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Influence of *S. carnosus* Bacteria as Biocollector for the Recovery Organic Matter in the Flotation Process

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***Abstract*—**The mineral bioflotation represents a viable alternative for the evaluation of new processes benefit alternative. The adsorption bacteria on minerals surfaces will depend mainly on the type of the microorganism as well as of the studied mineral surface. In the current study, adhesion of *S. carnosus* on coal was studied. Several methods were used as: DRX, Fourier Transform Infra-Red (FTIR) adhesion isotherms and kinetic. The main goal is to recovery of organic matter by the microflotation process on coal particles with biological reagent (*S. carnosus*). Adhesion tests revealed that adhesion took place after of 8 h at pH 9. The results suggest that the adhesion of bacteria to solid substrates can be considered an abiotic physicochemical process that is consequently governed by bacterial surface properties such as their specific surface area, hydrophobicity and surface functionalities. The greatest coal fine flotability was of 75%, after 5 min of flotation.

***Keywords*—**Fine Coal, Bacteria, Adhesion, recovery matter organic.

I. Introduction

CONVENTIONAL coal process beneficiation generates large quantities waste and these residues are stored in the tailings dams coal washing plants. Which for the mining sector is a serious problem of environmental pollution, because a part of this waste coal still contain a significant quantity of organic matter that find it hard to recover in conventional processes. Basically, the coal beneficiation process consists of separating the organic matter of coal inorganic matter that is associated with the impurities of this mineral as gypsum, kaolinite, quartz and others. The separation can be effected by various physicochemical or microbial techniques. The technique most commonly used in coal washing plants is Flotation process. A disadvantage of this process is that only can recover particle sizes of coal greater than 75 μm.

The combination between traditional flotation technology and biological microorganisms attracts attentions in all the word. In bioflotation process, bacterial replace the conventional reagents or work synergistically with them to produce high-grade concentrates with reasonable recovery [1]-[3]. One of the most important steps in mineral bioflotation is the adhesion of the microorganism onto the mineral surface [4], [5]. These microorganisms may act as bioreagents and induce hydrophobic properties once they have adhered to the mineral surfaces [6], [7]. The interactions that result in such adhesion include van der Waals forces and hydrophobic interactions, all of which are determined by the cell-wall and mineral surface properties [5], [8], [9].

Compared to conventional inorganic reagents, bacteria are non-toxic and environmentally benign, potentially providing an alternative to conventional flotation methods.

The aim of this study was the effect of recovery coal fine by using *S. carnosus* as biocolector flotation process. The main objectives were (i) optimization of operating parameters such as pH, flotation time, collector, and bacterial cell concentration and (ii) evaluation of the efficiency of biocolector bacteria.

II. Experiments

A. Coal Tailings Sample

Two samples of coal tailings were collected from two coal washing plants of different zones of the Carboniferous region of Coahuila Mexico. Selected coal samples were identified as CFPL derived from the Sabinas Basin and CFMK from Rio Escondido Basin. Each of the coal samples was wet sieved employing -100 +200, -200 +325, and -400 meshes. The fraction between -100 +200 (74μm), -200 +325 (40 μm) was separated for the flotation and adhesion tests. The proximate and ultimate analysis of the coal samples was carried out and the type of associated minerals was determined by X-ray diffraction (XRD) (JEOL JDX 8030). The surface area was determined by BET surface area analyzer.

The chemical reagents conventional usually used are Diesel as a collector and MIBC (methyl isobutyl carbonyl) as a frother. And in the bioflotation bacteria *S. carnosus* was used a biocollector and as a n-hexadecane as a frother. Solutions are prepared using distilled water.

B. Microorganisms

1) Bacterial Culture

The strain used in this study was ATCC 51365 *Staphylococcus carnosus*. The components of medium were 30 g of Trypticase Soy Broth and 3 grams of yeast extract (ATCC Medium No 1887), which were diluted in 1 liter of distilled water. The initial pH was adjusted to 7.0 using KOH.

