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Interaction between kaolinite and *Staphylococcus gallinarum* bacteria

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ABSTRACT

Kaolin plays a very significant role in the industrial aspects of life and new ones are still being discovered. It is a unique industrial mineral and very widely utilized in industry and its usage is influenced by its functional properties. However, the Egyptian kaolin is hard and massive. It is also low grade so that it needs beneficiation to be suitable for different industries. The kaolin used in most of the industrial applications should have very fine size distribution (80-90% by weight below 2 µm) and should be of high quality especially for applications like plastics, paints, paper industry, cosmetics, and pharmaceuticals. Moreover, great attention in recent years has been paid to the industrial applications of intercalated nano-composites where kaolin or clays are extensively used. Therefore, new technologies must be developed to achieve good separation, especially when treating these finely disseminated particles. One of such recent technologies is the bio-processing where micro-organisms could be used for removing these coloring materials. In this paper, the role of micro-organisms on the surface properties of the kaolin single mineral has been studied through zeta potential and adhesion measurements as well as the adsorption experiments. Complete characterization of both single mineral and bacteria isolated from its surface has been done using XRD, SEM, and FTIR as well as morphological and biochemical identification of bacterial isolates.

Introduction

Clay minerals are divided into four major groups. One of these is the kaolinite group. This group has a formula of $Al_2Si_2O_5(OH)_4$. The general structure of the kaolinite group is composed of silicate sheet (Si_2O_5) bonded to aluminum oxide/hydroxide layers ($Al_2(OH)_4$) called gibbsite layers, Figure 1. Pure kaolinite ($Al_2O_3.2SiO_2.2H_2O$) is white in color. Typical impurities present in kaolin ore are quartz, iron oxides, titanoferrous minerals, mica, feldspar, organic matter, etc. Ferruginous and titanoferrous minerals are the common coloring impurities present in kaolin. Iron stained titania (titanoferrous) gives dirty yellow color to kaolin. The main coloring impurity, particularly in the ultrafine size range, is titanoferrous minerals as anatase (TiO_2) which represents one of the major discoloring impurities in kaolin **[1]**.

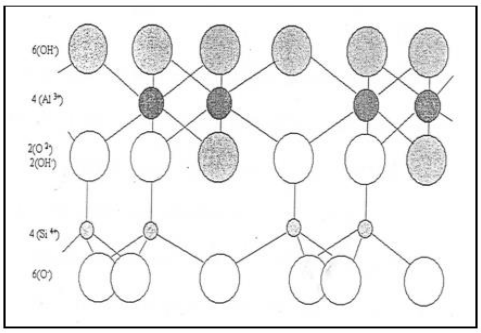

Fig.1. Structure of Kaolinite [2]

Kaolin is used in a multiplicity of industries because of its unique physical and chemical properties because it is chemically inert over a relatively wide pH range, is white, and has good covering or hiding power when used as a pigment or as an extender in coated films and filling applications. Shape, particle size, color, softness, and non-abrasiveness are physical properties that are especially important **[2]**.The main characteristic, which determines the utility of the clay for various applications, is its purity **[3]**. The oldest known use of kaolin is as a ceramic raw material. Presently, kaolin also finds application as a coating and filler pigment for paper, as a filler for paint, rubber, insecticide, plastics, fiberglass, fertilizers, food additive, formulation of medicine, cosmetics, etc. One of the highest value additions is achieved when the product kaolin meets the specifications of paint and paper coating grades where the critical properties are high brightness, low color values and small particle size, **[4, 5]**. The optical properties (brightness, Lab color, whiteness and yellowness) are improved only when the coloring impurities are removed. Purification of kaolin clay by conventional methods of beneficiation (such as attrition scrubbing, classification, and magnetic separation) can succeed in removing the majority of coarse particles of free silica and feldspars However, these methods of physical separation cannot remove such fine and ultrafine particles of associated coloring impurities (iron oxide and titanium oxide particularly those present as anatase particle) and in turn they reduce their brightness and quality. At the same time application of leaching technique to treat these fine and ultrafine coloring impurities is tedious and costly.

The most promising new approach based on integral green-chemistry methods that could be used to remove these coloring materials from kaolin is the biotechnological approach. This paper aims to study the role of interaction between one isolate of ***Staphylococcus gallinarum*** and kaolinite and its effect on the surface properties of kaolinite single mineral through the study of zeta potential, adsorption and adhesion measurements.

