



Bio-Surface Modification of Titanium Dioxide Mineral

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ABSTRACT

Titanium (Ti) is a major impurity in the economic Carboniferous and Cretaceous sedimentary kaolin deposits in Egypt. It is found that Ti is present as an independent mineral phase rather than in the crystal structure of kaolinite. Ti occurs mainly as uniform very fine-grained and rounded anatase crystals in pockets within the kaolin mass of all deposits. The optical properties of kaolin (brightness, Lab color, whiteness and yellowness) are improved only when the coloring impurities are removed. In this paper, the role of micro-organisms on the surface properties of titanium dioxide (anatase 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.

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1. Introduction

Natural titanium dioxide exists in nature in one of three crystalline forms, the two most important of which are anatase and rutile, the third being brookite. Although these minerals are essentially pure titanium dioxide, they do not appear white, because of the presence of impurities, such as iron, chromium, or vanadium, which darken them. Rutile is the thermodynamically stable form of titanium dioxide; Titanium dioxide anatase rapidly transforms to rutile above 700°C. Rutile melts between 1830°C and 1850°C [1-2].

Titanium is the ninth most common element in the earth's crust. TiO₂ is typically thought of as being chemically inert. Titanium dioxide has been used for many years in a vast range of industrial and consumer goods including paints, coatings, adhesives, paper and paperboard, plastics and rubber, printing inks, coated fabrics and textiles, catalyst systems, ceramics, floor coverings, roofing materials, cosmetics and pharmaceuticals, water treatment agents, food colorants and in automotive products, etc

Kaolinite group is one from the four major groups of the Clay minerals' groups. This group has a formula of Al₂Si₂O₅(OH)₄. The general structure of the kaolinite group is composed of silicate sheet (Si₂O₅) bonded to aluminum oxide/hydroxide layers (Al₂(OH)₄) called gibbsite layers. Pure kaolinite (Al₂O₃·2SiO₂·2H₂O) is white in color. Typical impurities present in kaolin ore are quartz, iron oxides, titaniferous minerals, mica, feldspar, organic matter, etc.

Ferruginous and titaniferous minerals are the common coloring impurities present in kaolin. Iron stained titania (titaniferous) gives dirty yellow color to kaolin. The main coloring impurity, particularly in the ultrafine size range, is titaniferous minerals as anatase (TiO₂) which represents one of the major discoloring impurities in kaolin [3-4]. 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 titanium dioxide and its effect on the surface properties of anatase single mineral through the study of zeta potential, adsorption and adhesion measurements.

2. Material and methods

2.1. Materials

Sample of anatase single mineral of was 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 [5].

2.2. Characterization

A Philips PW 1730 powder X-ray diffractometer with Fefiltered Co (K-alpha) run at 30 kV and 20 mA was used to examine single titanium dioxide 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 [5].

2.3. Isolation and Growing of Bacteria

Bacterial strain was isolated from surface of Egyptian 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 °C. Eighteen bacterial isolates were isolated, purified by streaking on nutrient agar plates, then transferred to nutrient agar slopes stored at 4°C and subcultured monthly. The efficiency of these isolates was screened using a laser particle size analyzer [5-7].

2.4. Bacterial Identification

2.4.1. Morphological and Gram Staining Identification

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

2.4.2. 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 biologi recommended agar media and incubated at 30°C. Inoculum was 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 biologi micro-plate. The plates were incubated for 36 hours at 30°C into the Omni-Log incubator/reader. The biology 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 Biologi's Microbial Identification Systems software through biology reader [8].

2.4.3. 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. Based on the later test, the most promising bacterial isolate has been selected to conduct this study

2.5. 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 2×10^{-2} M NaCl. The pH was then adjusted to the required value. The sample was shaken for 15 minutes. After shaking, the equilibrium pH was recorded and the zeta potential of the mineral particles was measured [7, 9].

2.6. 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 2×10^8 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.

