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Characterization and Antibacterial Activity of Naturally Synthesized Fe-O/Ti-O Composite from Ilmenite Ores in Abu Ghalaga, Egypt

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Abstract

Explorations into the potential application of Fe-O/Ti-O composite as antibacterial agents have been of interest due to their enhanced antibacterial activity. Fe-O/Ti-O composite was successfully synthesized using ilmenite ore as the initial titanium source. The rocks were cut and then the non-oxidized inner part was ground to -200 mesh size by using a pulverizer. Also, heat-treated obtained powder at 300 and 600°C for 2 h were prepared. This synthesis method was cost-effective and straightforward due to the absence of the traditional gravity, magnetic, electrostatic separation, ball milling, and heat up processes. The ilmenite and heat-treated powder were characterized by UV/Vis spectrophotometer, Energy-dispersive X-ray spectrometry (EDX) and Fourier transform IR spectroscopy. The antibacterial action of the prepared powders was investigated against Gram-positive bacterium; *Bacillus cereus*, and Gram-negative bacterium; *Escherichia coli*. Our results showed that the antibacterial activity of ilmenite decreased by increasing heat treatment temperatures. Also, the powders had strong antibacterial potential against Gram-positive than Gram-negative bacteria.

Keywords: Ilmenite, Ti-O, Fe-O, heat treatment, minimal inhibition concentration (MIC), minimal bactericidal concentration (MBC), antibacterial.

Introduction

The development of cutting-edge therapeutic alternatives to well-established anti-biotherapy is piqueing scientists' curiosity, with natural substances currently being the main subject of research. Natural products are still an important source for antibacterial research, according to Silver (2008). The primary benefits of these substances are their many mechanisms of action, which can combat bacterial resistance and minimise negative consequences (Pancu et al., 2021). Similar to this, metal oxides as Fe_3O4, TiO_2, CuO, and ZnO have lately become desirable antibacterial options due to their improved stability, selectivity, and reduced toxicity towards human cells (Reddy et al., 2007; Stankic et al., 2016). Ilmenite ore is an important natural source of metal oxides.

Weakly magnetic ilmenite is a mineral that mostly consists of titanium iron oxide. It is a metallic or submetallic-lustrous opaque iron-black mineral. The crystal structure of ilmenite is hexagonal, and it is found in igneous rocks and black sands all over the world (Anthony et al., 2001). Ilmenite has been the subject of numerous studies to investigate its potential as an antibacterial agent (Maulidiyah et al., 2019; Thambiliyagodage et al., 2022a; Thambiliyagodage et al. 2022b). According to Kalantari and Emtiazi (2016), ilmenite soil could be used to treat microbial pollution because it reduces bacteria by 50% when it's dark out. They also found that titanium and iron ions provide ilmenite its antibacterial qualities.

Due to its powerful photocatalytic activity, low price, photostability, chemical and biological stability, and high photocatalytic activity, TiO_2 is currently the most exceptional photocatalyst (Guesh et al., 2016). Several distinct uses for titanium oxide exist, including as antibacterial agents, a white pigment, food coloring and flavor enhancers, an organic compound degrader, an air and water sterilizer, and an odor neutralizer (Marino et al., 2017; Amini et al., 2015; Ghasemzadeh et al., 2014; Liou & Chang, 2012). Many microorganisms, including gram-positive and gram-negative bacteria, viruses, algae, fungi, and protozoa, can be eliminated by the photocatalytic properties of TiO_2 (Foster et al., 2011). The key advantage of TiO_2 is that it has an indefinite lifetime and doesn't decompose when bacteria and organic compounds do. Improved TiO_2 photocatalysts have been found to significantly reduce the quantity of bacterial cells and the activity of bacterial endospores when exposed to visible light by reducing their toxicity while maintaining their ability to germinate (Liou & Chang, 2012). Similar to how pH influences this process, iron has powerful antibacterial effects that are proportionate to iron levels. It is a harmful metal that combines with H_2O_2 to create the potent oxidizer hydroxyl radical, which can stop the growth of aerobic microorganisms (Sun et al., 2011).

In addition, iron oxide nanoparticles exhibit less noticeable antibacterial properties than other metal oxide nanoparticles, according to Gudkov et al. (2021). These effects depend on the microbial strain. They are also more biocompatible *in vivo* and less cytotoxic than CuO or ZnO nanoparticles. Iron oxide nanoparticles may develop into next-generation antibacterial agents, according to Gudkov et al. (2021). Moreover, antibiotics attached to magnetite nanoparticles (Fe_3O_4) improve cellular absorption and membrane fluidity, suggesting that they could be used in customised targeted delivery and controlled release nano shuttles for treating localised and systemic infections (Istrate et al., 2014). Similar findings were made by Gabrielyan et al. (2019), who discovered that Fe_3O_4 nanoparticles can be suggested as an alternative to antibiotics as an antibacterial agent.

