

Adsorptive removal of *Escherichia coli* bacteria by Natural and Modified kaolin: Characteristics, isotherm, kinetic and SEM studies

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ABSTRACT/RESUME

Abstract: The adsorption of *Escherichia coli* bacteria on natural and modified kaolin was investigated as a function of contact time, initial bacterial load, pH and temperature using batch studies. Maximum retention of *E.coli* was done at pH 4 and at a temperature of 30°C. The adsorption capacities of the modified kaolins KC (92%) and KS (98%) were greater than natural kaolin K08 (59%). The linear models of Langmuir and Freundlich were applied to describe the equilibrium isotherms and Freundlich equation was more in line with the experimental results. The adsorption processes followed pseudo-second order kinetics and a multilayer regime, which was confirmed by the SEM images which show that *E. coli* is adsorbed alone or in the form of aggregates on the clay or on mesoporous surfaces. The results obtained in this study will provide valuable information for a better understanding of the mechanisms of mineral-microorganism interactions in soil and associated environments. Experiences demonstrated also excellent hydraulic performance of these supports, which can make them an inexpensive alternative, eager for toxicity in the field of water treatment.

I. Introduction

Bacteria are adsorbed on the surface of minerals in soils, sediments and groundwater. Adhesion can significantly alter the activity and ecological distribution of bacteria [1], biomineralization [2], alteration of minerals and biodegradation of pollutants [3]. Therefore, understanding the sorption process of bacteria on minerals is ecologically and geologically important.

The surface of the bacteria usually carries many negative charges under physiological conditions from the weak acidic and basic functional groups of the polypeptides in bacteria [4]. Clay minerals are also negatively charged in most cases. Therefore, the physical contact of the bacteria with the mineral surface must overcome the electrostatic repulsion force.

Recently, a number of studies have examined bacterial adsorption on clay minerals. We cite as example the work of Jiang for *Pseudomonas putida*, which preferred adsorption on the surface of goethite, followed by kaolinite, then montmorillonite. Electrostatic interactions and hydrogen bonds were considered essentially to contribute to the adsorption of *P. putida* on these clays [5]. Chemical interaction is probably the main factor that makes some bacteria have selective adsorption on different mineral surfaces, in which the functional groups on the cell surface play an important role [6] because bacterial colloids' behavior in the environment is strongly influenced by their surface hydrophobicity. Most bacterial surfaces are generally hydrophobic, this results from the properties conferred by the membrane constituents, in particular lipid. However, certain membrane constituents exhibit

both hydrophobic and hydrophilic properties, which induces a high variability in the apparent hydrophobic / hydrophilic character of bacterial cells [7]. Surface proteins, lipoteichoic acids and LPS confer hydrophobic properties on surfaces [8]. Phospholipids have a hydrophilic "head" and hydrophobic "tails". In addition to these structural effects on the hydrophobic properties of bacterial cells, there are abiotic constraints such as pH, temperature or the ionic strength of the solution that control the reactivity of bacterial surfaces which, therefore, can also modify the apparent hydrophobicity of cells. The surface charge is not measured directly but it can be estimated either by measuring the electrophoretic mobility (speed of movement of particles subjected to an electric field), or calculated from the various models based on the concentrations of adsorbed species. In weakly concentrated media, a measurement of the electrophoretic mobility of the particles makes it possible to estimate the Zeta potential (ζ), representative of the effective charge of the particle ("particle + diffuse layer" charge). The pH (pHpzc), for which the surface charge is zero (Σ positive charges = Σ negative charges) is of particular interest, because there is no more electrical repulsion between the different particles. For "environmental" pH, between pH 4 and 8, most bacterial cells have a negative surface charge [8].

Despite these efforts, information on the surface properties of bacteria and clay minerals and their effects on adsorption behavior are still lacking, as different bacteria may in particular appear to have different affinities for various minerals.

In the present work, experiments were carried out to evaluate the adsorption properties of *Escherichia coli* bacteria (*E.coli*) on three kaolinic supports (K08, KC and KS). The mechanisms and parameters influencing bacterial adsorption on these supports have also been studied.