2.7. Adsorption Experiments

The adsorption density of bacterial isolate on the anatase mineral surface was determined by adding 1 g dry sample of titanium dioxide or anatase to the bacterial suspension in a 100 cm³ volumetric flask with a definite concentration of bacterial cells. The mixture was shaken 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 $25 \pm 2^\circ\text{C}$. 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].

2.8. 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 anatase single mineral was conditioned with selected bacterial isolate at pH 8 for 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].

3. Results and discussion

3.1. Bacterial identification

Microscopic examination revealed that cells of the selected bacterial isolate is non-spore-forming, gram positive cocci, and 0.5–1.8 µm in diameter, Fig.1. 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. 2.

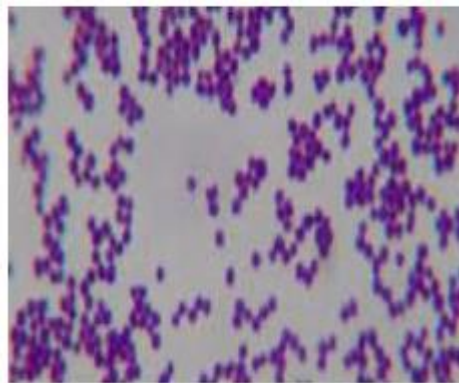


Figure.1. Gram Positive Cocci.



Figure. 2. Colony Morphology.

3.2. Biolog Micro-plate Results

The strain *S. gallinarum* utilizes 42 of the 95 substrates Table 1 represents a comparison between acid productions from organic compounds by isolated *S. gallinarum* (column A) and those reported by Schleifer and Bell, [7, 12], (column B).

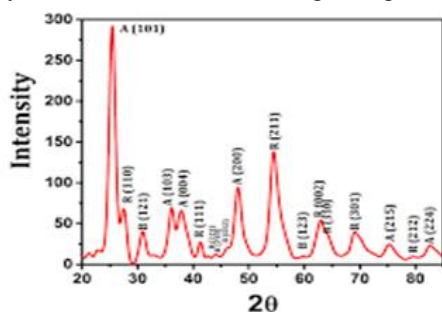
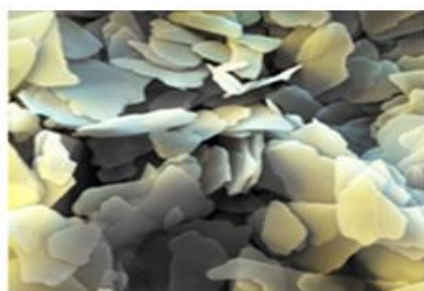
Table 1. Acids produced from organic compounds by isolated *S. gallinarum*.

Organic compound	A	B
Amygdalin	ND	+
Arbutin	ND	+
Cell obiose	+	+
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	+	+
B-Gentiobiose	+	+
B-Methyl-D-Glucoside	+	ND

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

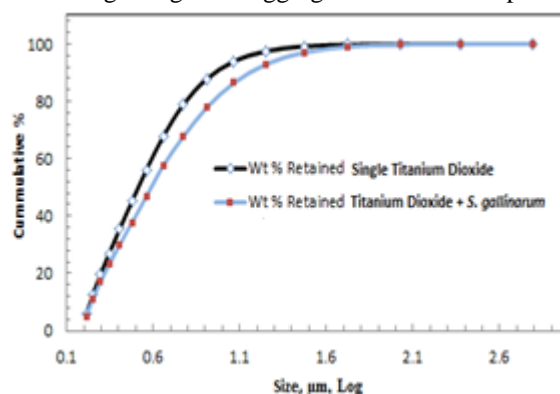
3.3. Characterizations

The results of XRD patterns exhibited strong diffraction peaks at 25° and 48° indicating TiO₂ in the anatase phase, Fig. 3. All peaks are in good agreement with the standard spectrum (JCPDS no.: 88-1175 and 84-1286). SEM was used to reveal the morphology and particle size of anatase crystals. SEM microimages indicated that titanium dioxide has a well-defined crystal structure with micro edges, Fig.4.

