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Abstract. $BiFeO_3$ nanoparticles was synthesized by sol gel auto combustion. The as-synthesized $BiFeO_3$ were characterized by X-ray diffraction (XRD), UV-Vis diffuse reflectance spectra (DRS) and Brunauer-Emmett-Teller (BET) analysis. The disinfection activities towards Gram-positive *Staphylococcus aureus* (*S.aureus*) were examined under visible light irradiation. The results showed a complete inactivation of 3 x 10^6 CFU/mL *S.aureus* was achieved within 20 min. The disruption of bacterial cell structure was observed by Transmission electron microscope (TEM). The cells were severely damaged after being exposed to $BiFeO_3$ under visible light irradiation. Hence, the results demonstrated the potential application of perovskite-type photocatalyst, $BiFeO_3$ in photocatalytic disinfection of various microorganisms.

1. Introduction

Clean and safe water access is necessary for the substance of all living organisms. The presence of pathogenic microorganisms play a major role in threating water safety. Removal of pathogenic microorganisms from contaminated water is crucially important to prevent potential negative effects on both human health and environment. Currently, various traditional disinfection techniques such as chlorination, ozonation, ultraviolet irradiation and advance filtration processes have been used for contaminated water treatment. These conventional techniques were found to suffer from some drawbacks such as formation of carcinogenic and hazardous byproducts (DBPs), regrowth of harmful bacteria [1], [2]. Escherichia coli (*E.coli*) are known as an indicator of water decontamination, have potential to many health problems such as serious diarrhea and can lead to fatality in humans [2]. It is of critical important searching for an alternative technology that are safe, low-cost and environmental-friendly methods in order to tackle the abovementioned problem. Photocatalysis have been extensively studied to circumvent the environmental problems including the inactivation of microorganisms by utilizing solar energy or visible light source [2]. Many studies have been conducted in photocatalytic

disinfection of various microorganisms by light-induced nanomaterials such as Cu-TiO_2 [3], Fe-doped $BiVO_4$ [2], Cd, Mo and W sulphide [4], Vanadium tetrasulfide (VS_4) [5], g-C_3N_4-AgBr [1], Pb-$BiFeO_3$/rGO [6].

Among various visible-light-driven photocatalyst, perovskite-type bismuth iron oxide ($BiFeO_3$) have been recognized as promising and efficient photocatalyst due to their attractive properties. $BiFeO_3$ has a small band gap (~2.0 eV) which appears to be sensitive to visible light and exhibit high efficiency in photocatalytic activity. $BiFeO_3$ also demonstrated a good thermal stability [7], high chemical stability [8], has magnetic properties [9], multiferroic at room temperature which helps in separation of charge carriers [10], showing a great potential in photocatalytic activity. Thus, this study will focus on photocatalytic disinfection of *Staphylococcus aureus (S.aureus)* under visible light irradiation.

2. Experimental

2.1. Materials

Bismuth nitrate pentahydrate, $Bi(NO_3)_3.5H_2O$, iron (III) nitrate nonahydrate, $Fe(NO_3)_3.9H_2O$ and 25% ammonia solution, NH_3 were purchased from Sigma Aldrich, while citric acid monohydrate, $C_6H_8O_7.H_2O$ was purchased from QRec Chemicals. Nutrient agar and Nutrient broth were provided by Merck. *Staphylococcus aureus* was obtained from DSMZ- German Collection of Microorganisms and Cell Cultures. The experiment will take place in a customized photocatalytic reactor under visible light irradiance using 100 W LED light source.