Thus, the present work's significant contribution is the new approach to produce high-purity Fe-O/Ti-O composite from ilmenite ore and study the effect of heat treatment of the naturally prepared composite based on the antibacterial activity.

Materials and Methods

Preparation of ilmenite

The Abu Galaga deposit of ilmenite ore is located on a hill overlooking Wadi Abu Galaga, 20 km west of the port of Abu Ghusun. When the rocks were split, the non-oxidized interior was pulverised to a size of 200 mesh. Afterwards, different powders were treated by heating at 300 and 600°C for 2 hours, respectively. The finished composite was generated after 4 hours of ball-milling the raw composite.

Characterization of ilmenite

Using a UV/Vis spectrophotometer, the optical characteristics of the ilmenite ore were investigated (Jasco UV/Vis Spectrometer v-630, Jasco, Japan). In the range of 200-900 nm, the electronic spectra of solid-state ilmenite ore were captured. Using a KBr disc (KBr pellet) and a JASCO FTIR-410 spectrometer, infrared (IR) spectra were taken in the 4000-400 cm^{-1} range. The elemental makeup of the ilmenite ore was ascertained using energy-dispersive X-ray spectroscopy (EDX), model JSM-7500F, Hitachi High-Technologies Corp., Tokyo, Japan.

Biological activity

Agar well diffusion Method

Testing of the produced composite's antibacterial properties included Gram-positive bacterium (*Bacillus cereus*) and Gram-negative bacterium (*Escherichia coli*). The agar well diffusion procedure was carried out in accordance with the Clinical and Laboratory Standards Institute's recommendations. Agar Mueller-Hinton (MHA) was made, and it was autoclaved. Thereafter, 100 µl cultures of each strain (0.5 McFarland standard (1-2 $\times 10^8$ CFU/ml)) were added to the cool, molted agar media for inoculation, and three identical Petri dishes were used for each. Following solidification, 200 µl of various powders containing 500 µg/ml were made and separately placed into the tiny wells (5 mm). For 48 hours, plates were incubated at 37°C. Zones of inhibition (ZOI) were measured in millimetres following the incubation (mm).

Minimal inhibition concentration (MIC)

In two sets of conical flasks holding various dosages (25-250 µg/ml) of powder concentrations, Mueller-Hinton broth (MHB) was made, distributed into 100 ml conical flasks, and autoclaved before being inoculated by 100 l of *B. cereus* and *E. coli* (0.5 McFarland standard (1-2 $\times 10^8$ CFU/ml)). For 24 hours, the inoculated flasks were incubated at 100 rpm and 37°C. In order to calculate the MIC value, the growth was evaluated spectrophotometrically at 600 nm against a negative control. Negative controls were also performed to exclude the composite (El-Zahed et al., 2023a).

Minimal bactericidal concentration (MBC)

To ascertain the least bactericidal concentration, the tested composites that completely inhibited the growth of the tested bacterial strains in the MIC testing (no apparent microbial growth) were subcultured and inoculated into solid MHA media (MBC) and then incubated for 24 hours at 37°C. MBC was defined as the dilution that fully prevented bacterial colony formation on the agar plates (El-Zahed et al., 2023b).

Statistical analysis

Using the software program SPSS version 18, the data were statistically examined. The mean standard deviation (SD) was used to express every value in the experiments.

Results and Discussion

The Abu Galaga deposit, located 20 km west of the port Abu Ghusun on a hill overlooking Wadi Abu Galaga, is where the ilmenite ore was extracted. The boulders were split in half, and the inside was then pulverised to a size of 200 mesh.

The ilmenite ore's elemental makeup was ascertained using energy-dispersive X-ray spectroscopy (EDX). Carbon (9.5%), oxygen (39.3%), titanium (15.1%), iron (24.0%), terbium (4.7%), molybdenum (2.6%), magnesium (1.8%), silicon (1.7%), and aluminium (1.3%) made up the majority of the key constituents in ilmenite matrix.

The chemical composition and functional groups of the sample of ilmenite ore were determined using FTIR. The stretching of -OH groups can be attributed to an absorption band at 3388 cm^{-1} in the FTIR spectra of ilmenite (Figure 1A). The O=C=O stretching of carbon dioxide was said to be the cause of the band at 2347 cm^{-1}. The -OH bending is represented by the characteristic band that is centred at 1598 cm^{-1}.