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The bacteria were incubated for 24 h at 37°C and after were put under rotation at a speed of 200 rpm for 12 h at 37°C.

2) *S. carnosus* Growth Kinetics

The kinetics of growth of the bacteria was carried out by measuring the number of microorganisms versus time (0 to 60 h). The change in cell number in the suspension was measured by microscopic counting using a Neubauer (Neubauer Chamber Reichert (Olympus BX53).The difference in the cell count was taken as the number of cells adhering to the coal surface

C. Adhesion Isotherms

For the adhesion isotherms 1 g fine coal sample was used in 50 ml of fresh bacterial culture. Aliquots were taken every 0, 2, 4, 8, 12, 24 and 36 h. The initial concentration of the bacteria and at time t was determined by direct counting in a Neubauer chamber. The amount of bacteria adhered at a certain time t is determined by the following formula:

$$B_{Adh} = \frac{(B_0 - B)V}{W * A_{sup}}$$

where B_{adh} is the adhering bacteria (cells/ml): B_0 and B are the concentration of free bacteria in time zero and t, respectively (cells/ml); V is the volume of sample in ml; w is the weight of the coal sample (g) and finally A_{sup} is the surface area of the mineral.

In this case the surface area for fine coal sample CFPL was 1.640 m^2/g, while for the CFMK shows the value was 9.980 m^2/g.

D. Microflotation

Changes in particle surface hydrophobicity were correlated with mineral recoveries from microflotation experiments. Each microflotation test was carried out in a modified Hallimond tube, which was placed on a magnetic stirrer. By the case of bioflotación (coal/bacteria) was necessary one gram of coal CFMK/CFPL sample and suspended in 70 ml of medium in a conical flask containing $1x10^9$ (cells/ml) of bacteria cells at pH 9. The flask was incubated on a rotary shaker for 8 h. After, the mineral and bacteria were conditioned with 70 µl n-hexadecano (froth) for 7 minutes in the modified Hallimond tube. Flotation of mineral/bacteria was carried out using nitrogen and oxygen bubbled to separate trough the flotation mixture at to flow rate of 150 ml/min and a pressure of 60 psi.

In the case of the conventional flotation, the same procedure was used, 1 gram of coal and mixed with 70 ml of distilled water, both were conditioned for 7 minutes with 70 µl of n-hexadecane (froth). Flotation of mineral was made at the same conditions that bioflotation and only was used oxygen bubble in the process.

All the above experiments were carried out in triplicate.

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

$$\%\ Flotability = \frac{C}{F} X\ 100$$

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

E. Fourier Transform Infrared Spectroscopy (FTIR) Analysis

Fourier transform infrared spectroscopy (FTIR) analysis was carried out on a Perkin-Elmer spectrophotometer model Spectrum FTIR System. The spectra were analyzed under the ATRI technique with an Amiths brand Model Durasampi IR IIII accessory, with a 1 rebound diamond window. The internal operation detector is a DTGS with a spatial resolution of 10mm. All FTIR spectra obtained were reported in the range 550 to 4000 cm^{-1} with a resolution of $4cm^{-1}$, and a co-addition of 16 scans to remove any noise signal relation. The spectra were displayed in terms of absorbance.

III. Results and Discussions

A. Coal Characteristics and XRD

CFMK and CFPL coals samples were characterized by XRD and chemical analysis. In Table I present results of proximate and ultimate analysis of fine coal CFMK and CFPL samples.

TABLE I
PROXIMATE AND ELEMENTAL ANALYSIS OF COAL SAMPLE IN ADDITION TO THE SURFACE AREA FOR THE COAL SAMPLE CFPL AND CFMK

Sample	CFPL	CFMK
Proximate analysis		
Ash content %	38.86	40.70
Sulfur %	0.94	1.38
Volatile matter (%)	23.24	17.21
Calorific value (kcal/kg)	4751	4061
Elemental analysis (%)		
Sulfur	0.28	0.26
Fierro	1.13	1.074
Carbon	37.24	44.06
Nitrogen	0.83	0.89
Hydrogen	3.25	3.56
Surface area (m^2/g)	1.640	9.980

As can be seen, both the coals are high in ash and volatile matter. On the other hand, XRD of the coal samples revealed that CFMK coal is more amorphous carbon that CFPL coal due to the presence of broad peaks between 2h=12° and 27°, in addition, quartz and Kaolinte are the main mineral matters (ash) associated with these (Fig. 1). And the surface area of each coal sample showed in Table I.

