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Preparation and Characterization of Bi_2MO_6 (M = Mo, W) for Antibacterial Activity

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Abstract

In recent years, Bi-based photocatalysts have begun to be applied in biological applications. However, the antibacterial ability of a Bi-based photocatalyst is still unclear. In this study, Bi₂MoO₆ and Bi₂WO₆ were successfully synthesized by a hydrothermal approach. The fabricated samples were characterized by X-Ray diffraction, FESEM, and UV-Vis spectra. Besides, the antibacterial activity of both photocatalyst samples toward E. coli as negative and S. aureus as positive pathogens were studied. Compared with antibacterial of Bi2WO6, the resultant Bi₂MoO₆ exhibited high susceptible S. aureus bacterial strain revealing large zones of 24 mm to 29 mm, while Bi₂WO₆ exhibited less susceptible of 17.5 mm to 21.5 mm as compared with the zone of inhibition against tested bacterial E. coli. Besides, a possible mechanism suggested the effect of nanosheet structure of samples to penetrate the cell membrane which results in leakage of interior cell and complete death and these results will provide some support for the applications of Bi₂MoO₆ and Bi₂WO₆ in antibacterial materials under common environments.

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1. Introduction

The deterioration of water quality caused by pathogenic has received public concern around the world. Besides, antibiotic accumulation in water has seriously become a great threat to environmental safety and human health [1]. As one of the most abundant bacteria in the environment, *E. coli* and *S.aureus* are always of great interest since they are pathogenic, harmless, and commonly found in food and water environment [1, 2]. Among the different fields, nanobiotechnology has represented an important research area with increasing the number of synthesis nanomaterials in several scientific studies as possible alternatives for resistance to pathogens [3]. In recent years, green, efficient, and cost-effective Bi-based semiconductor photocatalysts have emerged as a more promising methodology than conventional technology of bacterial inactivation methods such as UV disinfection and chlorination [4–7]. Because of its high stability, strong redox potential, low cost, and non-toxic nature, TiO₂ has been extensively reported as an effective bactericidal semiconductor photocatalyst [8]. However, its band

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gap of 3.2 eV allows light absorption up to 387 nm, which accounts for just over 4% of the total solar spectrum. Because visible light (47 %) in solar radiation is more abundant than UV, efficient visible-light photocatalysts are required to make the best use of this proportion [9]. Recently, the Bi-based semiconductor technology has been considered a promising alternative applicant to treat pathogenic microorganism pollution related to its various advantages like being clean, and, cost-efficient without generation of secondary pollution [3, 10]. Among these Bi-based photocatalysts, bismuth molybedates, Bi₂MO₆ (M=W, Mo) with the layered bismuth oxide family is of particular interest as a typical Aurivillius oxide due to its dielectric, ion-conductive, luminous, and catalytic properties [11-13]. A typical n-type semiconductor comprised of accumulating layers of alternating $(Bi_2O_2)^{2+}$ layers and $(MO_4)^{2-}$ octahedral sheets is also a promising visible-light-driven photocatalyst with good chemical and thermal stability, aside from its non-toxic and ecologically friendly nature [14, 15]. Moreover, Bi2MoO6 can degrade organic contaminants and exhibit high antibacterial effects under visible light irradiation [16-17]. Besides, Bismuth tungsten Bi₂WO₆, representing the Bi-based photocatalysts, can produce strong oxidizing free radicals that inactivate microorganisms and is very suitable for antibacterial treatment. Bi₂WO₆ has received much attention as active in visible light regions and is more useful for environmental treatment purposes [18]. Despite the catalyst's lengthy history as a potential solution, most research on Bi₂MoO₆ has concentrated on the photocatalytic breakdown of organic pollutants, with only a few studies looking into the photocatalytic inactivation of microorganisms. A pseudo-first-order process observed E.coli degradation on Bi₂MoO₆ nest-like structures in a few hours [19]. Both Bi₂MoO₆ and Bi₂WO₆ have outstanding visible light photocatalytic activity due to their superior geometrical structural features [14, 20]. Constructing a unique micro/nano hierarchical structure typically shortens the paths of water contaminants, absorbs incidental light more efficiently due to increased multiple scattering, and is easily separated from wastewater by filtering or sedimentation processes [15, 21]. In this paper, Bi-based semiconductors like Bi₂MO₆ (M=W,Mo) were synthesized by using the hydrothermal method. The antibacterial effects and the proposed mechanism of the resulting nanosheets against the standard and multidrug-resistant E.coli as gram-negative and S.aureus as grampositive bacteria were investigated.

