysaccharide-rich EPS selectively attaches to pyrite within 48 h, depressing its floatability and ensuring successful separation with a PIPX collector, resulting in Cu recovery of 95.8%. Natarajan et al. (2001) used *Bacillus polymyxa* as a collector and found that its metabolites can change the surface properties of quartz and kaolinite and increase the floatability of quartz and kaolinite. Mesquita et al. (2003) floated hematite with *Rhodococcus opacus* and found that this bacterium can improve the hydrophobicity of hematite. Carlos et al. (2016) used *Rhodococcus erythropolis* bacteria as a possible substitute of synthetic reagents used in hematite flotation, studies have shown that bacterial adhesion was higher at acidic pH, which also suggests an electrostatic attraction between the mineral surface and the biomass. Xiapeng (2001) studied through adsorption measurement and scanning electron microscopy of *Bacillus subtilis* and *Marburg phlei* adsorption on apatite and dolomite, compare these microbes oleate on the mineral flotation, the influence of the results showed that in the dolomite and anionic collector phosphate ore flotation, both inhibitor role, but the *Bacillus subtilis* more powerful inhibitors, especially for dolomites. Zheng (1998) studied the use of different bacteria as dolomite depressor in the flotation of apatite (*Bacillus subtilis* and *Mycobacterium phlei*). The results showed that the three bacteria had adsorption on dolomite and apatite. Under certain pH conditions, the adsorption of dolomite by *Mycobacterium phlei* is far superior to that of apatite, which can depress the dolomite flotation.

According to a preliminary study, *E. coli* had a better inhibitory effect on dolomite during the flotation of phosphate rock (Luo et al., 2016).

Therefore, the actual phosphate rock flotation test was carried out using *E. coli* as a dolomite inhibitor. At the same time, the effects of *E. coli* on the collophanite and dolomite floatability and adsorption experiments were carried out. Based on this, the effect of bacteria on the surface potential of the minerals and the results of infrared spectroscopy was analyzed to discuss the mechanism of bacterial selective depression of dolomite.

2. Test materials

2.1. Samples

The collophanite and dolomite samples were taken from China's Guizhou Province Weng'an County, crushing and dry grinding to -0.106mm. By X-Ray Diffraction (XRD) Phase Analysis and chemical analysis, the purity of the dolomite minerals is above 95%, and the theoretical purity of the fluorophosphate in the phosphate rock is above 90%.

The actual phosphorus ore is taken from China Fuquan City. X-Ray Fluorescence (XRF) spectrometer analysis of the samples results in Table 1.

Table 1. XRF analysis of phosphorus rock/%

component	P ₂ O ₅	SiO ₂	CaO	MgO	Fe ₂ O ₃	Al ₂ O ₃
content/%	25.35	15.06	38.59	3.22	1.33	3.53
component	MnO	TiO ₂	K ₂ O	Na ₂ O	SrO	
content/%	0.08	0.17	1.46	0.29	0.09	

Table 1 shows that the content of P₂O₅ is less than 30%; The loss on ignition of the sample is 10.83%; the content of SiO₂, MgO, Fe₂O₃, and Al₂O₃ in the samples is high. X-ray diffraction analysis and phase analysis show that the useful minerals in the sample are mainly fluorapatite, and the gangue minerals are mainly quartz and dolomite, followed by silicate minerals. MgO, Al₂O₃, and Fe₂O₃ exist mainly in dolomite, phlogopite, and iron oxides.

The grain size characteristic curve of actual ore is shown in Fig. 1. The grain size of the actual ore mostly belongs to the middle-grained uniform distribution type, and its grain size is concentrated in the range of 0.150-0.425mm. 0.425mm is the turning point of the characteristic curve of grain size

distribution from steep to slow, and its cumulative yield reached 75.72%. Among them, the cumulative yield of fine particles -0.075mm is 9.53%, the most gentle curve, accounting for a small proportion.

