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Bio-collector alternative for the recovery of organic matter in flotation processes

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Recovery of organic matter by change of electric charge caused by formation of monolayers.

Application of bacteria as a collector in flotation processes.

Enhance the flotability of fine particles of bio-modified coal.

Utilization of bacteria as biological alternative to chemical reagents collectors for coal flotation.

Possibility future for development of flotation processes minerals friendly environment.

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In this study a strain of Staphylococcus carnosus whose characteristics of hydrophobicity and ability to change the surface charge of some minerals such as coal, by excreting some exopolymers, has been used as bio-collector to evaluate the recovery of fine coal tailings mineral through micro-flotation, from the two sub-basins located in the Sabinas basin and identified as: Sabinas (CFP) and Rio Escondido (CFM), located in Coahuila, Mexico. Results show that in the absence of microorganism recovery is about 50% and that using S. carnosus recovery reaches values close to 90% in both samples of coal for a time of 12 h and a pH of 9. Mechanism has been investigated using techniques such as adhesion kinetics, adsorption isotherms, and infrared spectroscopy. It illustrates that biofilm consisted of excreted chemicals, such as fatty acids, improves the hydrophobicity of the coal surface.

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1. Introduction

Every year in Mexico, nearly 6 million tons of fine coal $($ <75 μ m) are generated during the washing process. These residues are commonly considered an economically and technically difficult waste to recover, with this waste in most cases stored in yards without being processed, Thus causing serious economic losses, and environmental pollution.

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In view of this fact, the mining industry has shown a strong interest in searching for new alternatives to improve the process of coal washing, as well as more effective reagents to improve the process and reduce operating costs. The recovery of organic matter present in coal tailings also represents an important source of energy that has both economic and environmental benefits for the mining sector [\[1\]](#page--1-0).

Bioprocessing fine coal is considered as an alternative to conventional processes, which have shown a great potential for coal cleaning. These extraction processes are mainly bioflocculation and bioflotation in which selective mineral removal is accomplished by the application of microorganisms, which change the surface properties of the minerals.

In the process of bio-flotation, bacteria replace conventional chemical reagents such as Methyl Isobutyl Carbinol (MIBC) and diesel, or they work in synergy with them to produce concentrates

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of a higher quality with a reasonable and selective mineral recovery [\[2,3\]](#page--1-0). A variety of species of bacteria are used to modify and improve mineral separation in the presence or absence of regular collectors [\[3,4\].](#page--1-0) The most commonly used microorganisms are: Acidithiobacillus ferrooxidans [\[5–7\]](#page--1-0), Leptospirillum ferrooxidans [\[8,9\]](#page--1-0) and, less commonly, Staphylococcus carnosus, Bacillus firmus, Bacillus subtilis and Bacillus polymyxa [\[10\].](#page--1-0)

Compared to conventional inorganic reagents, bacteria are non-toxic and environmentally benign, potentially providing an alternative to conventional flotation methods [\[11\]](#page--1-0). These bacteria selectively bind to mineral surfaces forming a biofilm or exopolysaccharides (EPS) layer [\[12\].](#page--1-0)

EPS are high molecular weight molecules consisting of polysaccharides, DNA, proteins, lipids and humic acids which are released by microorganisms, and then adhered to the cells surfaces [\[13\].](#page--1-0) According to previous studies, the production of EPS is influenced by the medium used (e.g. carbon source) [\[14\]](#page--1-0) growing conditions, operating conditions (e.g. Optical Density (DO)) [\[15\].](#page--1-0) Furthermore, the high surface hydrophobicity and bacterial loading have a tendency to allow EPS formation [\[16\]](#page--1-0).

The surface roughness and energy of the substrates are essential for the development of a biofilm and the attachment of microorganisms [\[17\].](#page--1-0) Many bacterial cells are more easily deposited on hydrophobic surfaces due to interactions of this type, while others are adsorbed on hydrophilic surfaces.

There are hydrophobic microorganisms that can be used as collectors and they adhere to the mineral surface during bioflotation due to changes in surface charge of the substrate/mineral. Such is the case of S. carnosus, which is a hydrophobic bacteria, a feature of its cell wall, which is composed of thin layers of two sugar derivatives, N-acetylglucosamine and N-acetylmuramic acid, and a small group of amino acids including L-alanine, D-alanine, Dglutamic acid and either lysine or diaminopimelic acid. These components are joined together to form a repetitive structure called tetrapeptide glycan. The use of S. carnosus as a collector could favor the modification of the surface charge on the fine coal particle and improve the extraction during the flotation process [\[19\]](#page--1-0).