2. Experimental Part

2.1. Synthesis of Bi₂MoO₆ and Bi₂WO₆ Nanoparticles

Based on prior research [22], a two-step hydrothermal technique was used to manufacture nanoplate Bi2MoO6 and Bi2WO6. Bi(NO3)3 (2 mmol) was first dissolved in 10 mL of nitric acid solution (HNO3), then a 10-mL aliquot of Na_2MoO_4 solution (1 mmol) was dissolved and dropped into the Bi(NO_3)³ solution while stirring. The blended solution's pH level was set at 4. The finished suspension was quickly transferred to a Teflon-lined 50-mL autoclave and cooked at 180° C for 15 hours in the second stage. Following that, the suspension was allowed to cool naturally at ambient temperature, and the solid result was cleaned multiple times before being dried in the air at 60° C for 24h. Bi_2WO_6 nanoparticles were made using identical processes and amounts as Bi_2MoO_6 nanoparticles, but instead of Na_2MoO_4 , Na_2WO_4 was used.

2.2. Characterization

The XRD analysis of prepared samples was performed by Shimadzu X-ray diffraction 6000 diffractometer with CuK α radiation (λ =1.542 Å). Data were recorded in the 2 θ range of 10°-70°. The band gap of the samples was recorded on a Shimadzu UV-1800 spectrophotometer and determined the optical properties. The surface morphology of samples was characterized by field emission scanning electron microscopy (FESEM, Mira3-XMU). The chemical composition of produced samples was determined by energy dispersive x-ray spectroscopy (EDS connected to FESEM).

2.3. Antibacterial Activity

The antibacterial activity of the resulting semiconductor like Bi_2MO_6 (M=W,Mo) was evaluated against *E.coli* as negative and *S.aureus* as positive pathogens by agar well diffusion assay [23, 24]. 25 µl of *E.coli* and *S.aureus* cultures suspension were prepared with initial concentration 10^6 (CFU/ml, McFarland tube No.0.5) and

incubated for 18h and spread on the Mueller Hinton agar surface poured in Petri plates. Holes of 6 mm were made, and each hole was packed with different test concentrations of samples ranging from 50,100 and 200 µg\ml for each sample, as well as using distal water (DW) as a negative control. The plates were wrapped with parafilm tape and incubated at 37°C overnight. Negative controls using only *E.coli* and *S.aureus* were used. The inhibition zones of bacterial growth were then measured in millimeters [25, 26].

3. Results and Discussion

3.1. Structural Properties

Figure 1(a,b) shows the X-Ray diffraction pattern of Bi_2MoO_6 and Bi_2WO_6 as generated by the hydrothermal method, respectively. As shown in Figure 1a, the (131), (200), (151), (202), (133) and (280) planes of the orthorhombic Bi_2MoO_6 phase were responding to well-defined peaks at $2\Theta=28^\circ$, 32.4° , 36° , 46.6° , 55.2° , 56° , respectively (JCPDS card no. 21-0102) [27]. Besides, there were no additional prominent peaks, confirming Bi_2MoO_6 purity. The peaks at 2Θ of 27.8° , 32.8° , 47° , 55.6° , 58.4° , 68.6° , and 75.6° are assigned to (131), (200), (202), (133), (262) and (400) planes of orthorhombic Bi_2WO_6 (JCPDS, No. 39-0256), respectively as shown in Figure 1b which are similar to patterns of Bi_2MoO_6 sample revealing the Bi_2WO_6 is also isomorphic [28]. Moreover, the diffraction peaks of Bi_2WO_6 in the range of $2\theta=30-60^\circ$ are higher than those of Bi_2MoO_6 . Besides, there were no other diffraction peaks that could have been caused by contaminants that indicate crystallinity [16].