Moreover, other peaks at 1086, 1009, 547, and 436 cm^{-1}, which define the Ti-O and Fe-O matrix, were appeared. The spectra of ilmenite after heating to 300 and 600°C (Figure 1B &C) were shifted to absorption band at 3429 and 3403 cm^{-1} for -OH groups. Bands at 2347 and 1607 cm^{-1} were ascribed to the O=C=O stretching and –OH bending. Several peaks were also observed at 1094, 1018, 546 and 444 cm^{-1} & 1103, 555 and 460 cm^{-1} which characterize the matrix of Ti-O and Fe-O.

Using a UV/Vis spectrophotometer, the optical characteristics of the ilmenite ore were investigated. In water, the electronic spectra of solid-state ilmenite ore were captured between 200 and 900 nm (Figure 2). They show that the ilmenite sample absorbs UV light at 234, 292, and 357 nm in an aqueous media quite significantly. It exhibits good absorbance at the UV area because it has greatest peaks at 354 and 357 nm wavelengths with absorbances of 0.3

and 1.2, respectively.

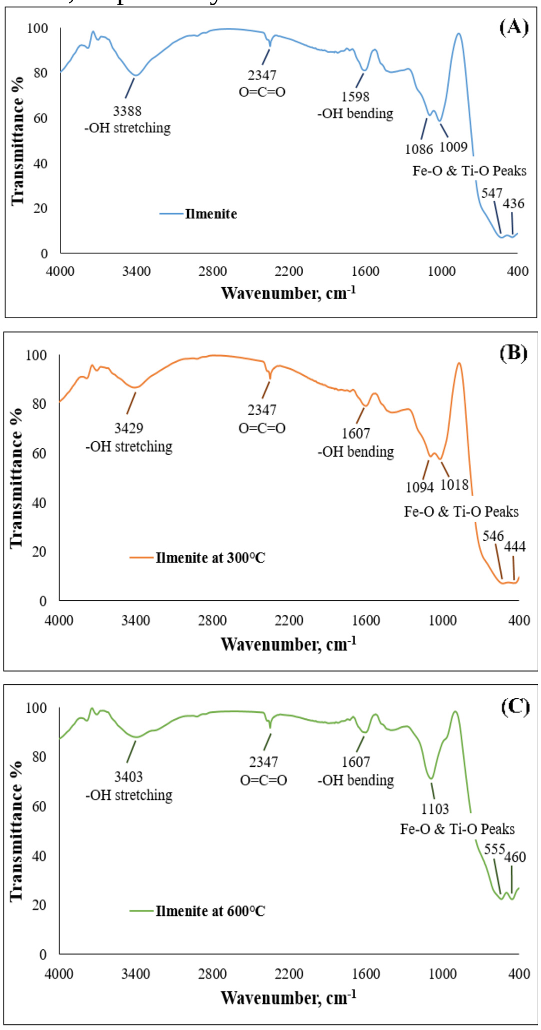

Figure 1. FTIR spectrum of ilmenite ore; (A) and the Fe-O/Ti-O composite at 300°C; (B) and 600°C; (C).

The material's energy band gap (E) has been determined using the formula below, where h is Planck's constant, c is the speed of light (hc ≈ 1.24 eV.µm), and λ is the wavelength of maximum absorption.

$$E = hc / \lambda$$

The ore's band gap energy was calculated using the aforementioned equation to be 5.30, 4.25, and 3.47 eV at 234, 292 and 357 nm, respectively. Also, this figure shows that titanium dioxide made up the majority of the ilmenite ore's composition.

According to that had been demonstrated in Table 1 and Figure 3, the produced composite had a good antibacterial effect, with highly significant differences ($P < 0.001$) between the tested microorganism and the diameter of the inhibitory zone. Fe-O/Ti-O composites (500 µg/ml) affected negatively on both Gram-positive and Gram-negative bacteria. The untreated ilmenite revealed superior antibacterial action against *B. cereus* and *E. coli* compared to the heat-treated composites. Ilmenite produced inhibition zones of 12 ± 0.03 and 21 ± 0.03 mm against *B. cereus* and *E. coli*, respectively while 300°C-treated Fe-O/Ti-O composite displayed inhibition zones of 11 ± 0.03 and 18 ± 0.06 mm against *B. cereus* and *E. coli*, respectively. These inhibition zones decreased when Fe-O/Ti-O composite treated by 600°C. It was also found that the obtained composites were more effective on Gram-negative bacteria than Gram-positive bacteria. The obtained results matched with Rani & Dehiya (2021) study which confirmed the stronger antibacterial action against Gram-negative bacterium *E. coli* with an inhibition zone of 17 mm than the other bacterial strains including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Shigella flexner* and *Salmonella typhi*. Ashraf et al. (2021) described the bactericidal activity of silver phosphate-ilmenite nanocomposites based by glycol chitosan mm against *S. aureus* and *E. coli*, with inhibition zones of 13 and 15 mm, respectively. Moreover, Maulidiyah et al. (2019) found that ilmenite ($FeO.TiO_2$) had good activity in inhibiting bacteria with a range of inhibitory diameter zone between 20 mm against *E. coli* and *S. aureus*.