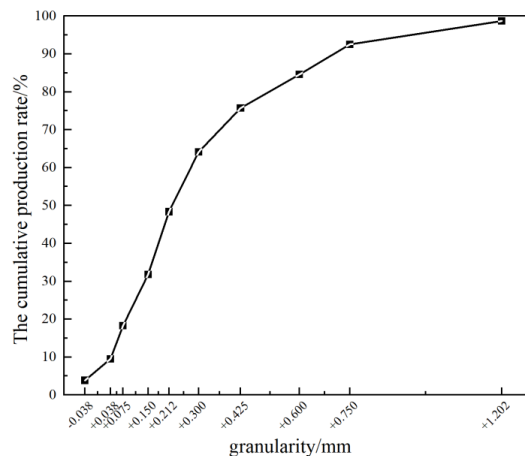


Fig. 1. Grain size characteristic curve

2.1. Bacterial culture and preparation

Escherichia coli strain is provided by the bacteria conservation center of the Chemical Engineering Institute of Guizhou University. *E. coli* medium formula refers to GB/T 1492.43-2001, *E. coli* medium formula: beef paste 3g, peptone 10g, sodium chloride 5g, distilled water 1L, pH 7.0~7.2. The medium was sterilized in a 0.1MPa 121°C autoclave for 20 minutes. After inoculation, the bacteria suspension was incubated in a thermostatic shaker at 34 °C for two days. The growth of *E. coli* in the 10~25h follows a geometric progression, and this period is the exponential growth phase of *E. coli*.

The culture medium of *E. coli* cultured for two days and centrifuged at 4000r / min for 10min, the supernatant discarded to obtain *E. coli* cells. Washing with distilled water after sterilization and centrifuge separately. Repeating three times and finally weighed the wet cell weight. The acquired cells were used for the test, or the cell suspension was stored in a refrigerator at 4°C.

3. Test methods

3.1. Flotation test method

3.1.1. Test method for actual ore flotation

The optimization test of the flotation roughing condition carried out without adding bacteria. Under the optimum roughing conditions, the actual ore flotation experiment carried out by adding bacteria as inhibitors.

Flotation tests conducted at room temperature. For each flotation test, the 0.2 kg sample is ground to the desired fineness by a ball mill of XMQ-240×90. Rougher flotation and cleaning flotation conducted in XFD 750cm³ flotation cell with speed 2004 r/min and charging air 0.3m³/h. The mixture of soap and ethyl phenylacetate (GYF) was used as a collector, other test chemicals were chemically pure, and test water was tap water. The flotation products are filtered, dried, weighed, and analyzed. The ore dressing indexes are calculated according to the analysis results.

3.1.2. Test method for the effect of *E. coli* on collophanite and dolomite floatability

The flotation test carried out in a 40cm³ flotation machine at room temperature. For each test, 4g samples were put into the flotation cell, add 40cm³ of deionized water, stir for 1 min. The pH adjusted with HCl or NaOH solution by stirring for 1 min. Adding the *E. coli* cell suspension, water glass, GYF, within the 3-minute interval, and then scraping of the foam manually. The products were dried and weighed. Mineral recovery was calculated by weight of the products.

3.2. Adsorption test method

The adsorption test was carried out in a shaker at a constant temperature. In the experiment, we put 50cm³ of a specified concentration of *E. coli* suspension was put in a 100cm³ triangular beaker, pH adjusted, 1g ore sample was added, and the size of ore sample was 0.074mm, After adsorption for 20 minutes, it was allowed to stand. The concentration of the suspension was determined by the turbidimetric method, and the adsorption rate was calculated.

The adsorption rate is calculated as:

$$Q = (A_0 - A) / A_0 \quad (1)$$

where: Q is the adsorption rate, A₀ is the absorbance value of the cell suspension before adsorption, and A is the absorbance value of the bacterial suspension after adsorption.

3.3. Determination of zeta potential

The Zetasizer 2000 Zeta Potential Analyzer was used for the determination of the potential. When the potential of the bacteria was measured, a particular concentration of the cell suspension poured into a 50cm³ beaker; the pH was adjusted with HCl or NaOH solution and vibrated for 5 min with ultrasonication. A syringe was used to take a certain amount of solution and added it to the Zeta potential sample cell for testing. The electrode inserted into the electrolyte (0.001mol/L KCl solution), room temperature was controlled at 25°C, and the surface potential was measured on the computer terminal according to the program operation. Each test was measured three times to calculate the average and standard deviation.