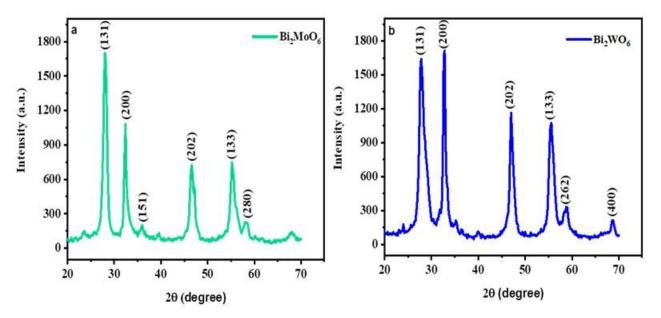


Figure 1: XRD patterns of the as-prepared a) Bi₂MoO₆ and b) Bi₂WO₆ products.

3.2. Morphological Properties

FESEM was used to characterize the microstructure and morphology of Bi_2MoO_6 and Bi_2WO_6 . As shown in Figure 2a, the FESEM image of Bi_2MoO_6 nanoparticles was made up of several uneven nanosheets or typical nanoplate like structures with smooth surfaces. Nanosheets were extremely thin with a thickness of 40-60 nm and an average width of approximately 300 nm. Furthermore, the nanosheets were tightly packed. Figure 2b, shows the FESEM image of the as-prepared Bi_2WO_6 . This resulted in aggregated irregular tiny flake-like Bi_2WO_6 that is made up of agglomerates of nanoplatelets with various orientations. The EDS spectrum of the Bi_2MoO_6 and Bi_2WO_6 samples were made to confirm the elemental composition and distribution homogeneity and are shown in Figures (c and d). Bi, M, and O components for Bi_2MoO_6 and Bi, W, and O components for Bi_2WO_6 are present throughout the entire samples. The homogenous distribution of each element in the matrix proves the samples' cleanliness.

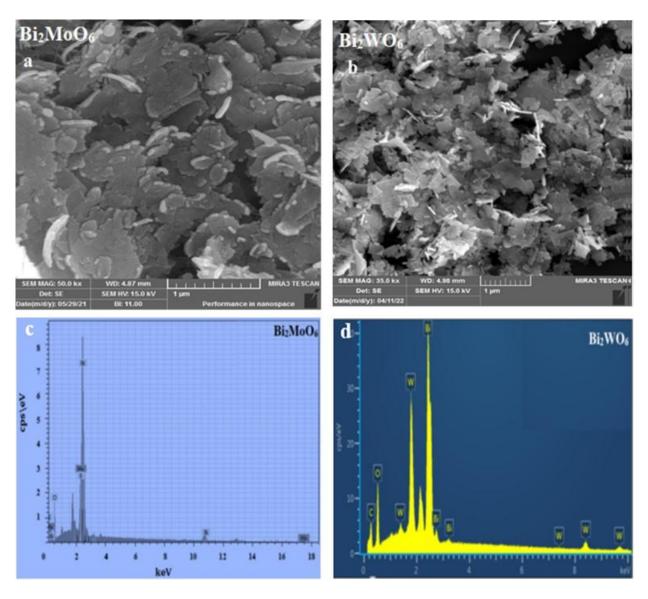


Figure 2: FESEM images (a) Bi₂MoO₆, (b) Bi₂WO₆ and EDS images (c) Bi₂MoO₆ and (d) Bi₂WO₆ samples of prepared hydrothermal technique.

3.3. Optical Properties

The UV-Vis spectra analysis was carried out in the range of 240-700 nm to determine the optical properties of Bi_2MoO_6 and Bi_2WO_6 , respectively. As displayed in Figures 3 (a, b) and 4 (a, b), it was obvious that the photocatalyst had a strong absorption capacity in the visible light region. As can be seen from Figures 3 (a, b) and 4 (a, b), the absorption edges of Bi_2MoO_6 and Bi_2WO_6 were observed at about 300 nm and 400 nm, respectively. The absorption edge of Bi_2MoO_6 is higher than that of Bi_2WO_6 . In addition, as shown in Figures 3b and 4b, the band gaps of resulted samples Bi_2MoO_6 and Bi_2WO_6 were calculated using equation h=A(hv-E_g)n where absorption coefficient, photon energy, and general constant are all defined, as α ,hv and A, respectively [29]. Using plotting α hv against hv (Tauc-plot), then a tangent onto the linear range and extrapolation can be used to determine the band gaps.