B. S. carnosus Growth Kinetics

In Fig. 2 shows the kinetics of growth of *S. carnosus* in relation to time of incubation. The figure shows that the exponential phase starts at 2 hours and ends at about 9 hours. And also it was determined that the optimal incubation time for the bacterium is no more than 24 h since the concentration

of bacteria after 20 at 60 h is almost constant and does not change much.

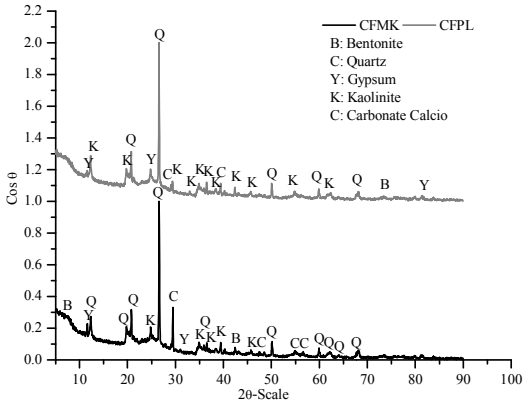

Fig. 1 XRD fine coal CFMK y CFPL sample

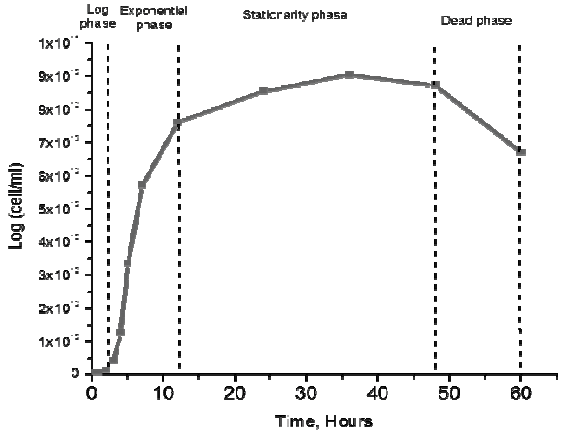

Fig. 2 Growth curve of *S. carnosus*

C. Adhesion Isotherms of S. carnosus on to Coal

Fig. 3 shows the adhesion isotherm of *S. carnosus* on the surface of coal CFMK and CFPL samples. The adhesion isotherms indicate that a large amount of bacteria were adhered onto coal. Coal CFMK samples at pH 6, 7, and 8 shows a less adhesion of bacteria compared to CFPL samples. The cause why this happens is that the CFMK sample shows a higher content of inorganic matter (ash).

On the other hand the adhesion of the bacteria on coal surfaces was carried out as a function of time and pH. The kinetics of adhesion revealed that most of the adhesion took place in about 12 hrs at pH 9. The cause for this is because the surface area of the CFMK>CFPL sample. In contrast, *S.carnosus* tends to self-protection by forming a biofilm under more basic conditions after 6 h, it shows more concentration on the coal surface. EL-Midany et al. [10] indicate that this biofilm has a hydrophobic nature such as that of amorphous carbon, and leads to bacterial adhesion. Moreover, the difference in adhesion between the two coal samples can be attributed to the difference in hydrophobicities in both samples.