II. Materials and methods

II.1. Bacteria and growth conditions

The bacterial strain used in the present work is *Escherichia coli* (*E.coli*) which was isolated and identified by previous work carried out in the Biology research laboratory (VRVSA: Laboratory for the Valorization of Plant Resources and Food Safety in semi-arid zones of southwest Algeria) at the University of Bechar. The nature of the bacteria was confirmed by a method called Observation in the fresh state, which is an observation using a microscope between slide and coverslip under different magnifications x10, x40 and x100 to confirm the purity, mobility and forms stumps. Then, we moved on to the Gram stain, which is a stain that allows to highlight the properties of the

bacterial wall and to use these properties to distinguish and classify them. Bacteria can be gram positive or negative microorganisms. *Escherichia coli* are gram negative. Growth procedures for *E.coli* were similar to those described earlier by Rong et al. [3]. In brief, the colony of *Escherichia coli* was initially inoculated into Luria Bertani nutrient broth and shaken at 150 rpm at 37 °C for 18 h. The cells were separated from the culture medium by centrifuging the suspension at 4000 rpm for 15 min, followed by three-time wash with sterilized distilled-deionized (ddH₂O). Previous work has indicated that the washing procedure does not significantly alter the structure of the cell wall [9]. Then, the bacteria were resuspended in (ddH₂O) to obtain the bacterial suspension. The bacterium concentration of aqueous solution was determined by measuring the optical absorbance (OD 620) of the suspension, which was then dried (105 °C) and weighed. Dry weights were plotted vs. measured absorbance to produce the standard curve that was linear and used in all subsequent experiments.

II.2. Clay Minerals

The natural mineral K08 used as an adsorbent was collected from a natural deposit, located in Tabelbala province, Bechar (Algeria) see Figure 1. It consists mainly of kaolinite in the presence of other mineral species which are illite and quartz. By providing that the incorporation of organic molecules in the clay layers will modify their adsorbent properties, thus improving their affinity for the retention of pollutants, we have opted to modify the surface of our clay by two different methods: cation exchange by cetyltrimethylammonium bromide (CTAB) to have KC and grafting with (3-aminopropyl) triethoxysilane (APTES) to have KS. The methods of modification and characterization of these new materials and natural kaolin are given in our previous works [10], [11] and [12].



Figure 1. Location of the Tabelbala commune in the wilaya of Bechar in Algeria

II.3. Bacteria adsorption on kaolins (K08, KC and KS)

Adsorption measurements were determined by batch experiments. For this purpose, in a series of tubes, 0.1 g of kaolin (K08, KC and KS) is brought into contact with 5 ml of bacterial solution at a well-determined concentration; these tightly closed tubes are shaken for a period using a thermostated shaker bath GFL-1083 Model. In order to separate the fraction of detached bacteria from those attached to the clay minerals, 5 ml of sucrose solution (60% by weight) was injected. The suspensions were then centrifuged at 4000 rpm by Eppendorf 5702 Model digital. Preliminary experiments indicated that the sucrose solution had little or no effect on determining the number of bacteria (Rong, 2008). The calibration curves and the amounts of bacteria present in the supernatant were determined by total bacterial analysis of protein by spectrophotometry at 620 nm using the spectrophotometer (UV.1700 Pharmrspac). The main advantage of this technique is its speed and ease of application [5] and [13]. The amount of bacteria adsorbed was calculated by the difference between the amount of bacteria added and that remaining in the supernatant.

The concentration of bacteria retained in the adsorbent phase, q_e (mg/g) and percentage adsorption (%) were calculated according to equations (1) and (2) below [14]:

$$q_e = \frac{(C_0 - C_e)}{m} \times V \quad (1)$$

$$\% \text{ sorption} = \frac{(C_0 - C_e)}{C_0} \times 100\% \quad (2)$$

Where C_0 (cells/L) and C_e (cells/L) are the initial concentration and the equilibrium concentration respectively, V is the volume of solution (L) and m is the mass of the support added.

To find the values of the adequate and optimal parameters for retention (contact time, pH, bacteria concentration and temperature), one parameter is varied while fixing the others. You find all these fixed and variable values in the next section Parameters affect *E. coli* adsorption on kaolins.

III. Results and discussion

III.1. Parameters affect *E. coli* adsorption on kaolins

A number of studies have shown that the adsorption of bacteria on minerals is a function of several

parameters such as pH, temperature, contact time and surface coating of minerals.

III.1.1. Effect of contact time

The study of the adsorption of *E. coli* on clay adsorbents requires the determination of the time that corresponds to the adsorption equilibrium or to state of saturation of the support by the substrate. In this case, the experimental procedure consists in contacting 0.1 g of clay with $2 \cdot 10^{19}$ cells/ml of bacterial solution at pH 6.5. Figure 2 shows the adsorption of *E. coli* on the three adsorbent supports (K08, KC and KS).