**Figure.3. XRD Mineralogical Analysis of Anatase Mineral.****Figure.4. Typical SEM Image of Anatase Mineral.**

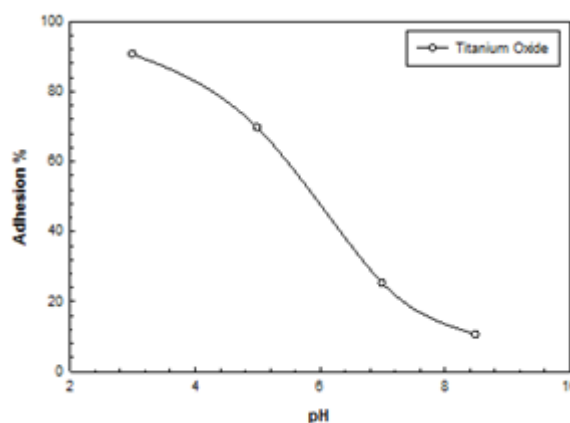
3.4. Particle size analysis

The change in size distribution of single pure mineral sample, titanium dioxide, after its treatment with *S. gallinarum* was taken as a measure for the selectivity. Successful adsorption of the *S. gallinarum* will cause, therefore, a degree of aggregation (or dispersion) for mineral particles leading to a change in their size distribution. This technique was successfully used to screen different microorganisms for selective adhesion onto different minerals' surfaces [13-16]. The results obtained in Fig. 5 show different degree of variation in the size distribution of samples after their treatment with *S. gallinarum* which indicates a slight degree of aggregation for anatase particles.

**Figure.5. Size Distribution of Titanium Dioxide Before and After Treatment with *S. gallinarum*.**

3.5. Bacterial Adhesion onto Minerals' Surface

Figure 6 shows the adhesion of bacteria onto the surface of Anatase mineral over a wide range of pH (3-9). The results showed that, the *S. gallinarum* can be adhered onto the mineral surface. The highest values for adhesion are obtained at pH from 3-5 and lower adhesion percentage was occurred at pH of 8.5 .

**Figure.6. Adhesion of *S. gallinarum* onto Titanium Dioxide Mineral Surface**

3.6. Surface Properties of Single Mineral and *Staphylococcus gallinarum* bacteria

Zeta potential was measured for of *S. gallinarum* bacteria as well as for TiO₂ single mineral at constant ionic strength of 2 X10⁻² M NaCl (as indifferent electrolyte). The results showed that the electronegativity of zeta potential increases gradually with increasing the pH value. The results illustrates that the values of zeta potential for *S. gallinarum* 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 around 1.5, Fig. 7.

These results are in agreement with literature [17-19]. The iso-electric point (IEP) of titanium Dioxide corresponds to pH of about pH 4. Conditioning of titanium oxide with *S. gallinarum* resulted in a significant displacement for the IEP of titanium oxide to that of bacteria, i.e., at about pH 3. Interestingly, The values of zeta potential were seen to be shifted to lower values in the entire pH range (1-7), i.e., the surface of titanium oxide became more hydrophobic.

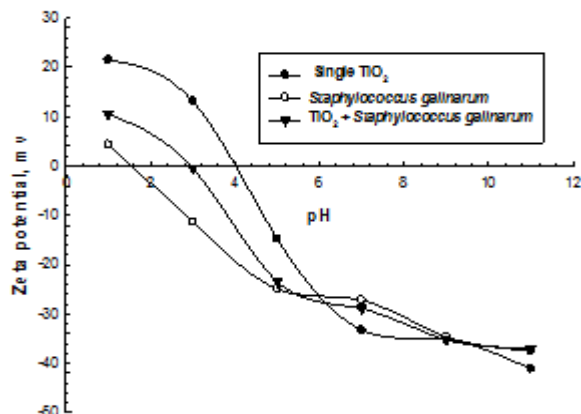


Figure.7. Zeta potential of *S. gallinarum*, single Titanium Dioxide, before and after Interaction with Bacteria

3.7. Adsorption Experiments

The adsorption density of bacteria onto each single mineral was also studied, the results of which are shown in Figure 8. The experiment was 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.