3.4. Determination of infrared spectrum

In this experiment, Fourier infrared spectrometer was used for testing. The ore sample prepared by 2g and the ore sample adsorbed by bacteria were weighed and added to 40 cm³ deionized water to the beaker. NaOH and HCl solution was utilized to adjust the suspension to the pH required for the experiment. After magnetic stirring for 10 min, the samples were filtered, washed thoroughly with distilled water, and dried in a vacuum oven at 40 °C for 24 h. Then the samples were mixed with KBr to record 4000-500 cm⁻¹ spectra at 25 °C.

4. Results and discussion

4.1. Flotation test

In this paper, the rough separation condition test was carried out, and the effects of collector GYF dosage, inhibitor tannin dosage, sodium silicate dosage, grinding fineness and *E. coli* dosage on flotation indexes were respectively studied by one-time one-factor method. The results of Effect of *E. coli* dosage on flotation were shown in Fig. 2.

Under the optimum rough conditions, the dosage of *E. coli* on the mineral separation was carried out. Based on this, a closed-circuit test was carried out too. The test results are shown in Fig. 2 and Table 2, respectively.

4.1.1. Effect of *E. coli* dosage on flotation

As can be seen from Fig. 2, the dosage of *E. coli* has a high-impact on the flotation results. The content of MgO in concentrate decreased with the increase of *E. coli* dosage. Therefore, *E. coli* has a strong inhibitory effect on the flotation of dolomite. The content of P₂O₅ in the concentrate was the highest when the dosage of bacteria was 1g/t and then decreased with the increase of the dosage of bacteria. The recovery of P₂O₅ in the concentrate increased first and then decreased and reached the maximum when the dosage of *E. coli* was 2.75g/t. At this time, the concentrate P₂O₅ grade was 29.63%, the recovery rate of P₂O₅ was 75.56%, and the content of MgO was 2.51%.

4.1.2. Closed-circuit flotation tests

The closed-circuit flotation flowsheet and the flotation agent scheme are shown in Fig. 3. After the

closed-circuit test was balanced, the final test results were shown in Table 2, and other sesquioxide in concentrate were shown in Table 3.

From Table 2, a closed-circuit flotation test yielded the phosphate concentrate with P_2O_5 grade of 30.75% and P_2O_5 recovery of 75.32%, the calculated concentrate productivity is 62.04%.

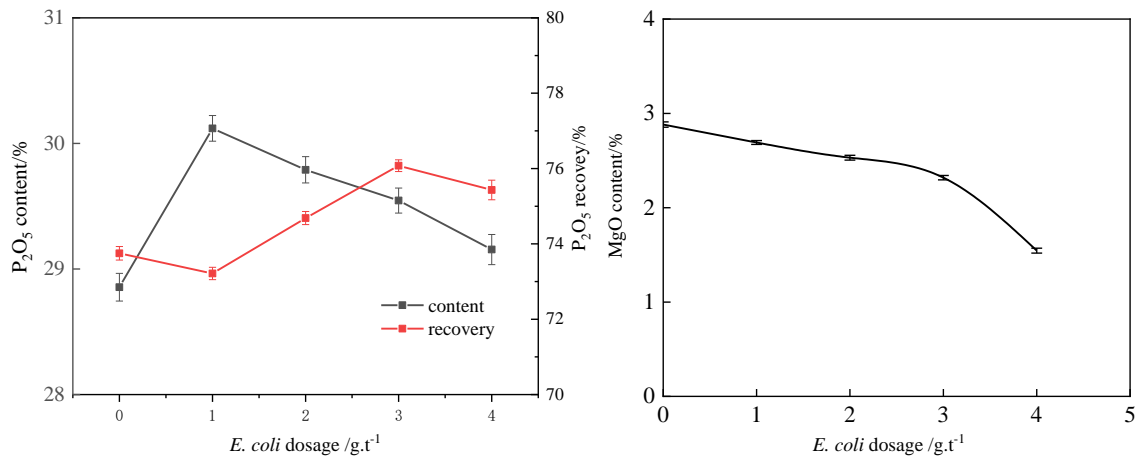


Fig. 2 . Effect of *E. coli* dosage on flotation (pH=10, sodium carbonate 2.5kg/t, water glass 3kg/t, GYF 2.5kg/t, aeration flotation 3 min. Left: Effect of *E. coli* on the flotation of phosphate. Right: Effect of *E. coli* on the flotation of MgO)