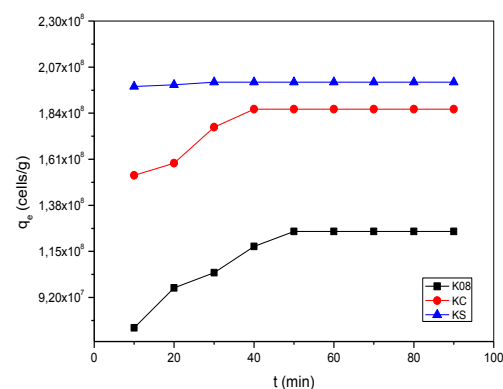


Figure 2. Effect of contact time on adsorption of *E. coli* onto K08, KC and KS

The follow-up of the retention of *E. coli* as a function of time has shown that it increases to equilibrium, resulting in the formation of a plateau that indicates the saturation of the supports. The three clays K08, KC and KS respectively reach their adsorption equilibriums after 50 min, 40 min and 30 min of contact time. The three kaolins are supports with different loads due to different modifications. This difference in charge means that the kinetics and the adsorption capacity of the *E. coli*, which is considered a negative charge, are not the same on the three supports.

III.1.2. Initial *E. coli* concentration effect

According to the literature, the initial charge of bacteria has a significant influence on the retention capacity. The influence of the initial charge of the bacterium on the adsorption process on kaolin is studied in the range of $1.4 \cdot 10^8$ to $4.1 \cdot 10^9$ cells at pH 6.5 and at ambient temperature. Figure 3 illustrates the effect of initial concentration of *E. coli* on its adsorption by the three types of kaolin (K08, KC and KS). It shows that there is a good affinity of the supports to bacteria.

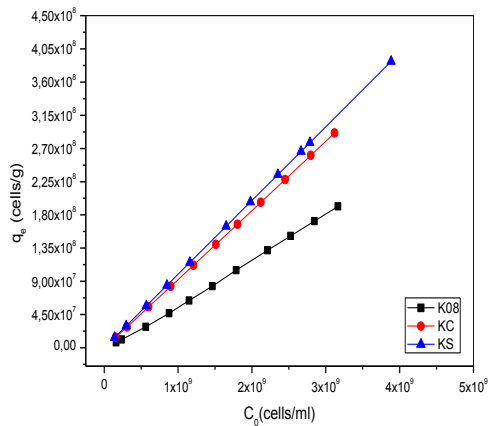


Figure 3. Effect of initial *E.coli* concentration on adsorption capacity of K08, KC and KS

Bacteria is composed of amino acids with different polarity. Intramolecularly non-polar amino acid side chains of the bacteria form hydrophobic region, whereas polar amino acids provide hydrophilic surface. The hydrophobic action between non-polar and polar amino acids plays a leading role in controlling the structural stability of bacteria in solution.

The retention effect due to bacterial load depends mainly on bacterial/support and Bacteria/bacteria interactions.

There are two possible cases:

- Increasing the load leads to an increase in bacterial retention.
- On the contrary, increasing the load leads to a reduction in retention.

When the bacteria / bacteria interactions are not favored, the rate of adsorption decreases with the increase of the *E. coli* load, this phenomenon is called the "blocking effect" in this case the support sites are saturated. Conversely, if the interactions between bacteria/bacteria are favored, retention *E.coli* increases with the increase of their office, it is called the phenomenon of "ripening effect" [15].

Rijnaarts observed that blocking on Teflon was more pronounced than blocking on glass, indicating that in addition to cell-cell interactions, hydrophobic cell-support interactions were important in controlling the maximum capacity [16].

The hydrophilic portion of the clay surface interacts with charged side chains of the protein. On the other hand, the hydrophobic character of clay surface could be removed by the charged side chains of the protein molecules. The adherence of bacteria spontaneously to the hydrophobic regions of clay minerals can reduce free energy of the system through a spontaneous thermodynamic process in order to remain structural stability.

Therefore, the hydrophobic interaction between the protein and the siloxane surface of clay mineral mainly governs adsorption, which can compensate the electrostatic repulsion of negative charge between bacteria and clay mineral surface. In addition, hydrogen bonding and van der Waal forces have been proved to be important in binding of proteins onto clay minerals. Van der Waals forces afford a partial overlap of the electrostatic repulsion[17].