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 titanium oxide can be explained based on these surface interactions. Such interactions between mineral surface and *S. gallinarum* are seen to result in significant surface chemical changes, not only on the cell surfaces but also on the interacted minerals.

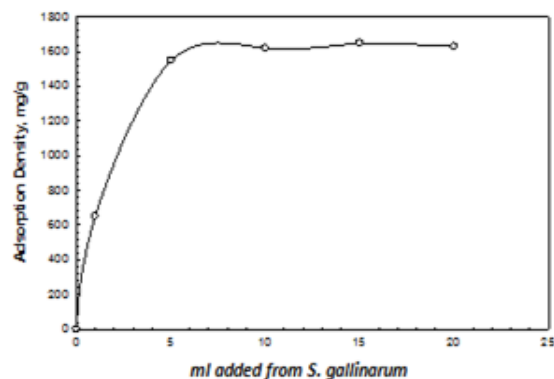


Figure 8. Adsorption of *Staphylococcus gallinarum* onto surface of titanium Dioxide.

3.8. FTIR Measurements

The interaction between mineral surface and *S. gallinarum* could be understood from FTIR measurements for the bacteria and single mineral. FTIR of bacteria showed the existence of O-H, C-C, CH₂, C-O, C-N and C=O bands in decreasing order, Figure 9. These bands reflect the general organic structure of bacteria which are mainly composed of polysaccharides, lipids, and 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 [20].

On the other hand, the FTIR of TiO₂ exhibited prominent peaks at 1578cm⁻¹, 1451cm⁻¹ and 1123cm⁻¹. After treatment with *S. gallinarum*, a broad peak at 3430 cm⁻¹ shows O-H stretching due to alcoholic group. Peak at 1578 cm⁻¹ indicates the presence of C-C ring stretching. The band observed at 1451 cm⁻¹ is due to bending vibration of the CH₂ in the lipids and proteins. The peak at 1123 cm⁻¹ is due to the formation of the amide linkages between the bacterial proteins and the TiO₂ formed during the reaction period. This means that the adsorption of bacteria onto titanium dioxide is weaker in terms of the intensities of different functional groups of polysaccharides part.

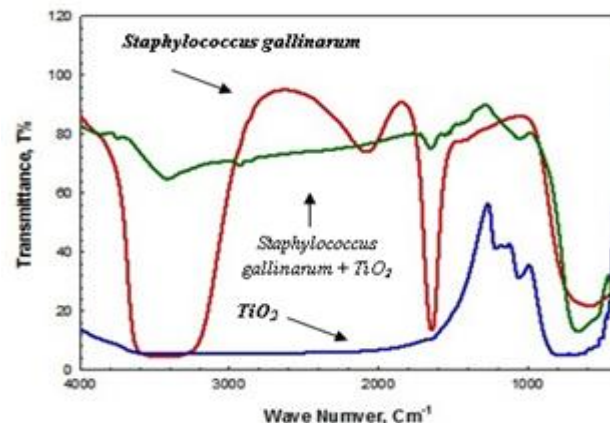


Figure.9. FTIR of *Staphylococcus gallinarum*, single titanium dioxide and titanium dioxide after treatment with bacterial isolates.

4. Conclusions

- A successful adsorption of the *S. gallinarum* bacterial isolate onto the surface of titanium dioxide caused a degree of aggregation for mineral particles leading to a change in their size distribution
- The values of zeta potential of *S. gallinarum* 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.
- Interaction between anatase mineral and bacterial isolates leads to a displacement for the IEP of single mineral to about 3 and the hydrophobic effect of *S. gallinarum* is appeared at pH range (5-11).
- The adsorption results indicated that there is a higher bacterial affinity of *S. gallinarum* to titanium dioxide mineral surface.

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