3.4. Antibacterial Activity

To investigate the antibacterial activity of resulting Bi_2MOO_6 and Bi_2WO_6 samples against test bacteria strain *E.coli* and *S.aureus* bacteria, three different test concentrations (50, 100, and 200) $\mu g/ml$ of each sample were incubated with bacterial strains at 37°C for overnight without irradiation, respectively. As shown in Figures 5a,

b and 7a, b, the results indicated that the tested bacterial strains, S.aureus revealed the highest zone of inhibition (24 to 29 mm) for Bi_2MoO_6 and (17.5 to 21.5 mm) for Bi_2WO_6 in the concentrations of 100 $\mu g/ml$, 200 $\mu g/ml$, respectively. While the less susceptible strain was found to be E.coli which revealed a smaller zone of inhibition (15.7 to 20.3mm) for Bi_2MoO_6 and (12.6 to 18.7 mm) for Bi_2WO_6 at concentrations of 100 $\mu g/ml$,200 $\mu g/ml$, respectively.

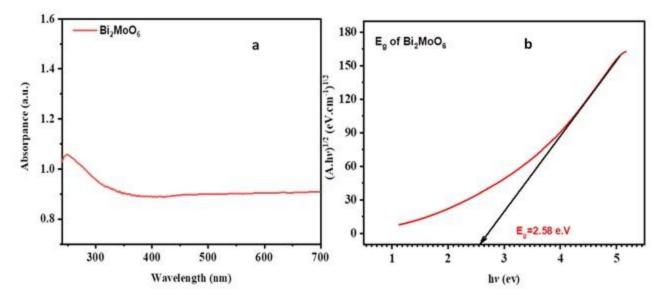


Figure 3: The UV-Vis analysis spectra of (a Bi₂MoO₆, (b energy gap Eg of Bi₂MoO₆

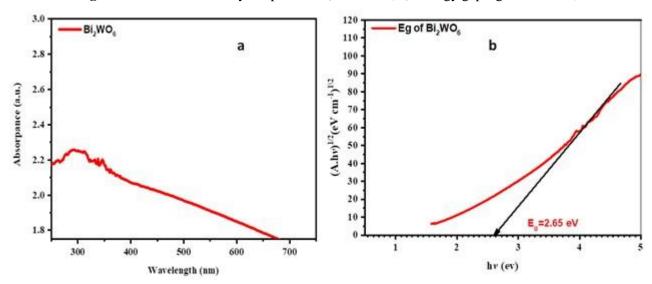
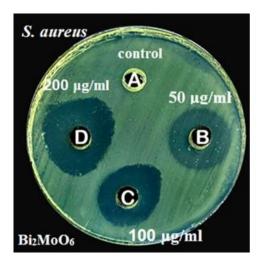


Figure 4: UV-Vis analysis spectra of (a Bi₂MoO₆, and (b energy gap E_g of Bi₂WO₆

No inhibition zone was detected for control (D.W) as shown in Figures 6 a,b and 8 a,b, respectively. Moreover, the results exhibited that the tested bacterial strain, S.aureus was the most susceptible bacterial strain revealing large zones of (24 mm to 29 mm) for Bi_2MoO_6 and becoming less susceptible (17.5 to 21.5 mm) for Bi_2WO_6 in the concentrations of 100 µg\ml, 200 µg\ml, respectively in comparison with the inhibition zone against tested bacterial E.coli as display in Figures 7 a,b and 8 a,b.