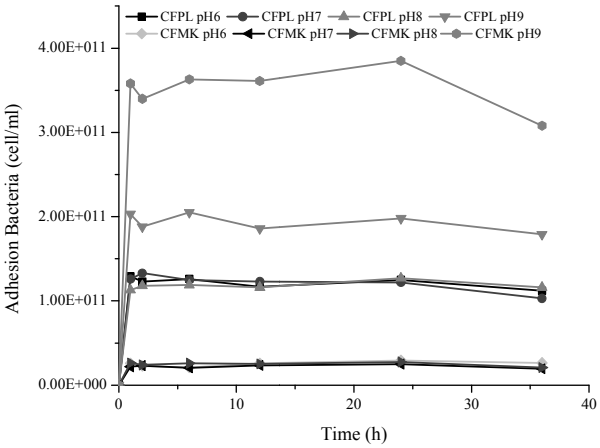

Fig. 3 Adhesion Isotherm of studied bacteria on fine coal CFMK and CFPL at different pH

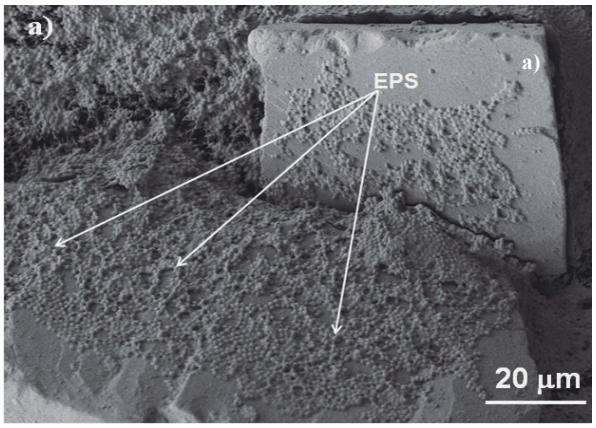

Fig. 4 (a) SEM monograph of *S. carnosus* on fine coal CFMK surface after 12 h at pH=9. Biofilm form on the coal surface during adhesion test

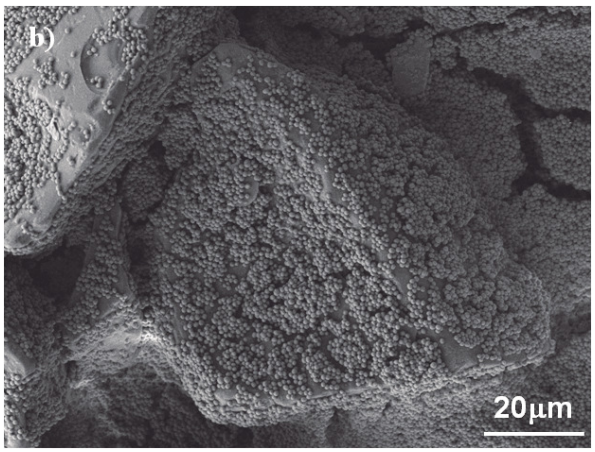

Fig. 4 (b) SEM monograph of *S. carnosus* on fine coal CFPL surface after 12 h at pH=9

A. SEM Micrograph and FTIR Spectra of S. carnosus on Fines Coal Samples

The micrograph of *S. carnosus* adhering to coal surface is shown in Fig. 4. A uniform layer of bacteria is formed on the surface of both coal samples. In the case of Fig. 4 (a) can be observed formation of biofilms onto the surface of the coal CFMK sample. While in Fig. 4 (b) is located a multi-layered formation of bacteria surrounding the fine coal CFPL particle.

Figs. 5 (a) and (b) depict the FTIR spectra of coal CFMK and CFPL samples, *S. carnosus* bacteria and their interactions product at pH 9. The FTIR spectra of *S. carnosus* show the characterizing peaks of bacteria: the band at 1639 cm^{-1} is characteristic of Amide I (C=O) in the protein, 1535 cm^{-1} is characteristics of bending vibrations of amide II (C=O) in proteins. The band at 1370 cm^{-1} can be assigned at the fatty acids and proteins groups. The bands between ranges of 1200-900 cm^{-1} can be assigned to complex vibration modes of polysaccharides. Finally at 854 cm^{-1} the bending bands or the aromatic –CH moiety appears.