2.2. Preparation of $BiFeO_3$ photocatalyst

The synthesis of $BiFeO_3$ was carried out by gel-combustion method based on the previous study carried out by Hassan & Islam (2015). According to stoichiometric preparation of bismuth ferrite, BiFeO3, the calculated amount of bismuth nitrate pentahydrate, $Bi(NO_3)_3 \cdot 5H_2O$ and iron (III) nitrate nonahydrate $Fe(NO_3)_3 \cdot 9H_2O$ were dissolved in citric acid solution at 60 °C of constant stirring for 30 minutes. The amount of citric acid needed was equal to the molar amount of metal nitrates in the solution. Subsequently, ammonia solution was added dropwise to adjust the pH to 7 and subsequently stabilize the solution. The nitrate-citrate solution was then heated to 130 °C for 2 hours to obtain viscous gel. The resultant gel evaporates all water molecules from the solution and vigorous fuming was occurred which subjected to combustion reaction. The powders were annealed at temperature of 400°C overnight to obtain homogenous $BiFeO_3$ nanoparticles. This activation process will transform the precursor into loose powder. The obtained powder was then collected as the $BiFeO_3$ nanoparticles.

2.3. Characterization of $BiFeO_3$ photocatalyst

The crystallinity and the phase purity of the samples were determined by powder X-ray diffraction (XRD) using an X-ray diffractometer (PANanalytical X'Pert PRO, Japan) with Cu Kα radiation (λ = 0.154056 nm). The prepared materials were characterized by UV–vis Diffuse Reflectance Spectroscopy (DRS) measurements using a UV–vis-NIR spectrophotometer (Lambda 1050 PerkinElmer). Brunauer-Emmett-Teller (BET) surface area were determined from nitrogen adsorption-desorption method.

2.4. Photocatalytic disinfection experiment

The strain *S.aureus* used in this study was purchased from DSMZ- German Collection of Microorganisms and Cell Cultures. All apparatuses utilized were sterilized by an autoclave at 121°C. *S.aureus* was grown in nutrient broth and incubated overnight at 37°C under constant shaking. The bacterial cells were centrifuged and washed with saline solution (0.9% NaCl). After that, the bacterial stock solutions were re-suspended in sterilized saline solution and initial bacterial concentration was approximately 3 X 10^6 CFU/mL. The experiment was conducted in a photocatalytic reactor under irradiation of 100 W visible light. Upon disinfection experiment, bacterial suspension was stirred in the dark for 30 min to attain the equilibrium. The photocatalyst (1 mg) was dispersed in bacterial

suspension under continuous stirring. The samples (1 mL) were collected at predetermined time intervals and cultivated onto the Nutrient agar plate to assess the viability of bacteria. Prior to colony count, the plates were incubated overnight at 37℃. Three replicate plates were used in each sampling time. Three control experiments procedure was conducted as stated: (i) light control (without photocatalyst), (ii) dark control (without visible light) and negative control (without light and photocatalyst).

2.5. Characterization of the morphology of S.aureus

The morphology of treated *S.aureus* by $BiFeO_3$ was examined by TEM analysis (Hitachi).

3. Results and discussion

3.1. Characterization of $BiFeO_3$ photocatalyst

The crystal structure of the prepared samples were investigated by XRD analysis. Figure 1 shows the XRD patterns of the $BiFeO_3$ nanoparticles. The synthesized $BiFeO_3$ demonstrated the formation of rhombohedral perovskite structure as all the diffraction correspond to JCPDS card no 82-2327. The most prominent peak of $BiFeO_3$ can be seen at 32.08˚ indexed correspond to (110) diffraction plane. The characteristic of this peak indicates that $BiFeO_3$ has the perovskite-type rhombohedral structure [12]. From the XRD pattern, sharp and high intensity with narrow width of the observed peaks signifies that the $BiFeO_3$ nanoparticles are well crystallized [13][14]. The average of crystallite size of nanoparticles was calculated using Scherrer's formula. The calculated crystallite size of $BiFeO_3$ was about 12 nm.