Our study confirms this finding. The results show that clay surface coatings and ionic strength can affect the maximum bacterial retention capacity of kaolin. We observed that the presence of organosilane increases the bacteria adsorption capacity on the surface of the kaolin; this increases the amount of bacteria able to remove from the aqueous phase. The results indicate also that clay surface modifications with hydrophobicity agents (carbon chains) can increase the capacity of the natural kaolin support (K08), which is 59% to 92% retention by KC and 98% by KS. In addition, the presence of protonated amine functions on KC and KS, which play an important role in electrostatic attraction.

III.1.3. Effect of temperature

To determine the experimental temperature that allows the best adsorption *E. coli*, all other parameters were set, such as the initial bacterial charge of 2.10^9 cells and pH 6.5. The tubes are held in an incubator-agitator where we increase the temperature each time. The study is carried out in a temperature range of 20°C to 60°C and it is shown in Figure 4.

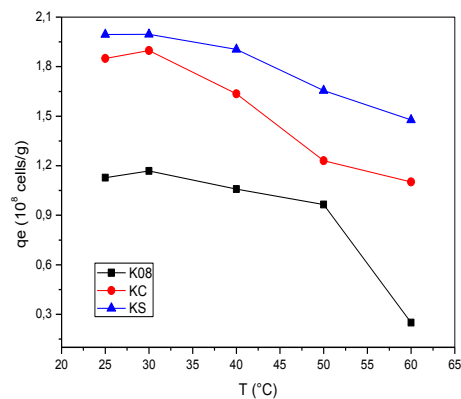


Figure 4. Effect of temperature on *E.coli* adsorption by K08, KC and KS

The maximum amount of adsorption was reached at 30 °C. The extent of bacteria adsorption on solid surfaces was affected by bacterial physiological state, and vigorous bacterial metabolism facilitates their adsorbabilities. Therefore, the greater adsorption of *E.coli* on minerals in the range of

temperature from 20 to 35°C may be related to the physiological state since its activity is optimum in this temperature range. It is also possible that, at lower or higher temperatures, agglomeration and entanglement of bacteria can occur in solution, which adversely affects their adsorption.

III.1.4. Effect of the pH

The bacteria can be adsorbed by these clay minerals by electrostatic interactions. This depends on the negative and positive charge states of clay surfaces and bacteria, which depend on the pH of the environment.

In this study, we followed the effect of pH on the adsorption of *E. coli* for an initial bacterial load of 2.10^9 cells. The medium was acidified by adding a few drops of HCl (0,1N) and NaOH (0,1N) was used to obtain basic pH. The change in the amount of bacteria adsorbed as a function of this parameter (Figure 5.) *shows that* the adsorption of *E. coli* on the clay surfaces depends strongly on the pH of the solution. When the pH increases from 4.0 to 8.0, the amount of bacterial adsorption decreases slightly; this decrease is greater in the range pH 8 to pH 12.

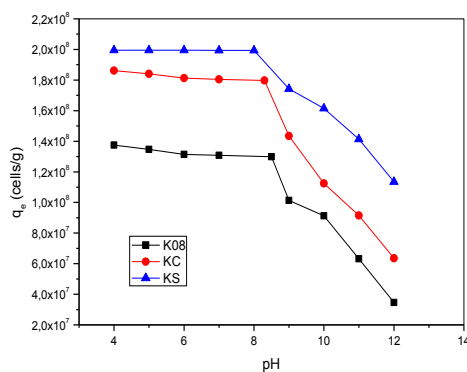


Figure 5. Effect of the pH on the q_e

Maximum retention of *E. coli* is made at an acid pH (pH 4), which is probably due to being the preferred medium of the bacteria, where they are more active [18].

We suggest that when the pH increases the positive charges decrease in the interfoliar space, also at higher pH (basic conditions), OH^- ions are more abundant and therefore an electrostatic repulsion between the negative charges which decreases the adsorption of bacteria. These results imply that the charges and the electrostatic properties of the clay surfaces play an essential role in the adsorption of *E.coli*.

Zhao obtained the same result during an adsorption of *E. coli* on kaolinite and montmorillonite from Shanghai in China [18].

III.2. Modeling of *E. coli* adsorption isotherms

Several adsorption isotherm models have been employed to interpret the adsorption behaviors on solid adsorbents. In this study, we have reproduced the experimental data using the Langmuir (Figure 6) and Freundlich models (Figure 7) given by equations (3) and (4) below [19] and [20]:

$$\frac{C_e}{q_e} = \frac{C_e}{q_m} + \frac{1}{k_L q_m} \quad (3)$$

$$\ln q_e = \ln k_F + \frac{1}{n_F} \ln C_e \quad (4)$$

q_m is the maximum adsorption capacity expressed in mg/g and k_L is the Langmuir constant expressed in L/mg. K_F and n_F are Freundlich constants, characteristics of the adsorbent efficiency. These parameters and the correlation coefficients (R^2) are summarized in Table 1.