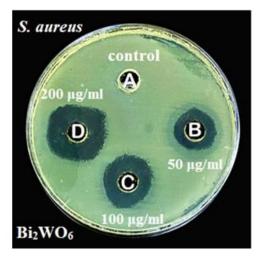
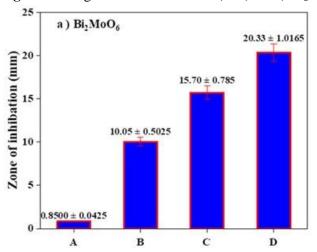


Figure 5: Images of inhibition Zone (mm) for a) Bi₂MoO₆ and of b) Bi₂WO₆ against *E.coli* bacterial strain.



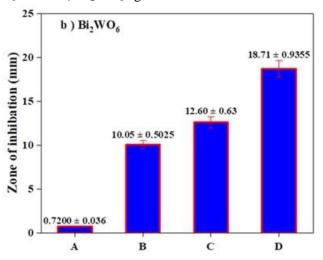
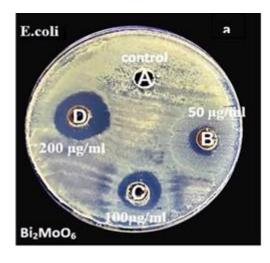


Figure 6: The Antibacterial activity of a) Bi₂MoO₆ and b) Bi₂WO6 against *E.coli* using different content A)control, B) 50 μg/ml, C) 100 μg/ml, D) 200 μg/ml



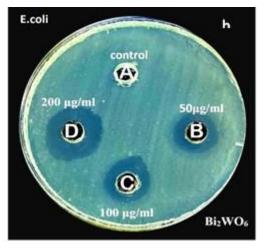


Figure7: Images of inhibition Zone (mm) for a) Bi₂MoO₆ and of b) Bi₂WO₆ against *S.aureus* bacterial strain

These results reveal that Bi₂MoO₆ has strong antibacterial activity against *E.coli* and *S.aureus* bacteria related to possible mechanism dealing with penetration of cell membrane by Bi₂MoO₆ nanosheets as compared with Bi₂WO₆, which results in oxidation of the bacterial membrane by the effective electrostatic force between Bi-based and bacterial surface and results in the leakage of the interior component as well as improved their resistance to biological contamination [29, 30]. In addition, the interesting point to underline is that the resulting samples Bi₂MoO₆ and Bi₂WO₆ exhibited a strong effect on gram-positive and gramnegative bacteria, which highlights the great clinical and technological application [24, 31].

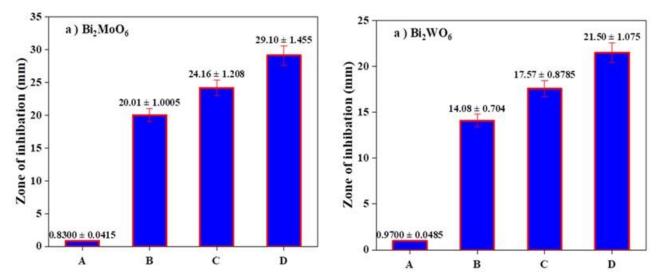


Figure 8: The Antibacterial activity of a) Bi₂MoO₆ and b) Bi₂WO₆ against *S.aureus* sing different content A)control, B) 50 μg/ml, C) 100 μg/ml, D) 200 μg/ml

4. Conclusions

Bi₂MoO₆ and Bi₂WO₆ were synthesized by a hydrothermal method. The structure and morphology characterization of fabricated samples by using FESEM, EDS, and XRD reveals the formation of a nanosheet or nanoplate like structure with a smooth surface of each sample and the forming of orthorhombic Bi₂MoO₆ and Bi₂WO₆ phases without defects. Besides, a strong absorption capacity in the visible light region was exhibit of resulted in Bi₂MoO₆ and Bi₂WO₆. Moreover, the antibacterial activity of Bi₂MoO₆ and Bi₂WO₆ was improved against S.aureus bacteria and became less susceptible against *E.coli* as well as the Bi₂MoO₆ became highly susceptible against *E.coli* and *S.aureus* bacteria related to oxidation of the bacterial membrane by the effective electrostatic force between the Bi-based and bacterial surface.

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Conflict of Interest

No conflict

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