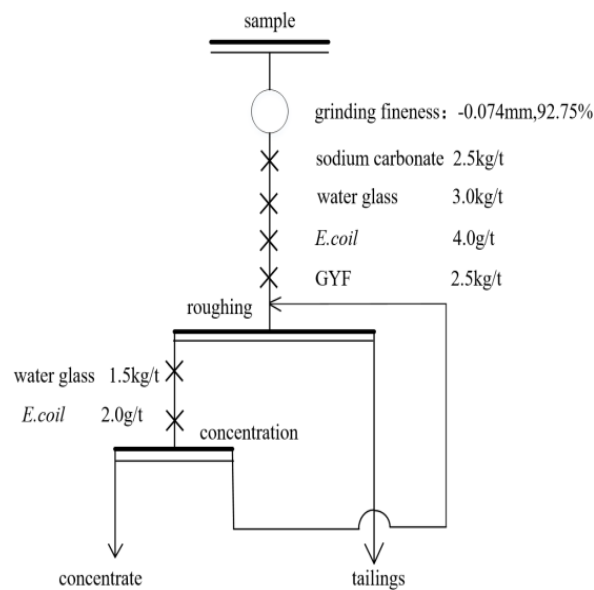


Fig. 3 . Flowsheet of close-circuit flotation

Table 2. Results of closed circuit experiment / %

product	productivity	ore grade			recovery		
		P_2O_5	MgO	SiO_2	P_2O_5	MgO	SiO_2
concentrate	62.04	30.75	1.15	9.11	75.32	21.86	37.66
tailings	37.96	16.47	6.72	24.66	24.68	78.14	62.34
raw ore	100	25.35	3.22	15.06	100	100	100

Table 3. Chemical analysis of concentrates / %

component	P_2O_5	SiO_2	CaO	MgO	Fe_2O_3	Al_2O_3
content / %	30.75	9.11	44.39	1.15	1.12	1.77

From Table 3, it can be seen that the content of SiO_2 in phosphate concentrate is 9.11%, the content of MgO is less than 1.2%, and the sum of the content of Fe_2O_3 and Al_2O_3 is less than 3%. The phosphate concentrate reaches the standard for phosphate ores for acid processing.

4.2. Selective depression and its mechanism of *E. coli* to collophanite and dolomite

4.2.1. Effect of *E. coli* on floatability of dolomite and collophanite

From the test results in Fig. 4 can be seen, with the increase in the dosage of *E. coli*, dolomite, and collophanite recovery rate showed a downward trend. The recovery rate of dolomite decreased, and the recovery rate of collophanite was. Therefore, *E. coli* depressed the flotation of collophanite and dolomite, but depressed dolomite more strongly.

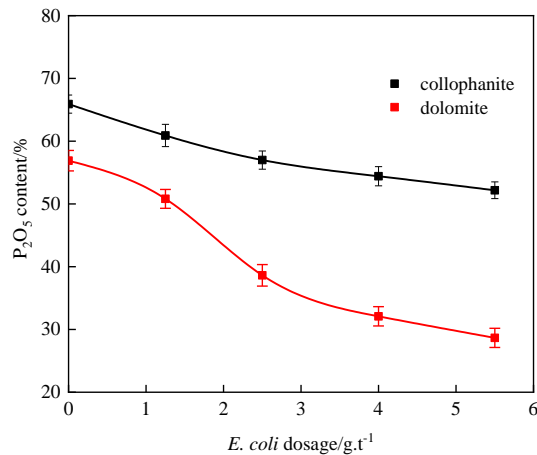


Fig. 4 . Effect of *E. coli* on floatability of dolomite and collophanite (pH=10, GYF 5kg/t, water glass 3.0kg/t, aeration flotation 3 min)