Table 1. Langmuir and Freundlich adsorption isotherm constants

Adsorbent	Langmuir		Freundlich		
	q_m (mg/g)	R^2	n_F	K_F	R^2
K08	-2.25	0.843	0.80	0.27	0.998
KC	-7.85	0.934	0.89	0.50	0.999
KS	-0.3	0.796	0.30	0.63	0.996

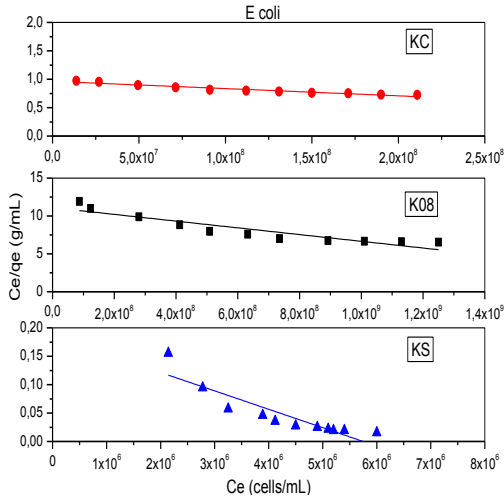


Figure 6. Langmuir isotherm plot for adsorption of *E. coli* on K08, KC and KS

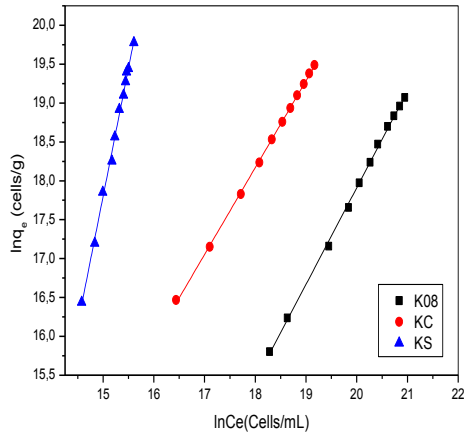


Figure 7. Freundlich isotherm plot for adsorption of *E. coli* on K08, KC and KS

The Langmuir model, often accompanied by monomolecular layer formation, revealed not only low correlation coefficients but also negative q_m values, which can never be the case; while Freundlich model which can consider the possibility of multilayer formation, correctly describes the adsorption of *E. Coli* on clay supports with high correlation coefficients. The values of K_F given by the modified supports KS and KC are higher than that given by the natural kaolin K08. These results suggest that the adsorption capacity of *E. coli* on these modified kaolins is greater and bacteria prefer to adsorb onto these supports that not only exhibit hydrophobicity but also protonated amine functions responsible for electrostatic attraction. These protonated amine functions are more liberated in the KS, which gives it the highest adsorbent character.

III.3. Adsorption Kinetics

Numerous models have been used in the literature to describe the adsorption processes; the most appreciated was the kinetic model of the pseudo-second order (Eq (5)) [21] which we adopted for our experimental results. Figure 8 shows linear plots and Table 2 gives very high values of the correlation coefficient, in addition to a good agreement between the experimental and calculated values of q_e . Therefore, *E.coli* adsorption on the three kaolinic supports is strongly represented by pseudo-second order kinetics. In many cases, this model is well correlated with adsorption studies. The second-order applicability to the adsorption data indicates that the concentration of bacteria and clay materials is involved in the speed determination step.

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \tag{5}$$

Table 2. Pseudo second order model rate constants

Adsorbent	$k_2(\text{g.mg}^{-1}.\text{min}^{-1})$	$q_e \cdot 10^8(\text{cells.g}^{-1})$	R^2
K08	9.33	1.38	0.997
KC	1.91	1.93	0.998
KS	5.16	1.99	1