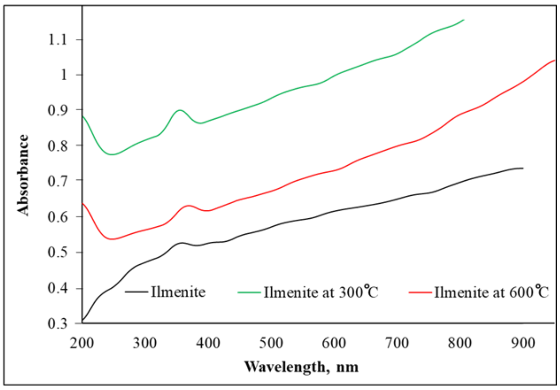

Figure 2. UV/Vis absorption spectra of ilmenite ore and the Fe-O/Ti-O composite at 300°C and 600°C in water medium.

Table 1. Agar well diffusion test of the prepared composite.

Antibacterial agent	Zones of inhibition (mm ± SD)	
	B. cereus	*E. coli*
Ilmenite	12 ± 0.03	21 ± 0.03
Fe-O/Ti-O composite at 300°C	11 ± 0.03	18 ± 0.06
Fe-O/Ti-O composite at 600°C	10 ± 0.03	15 ± 0.14

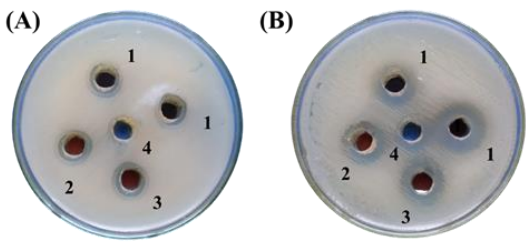

Figure 3. Antimicrobial activity of the prepared composites; (1) ilmenite, (2) Fe-O/Ti-O composite at 300°C and (3) Fe-O/Ti-O composite at 600°C against *B. cereus*; (A) and *E. coli*; (B). (4) dimethylformamide (DMF) as a solvent.

The antimicrobial action for 0-150 µg/ml of composites showed a gradual inhibition action against *B. cereus* and *E. coli* (Figure 4). Therefore, the antimicrobial behavior of composite showed a dose-dependent manner. Ilmenite, 300°C- and 600°C-treated Fe-O/Ti-O composite attained 100% inhibition (MIC values) at the concentrations of 100, 150 and 175 µg/ml against *E. coli*, respectively. While the MIC values were 175, 200 and 225 µg/ml against *B. cereus* for ilmenite, 300°C- and 600°C-treated Fe-O/Ti-O composite, respectively.

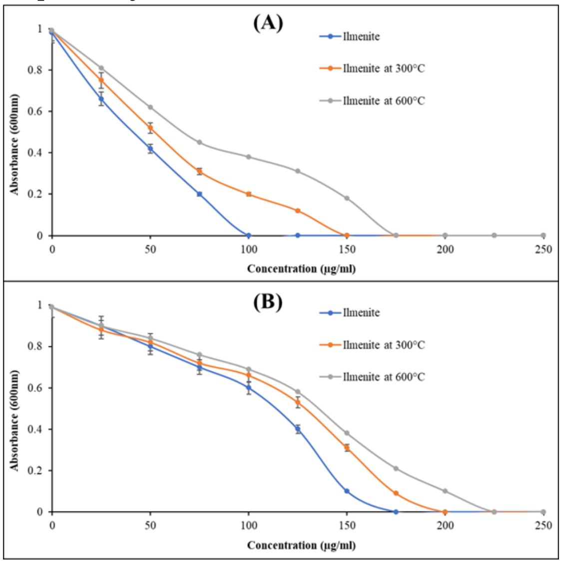

Figure 4. Minimum inhibition concentration of the prepared composites against *B. cereus*; (A) and *E. coli*; (B).