4.2.2. Factors affecting the adsorption of collophanite and dolomite by *E. coli*

The adsorption of bacteria onto minerals is affected by various factors such as adsorption time, pulp concentration, pH value, cell suspension concentration, stirring speed, temperature etc. (Wang et al., 2020). We investigated the effects of adsorption time and pH value on *E. coli* adsorption. The results of the adsorption time and the initial pH value of the solution on the bacterial adsorption effect are shown in Figs. 5 and 6, respectively.

As can be seen from the results in Fig. 5, the adsorption rate of *E. coli* onto collophanite and dolomite increased rapidly with time in the range of 0~5 min. After adsorption of 20min, the adsorption rate of bacteria almost did not change with the increase of adsorption time, and the adsorption of *E. coli* to minerals reached a balance. At this time, the adsorption rate of *E. coli* to dolomite is less than 50%, while the adsorption rate of collophanite is less than 20%. The adsorption capacity of *E. coli* to dolomite is much better than that of collophanite.

It can be seen from Fig. 6 that the adsorption of *E. coli* onto the surface of the collophanite and the dolomite decreased with the increase of pH, and the adsorption rate of *E. coli* to dolomite is always higher than that of the collophanite. The aggregation of bacteria occurs under acidic conditions. Moreover, the lower the pH value is, the more obvious the agglomeration phenomenon is. The aggregation and precipitation of bacteria make the calculated adsorption rate higher. When the pH value is about 7.8, the adsorption rate of *E. coli* on the surface of dolomite is close to 50%, while the adsorption rate onto the surface of the collophanite is below 30%. Under these conditions, the selective adsorption of *E. coli* to dolomite was the best. Under strong alkaline conditions, the amount of bacteria adsorbed onto minerals is not high. This phenomenon may be due to the competitive adsorption between -OH and bacteria (Tong et al., 2020).

4.2.3. Effect of *E. coli* on Zeta potential of collophanite and dolomite

Zeta potential can reflect the behaviour of particles in dispersed system to a certain extent and is an

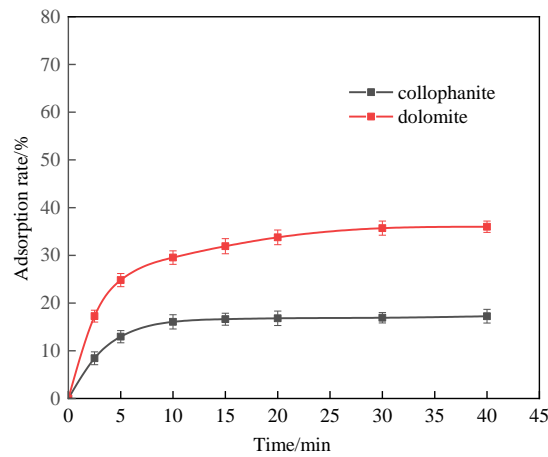


Fig. 5 . Effect of adsorption time on *E. coli* adsorption ($c_{\text{core}}=10\text{g/L}$, $c_{\text{bal}}=2.4\text{g/L}$, $\text{pH}=8.04$, $v=110\text{r/min}$, 25°C)

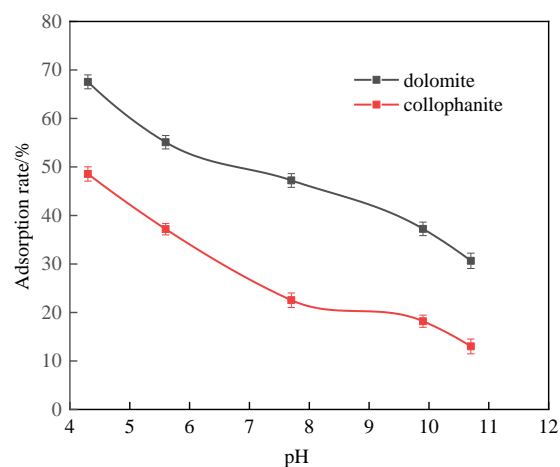


Fig. 6 . Effect of the initial solution pH value on *E. coli* adsorption ($c_{\text{core}}=10\text{g/L}$, $\text{pH}=8.04$, $v=110\text{r/min}$, 25°C , 20min)