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Material and methods

Materials

Samples of single minerals of kaolin were delivered from 'Wards' Company, USA. The purity 99% of the samples was confirmed using XRF. The –200 mesh fractions were used in adsorption. Analytical grade HCl and NaOH, from Aldrich, were used for pH regulations.

Characterization

A Philips PW 1730 powder X-ray diffractometer with Fe-filtered Co (K-alpha) run at 30 kV and 20 mA was used to examine single kaolinite mineral. Infrared vibrational spectra were recorded on a Nicolet Magna 750 Fourier-transform spectrometer. For each sample, 28 scans were accumulated over the 4000-400 cm^{-1} spectral range employing the transmittance mode and a resolution of 4 cm^{-1}. The pressed KBr disc employed for this purpose was prepared using 0.4 mg of sample and 200 mg of KBr. Selected samples were observed on fractured surface under a JSM-6400 scanning electron microscope (SEM) to examine the morphology of single mineral.

Isolation and Growing of Bacteria

Bacterial strain was isolated from surface of kaolin ore through vigorous agitation of kaolin sample with 0.4% sodium chloride, NaCl, solution for 30 min on a rotary shaker at 30°C, and allowed to settle. The supernatant obtained was serially diluted with sterile water and spread on the surface of nutrient agar plates which were incubated at 30 ºC. Eighteen bacterial isolates were isolated, purified by streaking on nutrient agar plates, then transferred to nutrient agar slopes stored at 4C° and subcultured monthly. The efficiency of these isolates was screened using a laser particle size analyzer **[6, 7]**. Based on the later test, the most promising bacterial isolate has been selected to conduct this study.

Bacterial Identification

Morphological and Gram Staining Identification

Microscopic examination and gram staining of the selected bacterial isolate were carried out.

Bio-Chemical Identification

The selected bacterial isolate was identified using the BIOLOG GEN III Micro-plate microbial identification system. A pure culture was grown on biolog recommended agar media and incubated at 30° C. Inoculuma were prepared where the cell density was in the range of 90-98%T. precisely 100 µl of the cell suspension was transferred by multichannel pipette into the wells of biolog micro-plate. The plates were incubated for 36 hours at 30° C into the Omni-Log incubator/reader. The biolog micro-plate tests the ability of an organism to utilize or oxidize a pre-selected panel of 95 different carbon sources. The dye tetrazolium violet is used to indicate utilization of substrates. A panel of 95 different substrates gives a very distinctive and repeatable pattern of purple wells for each organism in which the manufacturers literature terms a "Metabolic Fingerprint". Finally; micro plate was read using Biolog's Microbial Identification Systems software through biology reader **[8]**.

Measuring Selectivity of the bacteria to Mineral Surface

A laser particle size analyzer (FRITSCH Model Analyst 22) was employed for measuring size analysis of single minerals before and after treatment with the bacterium. Fixed volume 10 ml of the isolate under test was conditioned with one gram of each mineral for 15 minutes before recording the change in size distribution.

Zeta Potential Measurements

A Laser Zetameter "Malvern Instruments" model "Zeta Sizer 2000", was employed for zeta potential measurements. 0.01 gram of ground sample was placed in 50 ml double distilled water with definite dispersant or flocculants concentration at ionic strength of $2X10^{-2}$ M NaCl. The pH was then adjusted to the required value. The sample was shacked for 15 minutes. After shaking, the equilibrium pH was recorded and the zeta potential of the mineral particles was measured **[7, 9]**.

Adhesion Measurements

Adhesion of the bacterial isolate on the mineral surfaces was determined by dry weight difference before and after conditioning with the mineral particles. 0.5 gram of the ground mineral (-200 mesh) was added to 80 ml of the 48 hour bacterial suspension with a fixed initial concentration of the bacterial isolate $2x10^8$cell/ml, and conditioned for 60 minutes after adjusting the pH values. An additional time of 20 min. was allowed for settling of the mineral particles, after which 20 ml of the supernatant was collected in a porcelain crucible and dried on a hot plate at 40 – 45°C. Adhesion studies were performed as a function of difference in weight before and after drying.