The MBC is the minimum concentration of an antibacterial agent needed to eradicate a specific bacterium over a long period of time, for example 18 or 24 h. Antibacterial agents are regarded as bactericidal if the MBC is no greater than 4 times the MIC. The MBC results are shown in Figure 5. Composites showed greater bactericidal activity against the Gram-negative bacteria than the Gram-positive bacteria. It is obvious that heat-untreated composite demonstrated strong antibacterial activity against the Gram-negative bacterium *E. coli* when compared to other composites with MBC value reached to 125 µg/ml.

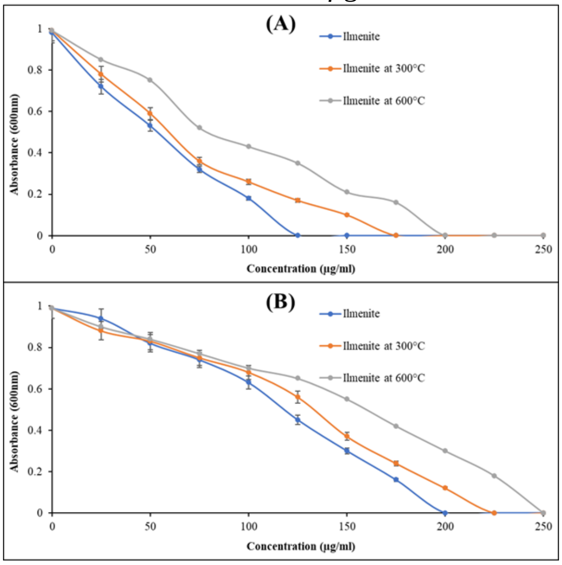

Figure 5. Minimum bactericidal concentration of the prepared composites against *B. cereus*; (A) and *E. coli*; (B).

Conclusion

Ilmenite, 300°C- and 600°C-heat treated Fe-O/Ti-O composite for two hours from naturally Abu Ghalaga black ilmenite, was prepared and examined for antibacterial activity based on agar well diffusion, minimum inhibition concentration and minimum bactericidal concentration tests. They also were characterized using an energy band gap, a Fourier-transform infrared spectrophotometer (FTIR), an energy-dispersive X-ray spectrometry (EDX), and a UV/Vis spectrophotometer. The unheated ilmenite showed the best antibacterial action compared to the other heated composites, especially Gram-negative bacteria. The Fe-O/Ti-O composite can be an outstanding contender for antibacterial applications due to its low cost and straightforward fabrication process as well as its quick and powerful antimicrobial capacity.

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الملخص العربي

عنوان البحث: التوصيف والنشاط المضاد للبكتيريا لمركب Fe-O / Ti-O المصنع بشكل طبيعي من خامات الإلمنيت في أبو غلجا بمصر

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احتلت الاستكشافات التطبيقية لمركب Fe-O / Ti-O كعوامل مضادة للبكتيريا موضع اهتمام كبيرا نظرًا لتعزيز وقوة نشاطها المضاد للبكتيريا. تم تصنيع مركب Fe-O / Ti-O من خام الإلمنيت كمصدر أولي للتيتانيوم. تم قطع الصخور ثم تم طحن الجزء الداخلي غير المؤكسد إلى حجم ٢٠٠ شبكة باستخدام مطحنة. كما تم تحضير المساحيق المعالجة حراريا عند ٣٠٠ و٦٠٠ درجة مئوية لمدة ساعتين. كانت طريقة التحضير فعالة، مباشرة وقليلة التكلفة وذلك بسبب عدم وجود الجاذبية التقليدية، الفصل المغناطيسي، الفصل الكهروستاتيكي، طحن الكرة أو الصهر. تم توصيف الإلمنيت والمساحيق المعالجة حرارياً بمقياس الطيف الضوئي للأشعة المرئية / فوق البنفسجية، مطياف الأشعة السينية المشتت للطاقة (EDX) والتحليل الطيفي للأشعة تحت الحمراء. تم اختبار التأثير المضاد للبكتيريا للمساحيق المحضرة ضد البكتيريا موجبة الجرام باسيلس سيريس والبكتيريا سالبة الجرام إيشيريكيا كولاي. أظهرت النتائج أن النشاط المضاد للبكتيريا للإلمنيت انخفض بزيادة درجات حرارة المعالجة الحرارية. وأيضا كان للمساحيق المحضرة نشاطا قويا مضادا للبكتيريا سالبة الجرام عن الموجبة لصبغة الجرام.