In the case of coal, the FTIR spectra, Fig. 5 exhibit a broad absorption band between the ranges of 3600-3200 cm^{-1} this band corresponds to the –OH groups. Oxygen functional groups found in coal are referred to presence of organic compounds such as: carboxylic acid and alcohols. The bands at 2922, 2917, and 2850 cm^{-1} corresponds at the stretching vibration of aliphatic – CH, -CH_2 and –CH_3 and band around 1437, 1436 cm^{-1} are assigned to aliphatic –CH bending vibration.

The bands at 1500-1600 cm^{-1} are assigned to the stretching vib8rations of C=C bonds in aromatic structure and probably as well as C=O stretching vibrations of carbonyl groups. In addition, there are prominent bands in the 1200- 1000 cm^{-1} region due to Si-O bending vibrations. Low intensity aromatic –CH bending bands were observed between 700 and 875 cm^{-1}. Finally the absorption bands at 912 cm^{-1} were assigned to Si-O-Si bending vibration.

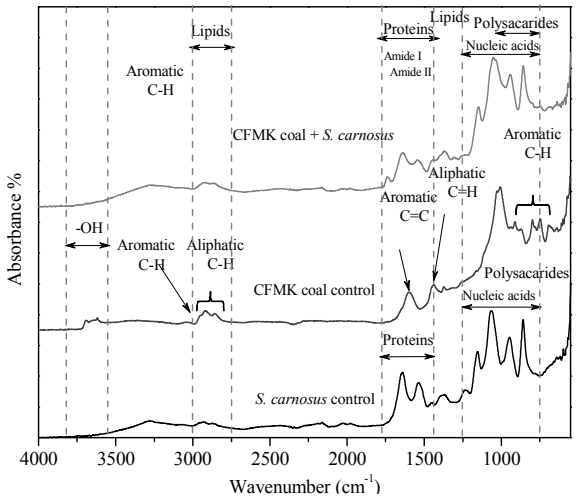

Fig. 5 (a) FTIR of CFMK coal and *S. carnosus* before and after their interaction

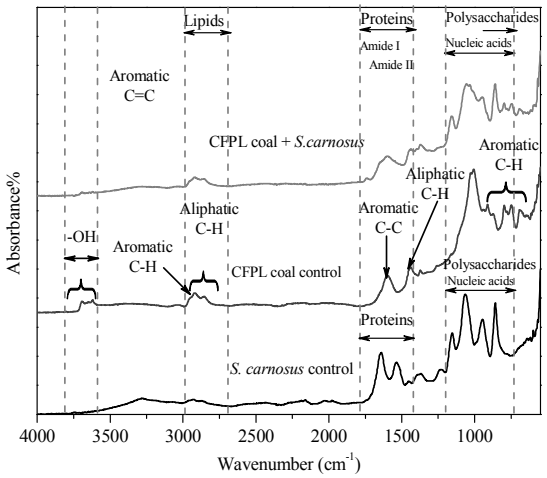

Fig. 5 (b) FTIR of CFPL coal and *S. carnosus* before and after their interaction

In Fig. 5 there is a reduction in fatty acid and OH groups peaks with no noticeable changes in the main peaks of both coal and bacteria. The reason behind the reduction of fatty acid and OH groups, indicating their significant role in *S. carnosus* adsorption on coal surface, is the effect of hydrogen bonding and the hydrophobic forces between the bacteria wall and the organic matter in coal, [11]-[13]. Or the –OH group presents in coal CFMK/CFPL controls corresponds to kaolinite present in the mineral matter of the coal.

Finally, the composition of the cell walls (lipid, glicolipid and phospholipids, polysaccharides and the carboxyl groups) controls the adsorption of bacteria to mineral surface, [14], [15].

B. Microflotation

The flotability studies were performed to evaluate the potential of using conventional reagents versus *S. carnosus* bacteria as biocollector for the fine coal samples. Fig. 6 shows the floatability results as function of time for coal CFMK and CFPL samples used different type of gases (N_2 or O_2) in flotation process.