Adsorption Experiments

The adsorption density of bacterial isolate on the mineral surface was determined by adding 1 g dry sample of kaolin or anatase to the bacterial suspension in a 100 cm^3 volumetric flask with a definite concentration of bacterial cells. The mixture was shacked for 15 minutes using a shaker at 150 rpm (Model JANKE & KUNKEL Type VX10). The pH was adjusted to the desired values using HCl and NaOH. A potentiometer (Orion Mod. 720A) equipped with a combined electrode was used to monitor the pH. The potentiometer was calibrated before each test by using buffer solutions of pH 4, 7 and 10. The solution was shaken at 150 rpm for 1 h at controlled temperature of 25 ± 2°C. Then, the samples were centrifuged at 15000 rpm for 20 min at room temperature to separate supernatant from the settled fraction. The total content of organic carbon, (residual concentration), in 40 ml of supernatant was determined using a 'Phoenix 8000' Total Carbon Analyzer" instrument. The average of three readings was taken as a measure for the residual concentration of organic carbon. The adsorbed amount was then calculated as the difference between initial and residual concentrations **[7, 10]**.

FTIR measurements

The adsorption density of the bacterial isolate under test on the mineral surface was measured using "PYE Unicam" Model "SP 1200 infrared spectra". A 0.2 gram of kaolinitre single mineral was conditioned with selected bacterial isolate at pH 8for 30 min. The samples were centrifuged at 15,000 rpm for 15 min. The supernatant was separated from the residual solid which was rinsed several times before drying at 30°C, **[7, 11].**

Results and discussion

Bacterial identification

Microscopic examination revealed that cells of the selected bacterial isolate is non-spore-forming, gram positive cocci, and 0.5–1.8 mm in diameter, Fig. 2. It occurs singly and forms pairs, short chains, and small groups. Colonies are yellow, yellowish, flat, opaque, and dry, with lobate or crenate edges, Fig. 3.

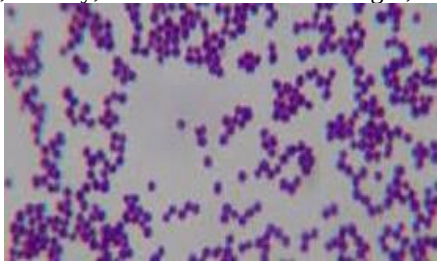

Fig.2. Gram Positive Cocci

Fig.3. Colony Morphology

Table 1 represents a comparison between acid productions from organic compounds by isolated ***S. galinarum*** (column A) and those reported by Schleifer and Bell, **[12]**, (column B).

Table 1. Acids produced from organic compounds by isolated *S. galinarum*

Organic compound	A	B
Amygdalin	ND	+
Arbutin	ND	+
Cellobiose	+	+
D-Arabitol	+	ND
D-Fructose	+	+
D-Fucose	-	+
D-Galactose	+	+
D-Glucitol	ND	+
D-Mannitol	+	+
D-Mannose	+	+
D-Raffinose	+	+
D-Ribose	ND	+
D-Sorbitol	+	ND
D-Xylose	ND	+
Glycerol	+	+
L-Arabinose	ND	+
L-Rhamnose		-
Maltose	+	+
Melezitose	ND	+
Melibiose	+	+
Myo-inositol	+	ND
N-Acetyl Neuraminic acid	+	ND
N-Acetyl-D- Glucosamine	+	ND
N-Acetyl-β-D-Mannosamine	+	ND
Salicin	+	+
Starchyose	+	ND
Sucrose	+	+
Trehalose	+	+
Turanose	+	+
β-Gentiobiose	+	+
β-Methyl-D- Glucoside	+	ND

(+) Positive, (-) Negative, (ND) No data are available.

Characterizations

The results showed that the natural kaolinite samples were composed of kaolinite in addition to quartz as an impurity, Fig. 4. In this figure, kaolinite is characterized by the marked 001 and 002 features and the presence of quartz in the sample is evident from the well known 101 and 100 peaks.

SEM was used to reveal the morphology and particle size of kaolinite crystals. SEM microimages indicated that kaolinite has a well-defined crystal structure revealed by its hexagonal plates with micro edges, Fig.5.

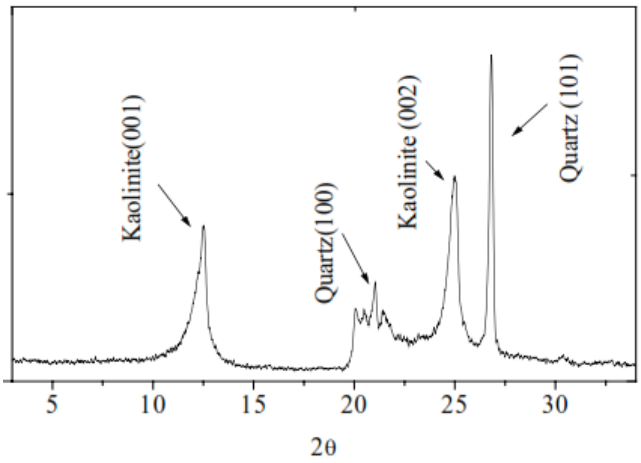

Fig.4. XRD-mineralogical analysis of single kaolinite.