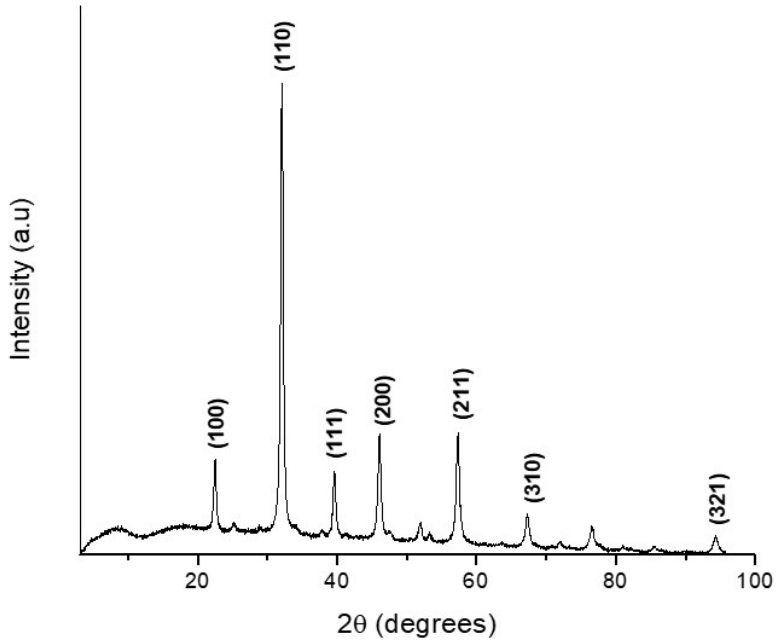

Figure 1. XRD pattern of $BiFeO_3$ nanoparticles.

Understanding optical properties of photocatalyst is one of the important key factor in photocatalytic studies. The UV-Vis diffuse reflectance spectra was performed to observe the optical absorption of prepared $BiFeO_3$ nanoparticles. As shown in Figure 2, the absorption edge of $BiFeO_3$ was found at approximately 580 nm implying that $BiFeO_3$ can be activated under visible-light source. Visible light wavelength range is between 400 nm to 700 nm [15]. Strong absorption edge in perovskite-type oxide can mainly be ascribed to the electronic transition from the valence band to conduction band [16][17]. The optical band gap energy (E_g) is a crucial parameter to

assess the activity of prepared photocatalyst, which the low band gap values of materials often exhibit the best performances in photocatalytic activity [18]. Generally, the common acceptable band gap value of the most photocatalyst are below 3 eV, making it desirable to be activated under visible-light irradiation [19]. The band gap value of the materials was observed by plotting $(\alpha hv)^2$ versus photon energy (hv), where α, h and v were absorption coefficient, Planck constant and light frequency, respectively. The calculated band gap value of the $BiFeO_3$ was shown in Figure 2 (b) below. The band gap value for the synthesized samples was 2 eV, thus making it desirable materials to be activated under visible light. Hence, $BiFeO_3$ photocatalyst can be considered for further study as a great visible light photocatalyst.

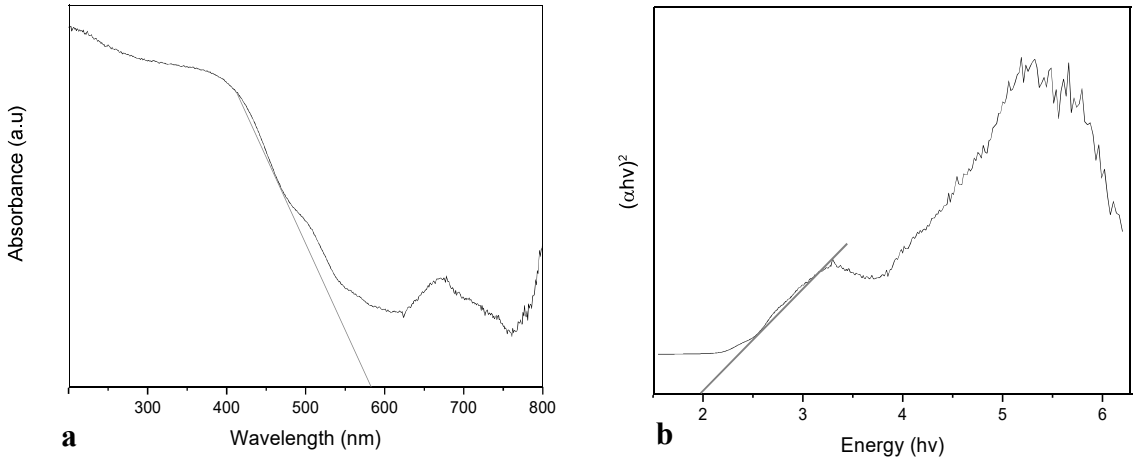

Figure 2. (a) UV-Vis absorption spectra of $BiFeO_3$ (b) Kubelka-Munk function $BiFeO_3$ to estimate band gap value.

