Hydrothermally synthesized Al-doped BiVO₄ as a potential antibacterial agent against methicillin-resistant *Staphylococcus aureus*

Charles Sundar Vicas^{1,2}, Namratha Keerthiraj², Nayan Byrappa³, Kullaiah Byrappa^{1,4*}

¹Department of Materials Science, Mangalore University, Mangalagangothri, Mangalore 574199, India

²Center for Materials Science and Technology, Vijnana Bhavan, University of Mysore, Mysore 570006, India

³Department of Studies in Environmental Science, University of Mysore, Mysore 570006, India

⁴Adichunchanagiri University, N.H. 75, B.G. Nagara, Mandya District - 571448, India

ABSTRACT

One-pot hydrothermal route was adopted to synthesize Al:BiVO₄, at 4 h and 8 h reaction durations, by adding 1% aluminiumoxide powder (w/v) to the precursors. The products were investigated using several characterization techniques that conform a significant morphological change and a decrease in bandgap energy of the materials upon Al modification of scheelite monoclinic bismuth vanadate matrix at both hydrothermal durations. Antibacterial experiments were performed against methicillin-resistant *Staphylococcus aureus* in visible light condition to harness the photooxidation property of Al-doped BiVO₄ and compare to that of unaltered BiVO₄. Minimum inhibitory concentration of the synthesized materials was identified. The results indicate that Al-doping on BiVO₄ has a significant effect on its photocatalytic antibacterial performance. Al:BiVO₄ synthesized at 8 h hydrothermal treatment parades excellent sunlight-driven photocatalysis compared to the one synthesized at 4 h.

Keywords: Bandgap energy, Hydrothermal method, Methicillin-resistant *Staphylococcus aureus*, Photo-oxidation, Selective doping

1. Introduction

Given extreme energy crisis, semiconductor technology offers a perfect solution in harnessing solar energy and effectively dealing with the environmental pollution [1]. Titanium dioxide (TiO₂) is widely studied and is most popular among photocatalysts that are being used. It is also known to be non-toxic with high oxidation capacity and chemical stability [2]. Henceforth it is extensively used in creams and cosmetics [3]. A major drawback with TiO₂ is its wide bandgap, which facilitates it to respond only to ultra-violet light, i.e., about 4% of total solar energy [4]. Thus, visible light photocatalysts like bismuth vanadate (BiVO₄) have come to limelight. BiVO₄ is widely studied due to their lower bandgap of 2.4 eV [5]. Photocatalytic property of BiVO₄ is identified to vary with their crystal structure. They exist in tetragonal zircon, monoclinic and tetragonal scheelite structures, among which the most active phase under visible light radiation is known to be

monoclinic scheelite structure [6-8]

Staphylococcus aureus (*S. aureus*) by far a major infectious agent among hospitals and within the community. Methicillin, a β -lactam antibiotic, acts by inhibiting penicillin-binding proteins (PBPs) that are unit concerned within the synthesis of peptidoglycan, a necessary mesh-like chemical compound that surrounds the cell. *S. aureus* will become proof against methicillin and different β -lactam antibiotics through the expression of a far off PBP, PBP2a, that's proof against the action of methicillin however which might perform the functions of the host PBPs. Methicillin-resistant *S. aureus* isolates are unit typically proof against different categories of antibiotics (through completely different mechanisms) creating treatment choices restricted, and this has light-emitting diode to the rummage around for new compounds active against these strains. Associate understanding of the mechanism of methicillin resistance has light-emitting diode to the invention of accent factors that influence the amount

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Copyright © 2019 Korean Society of Environmental Engineers

Received July 9, 2018 Accepted November 23, 2018

* Corresponding author

Email: kbyrappa@gmail.com

Tel: +91-7259667666

and nature of methicillin resistance. Several researchers round the world have experimented the chance of activity of nanoparticles such as silver, gold, zinc oxide, TiO_2 and so on, in terminating the growth of MRSA [9-11].

The synthetic routes available for the synthesis of BiVO_4 include majorly sol-gel method, hydrolysis of metal alkoxides, hydrothermal method, etc. [12]. Among these, the hydrothermal route is known to be most effective for processing advanced functional materials as it facilitates control over the size and morphology of the nanoparticles and does not involve in post-synthesis treatments [13]. In the present work, the preparation, characterization and photocatalytic efficiency of Al-doped BiVO_4 materials synthesised by hydrothermal method at 180°C for 4 h and 8 h duration, keeping pH 7 as constant, without the addition of any structure enhancing agents have been reported. Methicillin-resistant *S. aureus* (MRA, MTCC - 6908) is gram-positive cocci that is a significant infective agent that causes dental caries, skin and soft tissue infections (SSTIs), respiratory organ infection and so on [10]. It is an associate opportunistic parasite and statistically two thirds of the cocci in domestic water is of *S. aureus*. Antibacterial activity of aluminium doped BiVO_4 crystals against *S. aureus* has been completely investigated in this research.