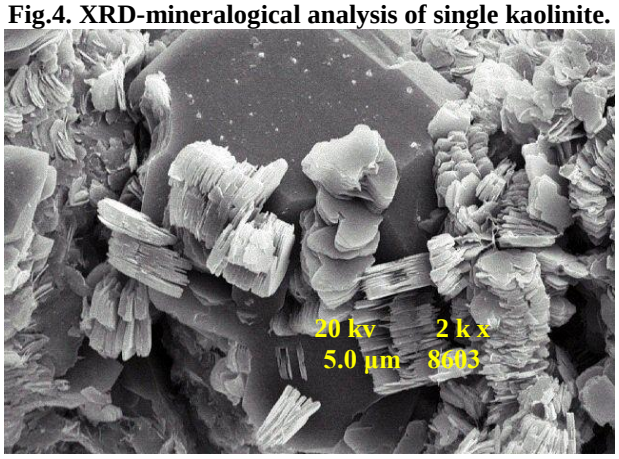

Fig.5. A typical SEM image of single kaolinite

Particle size analysis

The change in size distribution of single pure mineral sample before and after its treatment with ***S. gallinarum*** was taken as a measure for the selectivity. As shown in Figure 6, a successful adsorption of the bacterial isolate caused a degree of aggregation for mineral particles leading to a change in their size distribution which indicates the largest degree of selectivity of ***S. gallinarum*** for kaolin. This technique was successfully used to screen different microorganisms for selective adhesion onto different minerals' surfaces **[7, 13]**.

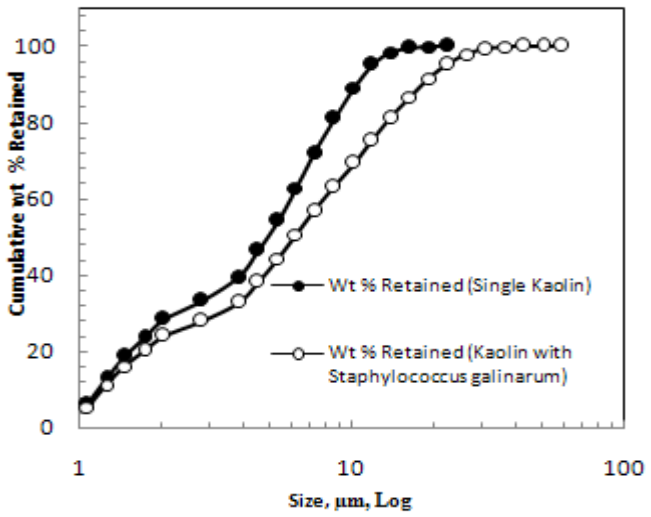

Fig 6. Size distribution of kaolin before and after treatment with *S. gallinarum*

Bacterial Adhesion onto Minerals' Surfaces

Figure 7 shows the adhesion of bacteria onto the surface of single mineral over a wide range of pH (3-9). The results

showed that, the ***S. galinarum*** can be adhered with a higher affinity to kaolin surface. Also, it is noticed that the highest values of adhesion for kaolin was obtained at pH from 3-5.