The specific surface area and pore size distribution are of important role in determining its capabilities to absorb and degrading pollutants or microbial inactivation. $BiFeO_3$ exhibited BET surface area, pore size and total pore volume of 27.61 m^2/g, 16.40 nm and 0.11 cm^3/g, respectively. These findings are consistent with previous reported studies [8]. High surface area provides more active adsorption sites and promotes better adsorption capacities in photocatalytic activity [20]. Figure 3 showed nitrogen adsorption-desorption isotherms and the pore size distribution curves of $BiFeO_3$. According to IUPAC classification, $BiFeO_3$ shows a typical type III isotherm with H3 hysteresis loops, implying the presence of certain amount of macroposity in the materials.

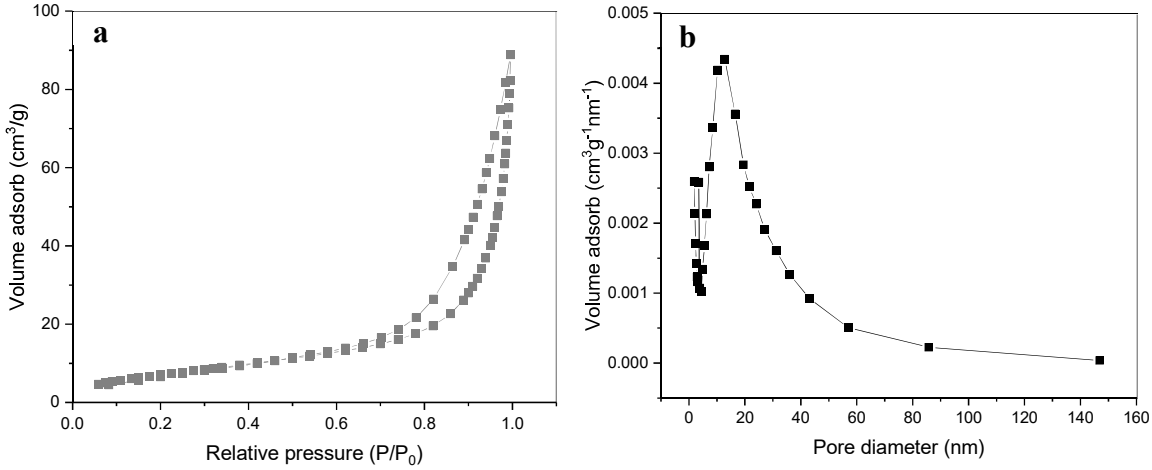

Figure 3. (a) Nitrogen adsorption-desorption isotherm (b) pore size distribution curves of $BiFeO_3$.

3.2. Morphological change of S.aureus exposed to $BiFeO_3$

The change of morphological bacterial cells during the disinfection process were observed under TEM images. Figure 4 (a) shows a smooth morphology of cells which indicated a normal display of bacterial cells. After 10 min reaction with $BiFeO_3$ under visible light, the cells become lysed as shown in Figure 4 (b). After 20 min of light irradiation, cells were irregularly shaped and become severely damaged as shown in Figure 4 (c). The outer membrane of the cells was ruptured which then results in release of intracellular components of the cells. Cells that have been exposed to photocatalyst demonstrated the production of reactive oxygen species during photocatalytic disinfection and interrupted all the unsaturated lipids as well as organic covalent bonds of the cells [1]. This eventually lead to the cellular death.