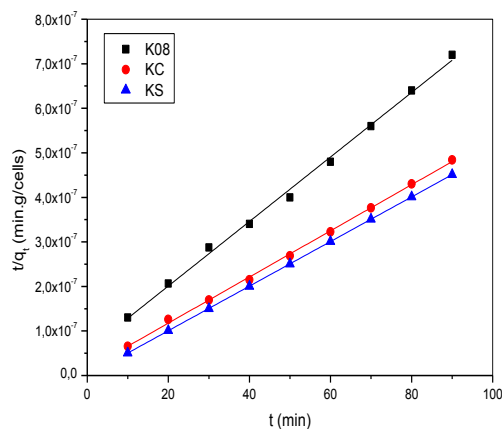


Figure 8. Pseudo-second order plot of *E.coli* adsorption onto K08, KC and KS

III.4. Scanning electron microscopy

Scanning electron microscopy (SEM) was employed to observe the attachment of bacterial cells with clay surfaces. After adsorption, the clay samples were gently washed twice with distilled water to remove loosely attached bacteria. The samples were freeze-dried and gold coated under vacuum. The SEM images were obtained by HITACHI S-4800 scanning electron microscope.

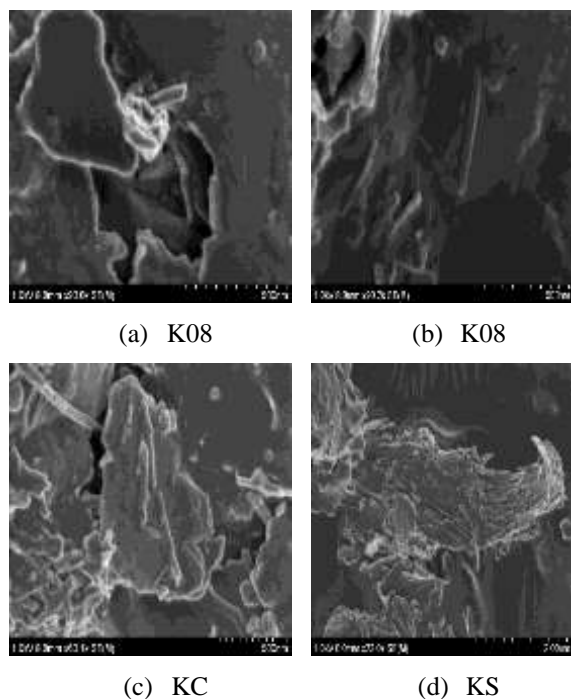


Figure 9. SEM images of kaolins after bacteria adsorption

These SEM images show that *E. coli* with a stem shape is adsorbed alone or in the form of aggregates on the clay or on mesoporous surfaces, which confirms the phenomenon of multilayers adsorption. It is clearly observed that the two supports KS (Figure 8 (d)) and KC (Figure 8 (C)) have a capacity to adsorb *E. coli* in aqueous solution greater than natural kaolin K08 (Figure 9 (a and b)) where (a) represents mesoporous retention and (b) surface retention.

The SEM images are quite in agreement with the results obtained by equilibrium studies and the good adaptation of the Freundlich model for this type of multilayer adsorption.

IV. Conclusion

Removing microbial pollution by conventional treatment is not sufficient. The processes using adsorption then constitute a technique of choice, complementary to the basic sector. The objective of our study was to study the possibilities of using the specific properties of the *E. coli* bacteria adsorption onto kaolin in the natural state (K08) and modified (KC and KS). The results obtained during this study have shown that kaolin is an effective adsorbent. The efficiency of retention also seems to be strongly influenced by the parameters studied, namely contact time, pH, initial charge and temperature. It emerges from the experimental results that the Freundlich adsorption isotherm better describes the nature of the adsorption phenomenon that was carried out in the multilayer regime, which was confirmed by the SEM images that allowed us to visualize the interaction between the bacteria and clay particles (K08, KC and KS). Our modified supports exhibited a stronger adsorption capacity than natural kaolin. KS has the highest adsorbing power due to the presence of hydrophobicity on its surface; the presence of protonated amine functions plays an important role in the electrostatic attraction, without forgetting its most important interfoliar spacing among the three supports. Therefore, the affinity of bacteria to this support was higher.

Kinetic studies have shown that the adsorption follows a pseudo-second order regime, which implies that the mechanism of adsorption of these bacteria depends both on their nature and on the nature of the support. The surface characteristics of cells play an important role in bacterial adsorption to clays. Under physiological conditions, bacterial cell walls are negatively charged due to functional groups such as carboxyl, phosphate and hydroxyl groups

present in lipoproteins on the surface. This suggests that the interaction between *E. coli* and adsorbents can be controlled not only by surface tension and van der Waals forces, but also by other factors such as electrostatic attraction. Therefore, the electrostatic properties of both bacterial and clay surfaces have been considered to govern the extent of adsorption of bacteria to mineral surfaces.

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