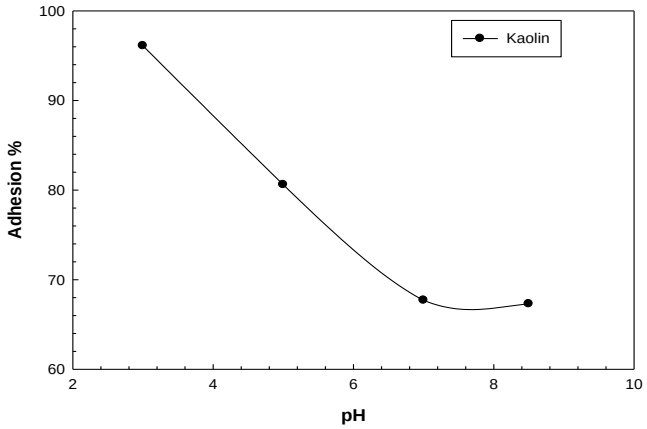

Fig 7. Adhesion of *S. gallinarum* onto Single Kaolin's Surface

Surface Properties of Single Minerals and S. gallinarum isolate

Zeta potential of each single mineral–bacteria system was studied. Measurement of zeta potential of ***S. galinarum*** alone as well as for single mineral, kaolinite, in absence and presence of bacteria has been performed, Figure 8. These measurements were done at constant ionic strength of 2 $X10^{-2}$ M NaCl. The results indicate that NaCl acts as indifferent electrolyte for kaolin. Generally, the value and sign of zeta potential depend upon the pH of the medium, indicating that both H^+ and OH^- are potential determining ions for kaolinite. The results showed that the electronegativity of zeta potential increases gradually with increasing the pH value. The zeta potential increases in magnitude with decreasing ionic strength of counter ions (NaCl) due to the increase in thickness of the diffuse layer, as well as the columbic interactions which is a dominant role in adsorption process, **[14]**.

Figure 8 illustrates the zeta potential the ***S. gallinarum*** in which the values of zeta potential are varied from (+5 to -35 mv) over the entire range of pH (1.0-12). This means that such type of bacterial isolate is hydrophobic in nature with iso-electric point (IEP) corresponding to pH of 1.8. These results are in agreement with literature **[15, 16].**

On the other hand, the zeta potential of kaolinite as a function of pH before and after interaction with bacteria was measured. The results show that the iso-electric point (IEP) of kaolinite corresponds to pH of about 3. However, conditioning of kaolin with bacteria resulted in a displacement for the IEP of kaolinite to about 2.5, i.e., near to that of ***S. gallinarum*** itself. Moreover, the values of zeta potential of kaolin after interaction with bacteria became less negative over the entire pH range. The hydrophobic effect of ***S gallinarum*** is appeared at pH range (4.5-11)

Adsorption Experiments

Figure 9 represents the results of adsorption density of bacteria onto kaolinite single mineral. The experiments are performed at pH range (8-9). These results indicate that the adsorption density is generally increases with increasing the concentration of bacteria over the entire range of concentration at fixed concentration of ***S. gallinarum.***.

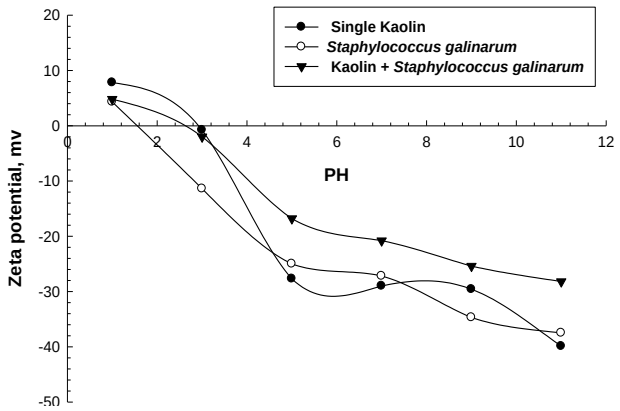

Fig 8. Zeta potential of *S. gallinarum*, single kaolin, before and after interaction with bacteria .

Such higher bacterial affinity to kaolin mineral surface is readily evident. The increased adsorption tendency of bacterial isolates of ***S. gallinarum*** onto kaolin can be attributed to electrostatic forces besides hydrogen bonding and chemical interaction. FTIR studies on bacterial cells and minerals before and after interaction have strongly indicated the role of hydrogen bonding and chemical interaction. Therefore, the shift in iso-electric points of kaolin can be explained based on these surface interactions. Such interactions between mineral surface and microorganisms are seen to result in significant surface chemical changes, not only on the cell surfaces but also on the interacted minerals.