efficient method to interpret the trend of the flotation efficiency and the modification performance caused by the presence of reagents (Tian et al., 2017; Li et al., 1966). The pH value of flotation pulp directly determines the charge of mineral particles and then affects the adsorption of *E. coli* on the mineral surface. Therefore, zeta potentials of samples, *E. coli* and samples adsorbed by *E. coli* were measured at different pH. The results are shown in Fig. 7 and Fig. 8.

It can be seen from Figs. 7 and 8 that PZC of *E. coli*, collophanite, and dolomite is very close, and their PZC is $\text{pH}=4.3$, $\text{pH}=4.5$, and $\text{pH}=5.6$, respectively. Under the condition of weak acid, the surface potential of *E. coli*, collophanite and dolomite is close to 0, Bacteria, ore and minerals coagulate easily with each other and can maintain a certain adsorption rate. When the pH is more significant than 5.6, *E. coli*, collophanite, dolomite surfaces are negatively charged, which was correlated with previous reports (Hakim et al., 2019; Zhou et al., 2017; Zhang et al., 2018). Under this condition, the adsorption of *E. coli* increases the negative potential of collophanite and dolomite. Therefore, the adsorption of *E. coli* on the surface of collophanite and dolomite is not physical adsorption caused by electrostatic adsorption, but chemical adsorption caused by other effects.

4.2.4. Infrared spectra of minerals adsorbed on *E. coli*

The infrared spectrum results are shown in Figs. 9 and 10. The *E. coli* cell surface component contains active groups such as $-\text{OH}$, $-\text{NH}$, $\text{C}=\text{O}$ and $\text{P}=\text{O}$. The N, O and P elements in these groups can provide lone pair electrons, which can bond with metal elements on the mineral surface and play an essential role in the adsorption process (Ci et al., 2007; Nie et al., 2002; Wang, 2017). In comparison with the spectral lines in Fig. 9, a new absorption peak is generated at 1654.03 cm^{-1} in the (a) line, corresponding to the $\text{C}=\text{O}$ (Liu et al., 2000) stretching vibrational absorption band in the protein amide

I band. At 1454.39cm^{-1} , the peak absorption peak shifts and moves toward the low wave. The absorption peak is C-H flexural vibration absorption band in CH_3 (Wang et al., 2011). These results indicate that *E. coli* was chemically adsorbed onto the surface of collophanite, and formed hydrogen bond with *Escherichia coli* on collophanite surface.