It can be observed, for both coal samples, that the highest flotability was attained at pH 9 and using gases (N_2 or O_2). And the coal samples floatability achieved recovery of organic matter with *S. carnosus* bacteria ≥75%, whereas in the conventional process were ≤ 35%. This means that the use of this organism favors the recovery of fine coal particles 45 μm. This means that the recovery of organic matter is increased due to biomodification, thus confirming the hydrophobic character of the bacterium *S. carnosus*. The cause of these results can be attributed to the presence of fatty acids of the functional group -CH_2 on the surface of the bacteria, which causes selective adhesion of the particles of hydrophobic character such as coal. Another possibility is that the improved hydrophobicity of coal might be due to the secretion of the proteins induced by bacteria in the cell-free extract during their growth in the presence of coal. Hanumantha et al. 2010 [15] also mention that the adhesion of microorganisms on the

surface of coal is accompanied by the expression of extracellular polymeric substances (EPS), which allows adhesion and this way the charge of the mineral surface changes.

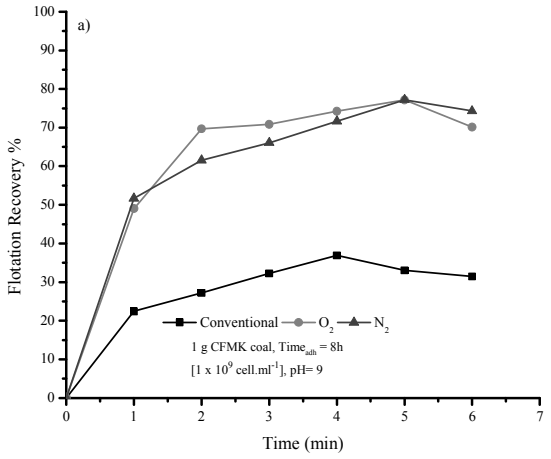

Fig. 6 (a) Microflotation using *S. carnosus* as collector at pH 9 in CFMK coal sample

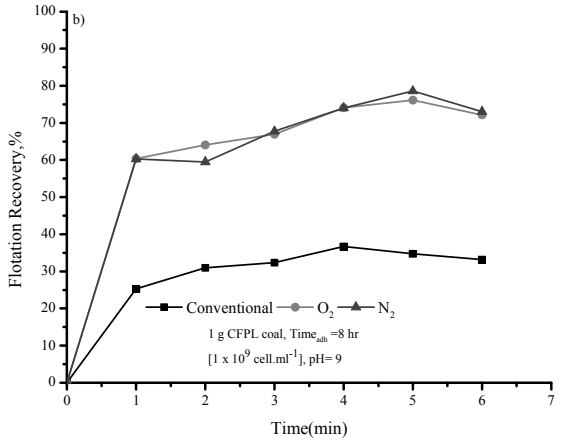

Fig. 6 (b) Microflotation using *S. carnosus* as collector at pH 9 in CFPL coal sample

Finally it can be said that *S. carnosus* has a hydrophobic effect on coal, which means that the microorganism may act as a natural collector and promote selectivity of valuable ore in coal cleaning processes. Besides, the application of this process by injecting an air flow in the process is more economical than the use of N_2 gas.

IV. Conclusions

The results indicated that the best conditions of adhesion were obtained at pH 9 in both coal samples and used *S. carnosus* bacteria as biocollector.

- The capacity that has *S. carnosus* to form bio-films favors the recovery of fine coal particles (CFM and CFP) during bioflotation, because it generates a change in the surface charges on the surface of the particles thereby contributing to the flotation.
- The use of *S. carnosus* as collector in addition to being a more friendly process with the environment turns out to be very efficient for the recovery of fine coal, with recoveries up to 90%.
- FTIR spectra results indicated that the adsorption of bacteria on the coal surface is physical and the main affecting forces are the electrostatic forces in addition to hydrogen bonding and long term hydrophobic forces.

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