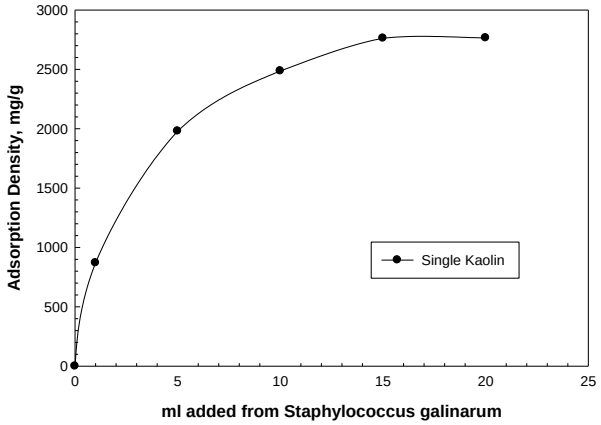

Fig 9. Adsorption of *S. gallinarum* onto surfaces of kaolinite

FTIR Measurements

The interaction between mineral surface and ***S. gallinarum*** could be understood from FTIR measurements for the bacteria and single minerals. FTIR of bacteria showed the existence of O-H, C-C, CH2, C-O, C-N and C=O bands in decreasing order, Figure 5. These bands reflect the general organic structure of bacteria which are mainly composed of polysaccharides and lipids (protein). Polysaccharides are defined from their hydroxyl bands at 3600-3200 cm^{-1} and carboxyl group bands at 1210-1740 cm^{-1} whereas the protein is characterized by its amino group bands at 3460 – 3150 cm^{-1} and 1650 – 1500 cm^{-1} respectively **[17].**

Table 2 represents the position and assignment of various absorption bands in the FTIR spectra. As noticed, the characteristic bands for kaolin, Figure 10, are at 471cm^{-1}, 538cm^{-1}, 752cm^{-1}, 789cm^{-1}, 1030cm^{-1}, 1115cm^{-1}, and the three characteristic bands for inner hydroxyls at 3620 cm^{-1}, 3655 cm^{-1} and 3691 cm^{-1} **[17, 18]**. Sharp bands at 1030 and 912 cm^{-1} suggest the presence of Al–O–H bands. The band at 1650 and

538 cm^{-1} were reduced after bio-treatment and could be associated with the octahedral coordination of Al^{3+} in kaolinite changing to an anorthic lattice structure in $[Al_2Si_2O_5(OH)_4]$ **[19]**. This is beside the appearance of some peaks for hydrogen bonds formed at wave numbers of about 1100 cm^{-1}, 1150 cm^{-1} and 1200 cm^{-1}. Such occupation of the bacteria to some of the positively adsorption sites of kaolinite lead to a reduction in the zeta potential of its surfaces to be close from that of the bacteria itself.

Table 2. Position and assignment of various absorption bands in the FTIR spectra

Position and assignments of bands (Cm^{-1})	Kaolin
OH stretching of inner surface hydroxyl groups (in plane vibration with a transition moment nearly perpendicular to the (001) plane)	3691
OH stretching of inner surface hydroxyl groups (anti-phase vibration with a transition moment lying in the (001) plane)	3655
OH stretching of inner surface hydroxyl groups	3620
Si-O stretching (longitudinal mode; shoulder of absorption band)	1115
In plane Si-O stretching	1030
In plane Si-O stretching	1006
OH deformation of inner hydroxyl groups	913
Si-O	789
Si-O perpendicular	752

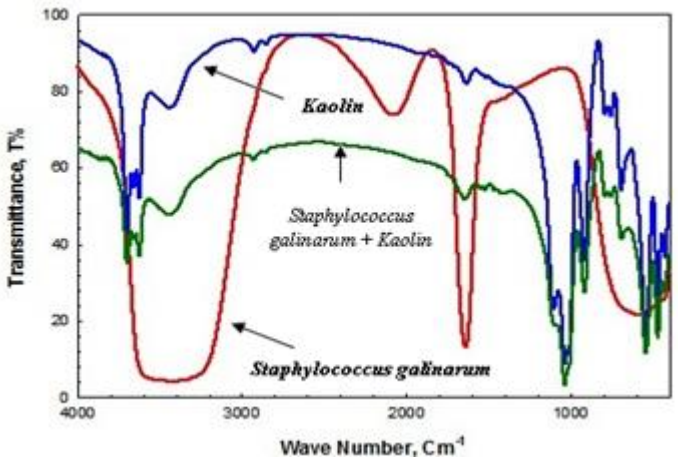

Fig 10. FTIR of *S. gallinarum*, single kaolin and kaolin after treatment with bacterial isolate

Conclusions

- A successful adsorption of the ***S. galinarum*** bacterial isolate onto kaolin surface caused a degree of aggregation for mineral particles leading to a change in their size distribution which indicates the largest degree of selectivity for kaolin.
- The values of zeta potential of ***S. galinarum*** are varied from (+5 to -35 mv) over the entire range of pH (1.0-12) which means that such type of bacterial isolate is hydrophobic in nature with iso-electric point (IEP) corresponding to pH of 1.8.
- Conditioning of kaolin with bacterial isolates leads to a displacement for the IEP of kaolinite to about 2.5 and the hydrophobic effect of ***S. gallinarum*** is appeared at pH range (4.5-11)
- The results of adsorption experiments indicated that there is a higher bacterial affinity of ***S. gallinarum*** to kaolin mineral surface.

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