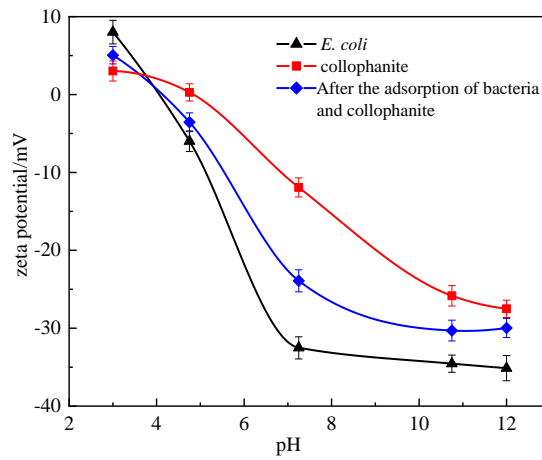


Fig. 7. Effect of *E. coli* on zeta potential of collophanite

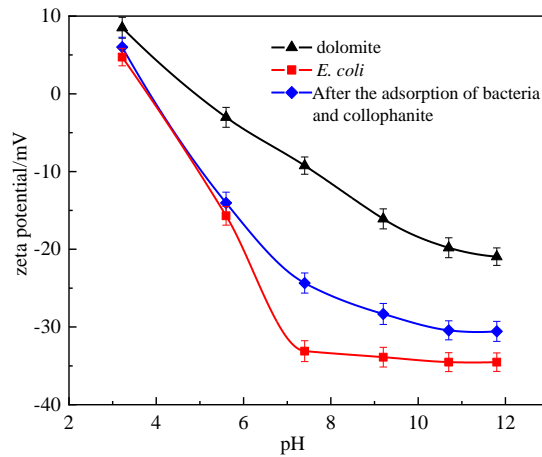
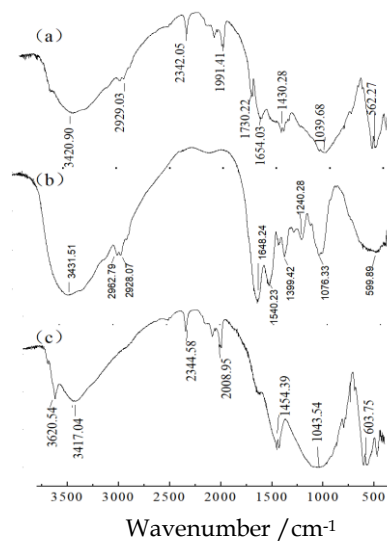
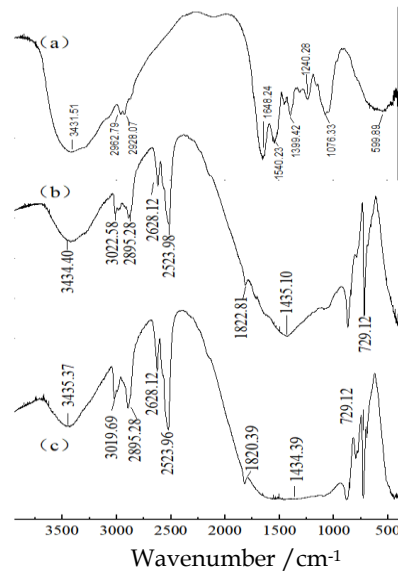


Fig. 8. Effect of *E. coli* on zeta potential of dolomite



(a) After the adsorption of bacteria and collophanite (b) *E. coli* (c) collophanite

Fig. 9. Infrared Spectra of *E. coli* and collophanite



(a) *E. coli* (b) collophanite (c) After the adsorption of bacteria and dolomite

Fig. 10 . Infrared Spectra of *E. coli* and dolomite

In Fig. 10, the characteristic absorption peak of dolomite is at 1434.39cm^{-1} in the spectral line (c). In comparison with each of the lines in Fig. 10, there is no new absorption peak in the spectral line (b). This may be because the specific absorption peak of dolomite is too sharp, and the absorption peaks of other chemical bonds are shielded. The peak value at 1435.10 cm^{-1} in the spectral line (b) is significantly weaker than that of line (c), because of the influence of hydrogen bonds or other chemical bonds between bacteria and minerals.

5. Conclusions

Escherichia coli can depress the flotation of collophanite and dolomite. However, the inhibition of dolomite is more intense. *E. coli* has a robust selective depression on the flotation of dolomite.

With *E. coli* as a depressant of dolomite, a useful beneficiation index of phosphorus concentrate with P_2O_5 grade of more than 30%, and MgO content less than 1.2% can be obtained by a closed circuit of one-stage roughing separation and one-stage scavenging. The content of sesquioxide in the concentrate is less than 3%. The use of *E. coli* as a depressant for the direct flotation of phosphate rocks has an effect of removing magnesium and sesquioxide.

The adsorption ability of *E. coli* to dolomite is more potent than that of collophanite. When pH is greater than 6, the bacteria are chemically adsorbed onto the surface of the collophanite and dolomite, which also increases the negative charge on the surface of the two minerals.

When the pH value is about 7.8, the adsorption rate of *E. coli* onto the surface of dolomite is less than 50%, while the adsorption rate on the surface of the collophanite is below 30%. Under these conditions, the selective adsorption of *E. coli* to dolomite was the best.

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