Therefore, this study is aimed at evaluating the effect on flotability of fine coal through the use of S. carnosus.

2. Materials and methods

2.1. Coal sample

Two samples of coal tailings were collected from two coal washing plants of different zones of the Carboniferous Region of the State of Coahuila. Selected samples were identified as CFP derived from the Sabinas Basin and CFM from Rio Escondido Basin. Each of the fine coal samples was wet sieved employing -100 +200, -200 +400, and -400 meshes. The fraction between -100 $+200$ (-150 µm to $+74$ µm), -200 $+325$ (-74 µm to $+44$ µm) was separated for the flotation and adhesion tests. These particle size fractions were selected because it was found where a higher percentage and content of coal exist.

The chemical reagents usually used are Diesel as a collector and MIBC (methyl isobutyl carbonyl) as a frother. Solutions were prepared using distilled water.

And the surface area for fine coal sample CFP was 1.640 m²/g, while for the CFM shows the value was 9.980 m²/g.

2.2. Microorganisms

2.2.1. Culture of microorganisms

The strain used in this study was ATCC 51365 S. carnosus. The choice of bacterium is for their characteristics of ability to modify hydrophobicity and the mineral surface. One of the bacterium S. carnosus features formed as grapes, in the form of cocci. The cell wall is peptidoglycan which is composed of a polymer of sugars and amino acids. Other constituents that extend from peptidoglycan are teichoic acids, lipoteichoic acids and proteins. The cells composed of higher amounts of protein contribute to the hydrophobic property while the polysaccharides on the cell surfaces impart a hydrophilic character.

The organism was cultured using: 30 g of Trypticase Soy Broth and 3 g of yeast extract (ATCC Medium No 1887), which were diluted in 11 of distilled water. The initial pH was adjusted to 7 using KOH. The medium was subjected to a sterilization process in a Model 25X ALL AMERICAN autoclave under standard conditions. Once at room temperature the broth was inoculated with the freeze-dried bacteria and incubated for 24 h at 37 \degree C. Subsequently, an inoculum was put under rotation at a speed of 200 rpm for 12 h at 37 \degree C.

2.2.2. S. carnosus growth kinetics

The kinetics of bacteria growth was carried out by measuring the number of microorganisms versus time (0–60 h). Microorganism counting was done in a Neubauer chamber (Neubauer Chamber Reichert) in the phase contrast microscope (Olympus BX53).

2.3. Microflotation

Microflotation testing was carried out in a modified Hallimond tube, which was placed on a magnetic stirrer. Flotation was divided into two categories: conventional flotation and bioflotation.

2.3.1. Control (conventional flotation)

One gram of fine coal sample (CFM/CFP) was weighed and mixed in 70 ml of aqueous solution at pH 9 (distilled water at a controlled pH by addition of NaOH) for 10 min in a Bunsen beaker of 200 ml capacity. As chemical reagents Diesel (collector) and MIBC (frother)) were used. The mixture (water/coal) was poured into the Hallimond cell in which 150 μ L of diesel and 70 μ L MIBC were added. The mineral was conditioned for 7 min at 150 rpm. Air was introduced into the cell at a flow rate of 200 ml/min with a pressure of 4.13×10^5 Pa.

2.3.2. Bioflotation

Prior to microflotation an inoculum with S. carnosus bacteria (bacteria + medium) was prepared, at a pH of 9. The incubation time was 12 h at 37 \degree C and 200 rpm. Afterwards, the inoculated medium was adjusted at a concentration of 1×10^9 cell/ml. 1 g of fine coal (CFM/CFP) was weighed and added along with the inoculated mixture in a 250 ml shake flask. Afterwards, the culture and coal were for 8 h stirring at a speed of 200 rpm at room temperature.

In order to secure an initial inoculum, the coal sample was taken 12 h after the process was initiated. During flotation the mixture bacteria/coal was poured in the flotation cell, which was regulated to a pressure of nitrogen and oxygen at 4.13 \times 10⁵ Pa and an air flow of 200 ml/min.

The flotation times were from 0 to 6 min. The settled and floated fractions were carefully separated, washed, dried and weighed. The flotability was then calculated as the ratio of floated and non-floated mineral by using the following formula:

%Plotability =
$$
\frac{C}{F}
$$
 × 100

where F is the weight in grams of mineral fed and C is the weight in grams of concentrated mineral (floated).

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