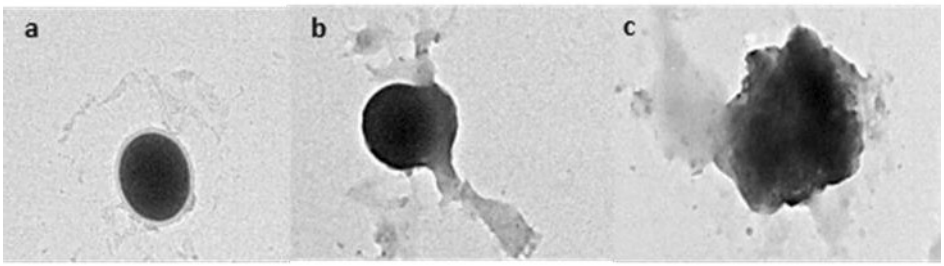

Figure 4. TEM images of S.aureus exposed to $BiFeO_3$ photocatalyst under visible light irradiation at time (a) 0 min (b) 10 min (c) 20 min.

3.3. Disinfection efficiency of photocatalyst

Photocatalytic disinfection activity of $BiFeO_3$ towards *S.aureus* was investigated at 1 mg of photocatalyst under visible light irradiation as shown in Figure 5. The results clearly show that no noticeable decrease in the viable cell density for all three control experiments, indicating that the bacteria cells were not affected by the absence of photocatalyst and light irradiation (negative control). Also, the presence of photocatalysts without light irradiation (dark control) and the presence of visible light without photocatalyst (light control) were not toxic to the cells. The inactivation of *S.aureus* can be clearly seen when the cells were exposed to $BiFeO_3$ nanoparticles under visible light irradiation. The cell density of *S.aureus* significantly decreased, suggesting that the death of bacterial cells.

Complete inactivation of *S.aureus* at initial concentration 3 x 10^6 CFU/mL was successfully observed in 20 min by $BiFeO_3$ nanoparticles under visible light irradiation.

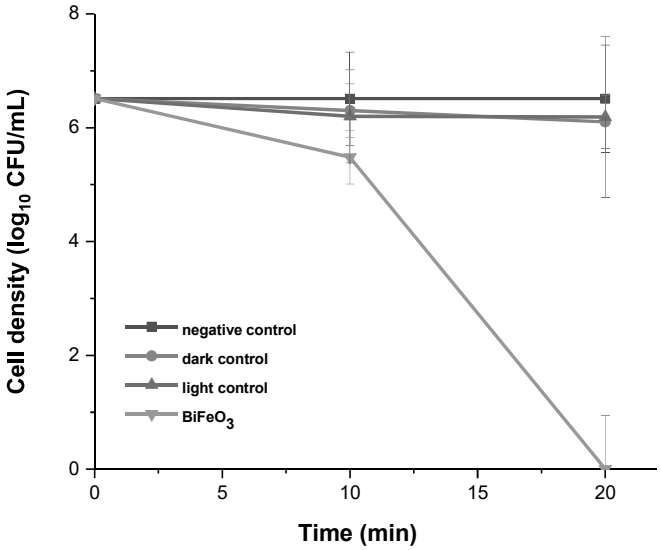

Figure 5. Disinfection efficiencies of $BiFeO_3$ under visible light irradiation with bacterial density 3 × 10^6. Error bars represent standard deviations from triplicate experiments (n=3).

4. Conclusion

In this study, $BiFeO_3$ nanoparticles was synthesized by sol-gel auto combustion method. Visible-light driven photocatalyst, c The absorption intensity of $BiFeO_3$ was found to be in the visible light range. Moreover, high surface of $BiFeO_3$ helps to increase the efficiency of photocatalytic disinfection activity. The as-synthesized $BiFeO_3$ exhibited strong antibacterial activity towards *S.aureus.* Direct contact between the nanoparticles and bacterial cells play a crucial role for the cell inactivation under visible light irradiation. Complete inactivation of *S.aureus* was achieved within 20 min and the damage of cell membrane of the cells led to bacterial inactivation. Taking into consideration, $BiFeO_3$ nanoparticles would be a great potential photocatalyst for an excellent photocatalytic disinfection of microbes.

Acknowledgement

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