2. Materials and Methods

2.1. Synthesis of Al-doped BiVO_4

Precursors used for the synthesis include 4M bismuth nitrate ($\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$, Rankem) dissolved in nitric acid (2M HNO_3 , Rankem) and 4M ammonium vanadate (NH_4VO_3 , Rankem) dissolved in ammonia (2M NH_4OH , Rankem). Precursor solutions were then mixed in equal volumes under constant stirring to form a clear yellow precipitate [14] while adding 1% aluminium oxide powder (w/v). pH of the mixture was adjusted to 7 by adding drops of conc. NH_4OH and the contents were transferred into two Teflon liners (20 mL in each) in general purpose autoclaves (SS 140). An amount of 20 μL of 1% sodium dodecyl sulphate (SDS) aqueous solution was added as a surfactant into each liner, before the treatment. Autoclaves were placed at 180°C (autogenous pressure) for 4 h and 8 h treatment, respectively (Thermotek® Hot-air Oven, India). The resultant products were repeatedly washed using ultrapure water (Purelab® Option Q7), filtered and dried overnight. Similar steps were followed to synthesise BiVO_4 without the addition of Al as a standard for comparison.

2.2. Materials Characterization

A series of characterization techniques were performed, such as X-ray diffraction (Rigaku® Mini-Flex II, Japan) for analysing the phase purity of the materials, Fourier transformation infrared spectroscopy (Jasco®, Japan) for identifying the functional groups, UV-Vis spectroscopy (Elico®, India) for obtaining absorption wavelength, field emission scanning electron microscopy (Quanta 200 FEG) for morphological studies and energy-dispersive X-ray spectroscopy (Thermo-scientific® UltraDry EDS detector, USA) for elemental analysis.

2.3. Bacterial Inactivation Test

Fresh overnight culture of *S. aureus* (gram-positive facultative bacteria, MTCC 6908) was made ready in Luria-Bertani broth (HIMEDIA) and therefore the cell suspension was diluted and adjusted to McFarland's customary 0.5. This cell suspension was collected into five sterilized flat bottom culture tubes (30 mL, Borosil®) aseptically in laminar air flow chamber and the prescribed concentrations (0.02, 0.04, 0.08, 0.1, 0.2 g/L) of the photocatalysts (Al: BiVO_4 (4 h) and Al: BiVO_4 (8 h)) were added into them. Two more tubes were taken together with these. One for 0.1 g/L of Degussa P25 titania, which is used as reference and another for 0.1 g/L of BiVO_4 (pure) which is used for comparison.

Culture tubes were capped and placed within the visible light for 9 h in a shaker. A 150 W short arc lamp with a 420 nm UV cut-off filter was used as visible light source. For every half an hour, percentage transmittance readings were taken at 580-600 nm and a graph was plotted against time.

2.4. Determining MIC of the Nanoparticles

Minimal inhibitory concentration is defined as the most minimal amount of material that can hinder the development of any microorganism. Experiment was conducted in multi-well culture plates of 96 wells as per standard protocol. MRSA were developed in Luria-Bertani (LB) broth medium at 37°C and 250 rpm overnight. The turbid broth was then diluted to get 7×10^6 CFU/mL as the inoculum. It was then added into wells, to which Al: BiVO_4 (4 h) and Al: BiVO_4 (8 h) nanoparticles (0.5, 1, 5, 10, 20, and 50 $\mu\text{g}/\text{mL}$) were added, respectively. The plate was placed on a rotating shaker at 200 rpm under visible light source for 9 h. Multiplate reader was used to measure OD at 595 nm and the values were plotted as shown in Fig. 6.

3. Results and Discussion

XRD spectra of the synthesised photocatalysts show the formation of well crystalline scheelite monoclinic structures, at both the durations of hydrothermal treatment. The graphs obtained are in good agreement with JCPDS card No. 14-0688 as shown in Fig. 1, which corresponds to I2/a space group of monoclinic bismuth vanadates with $a=5.195$, $b=11.70$, $c=5.092$ and $\alpha=\gamma \neq 90^\circ$, $\beta=90^\circ$. Although, notable differences were observed upon Al doping (Al^{+3} from Al_2O_3). An occurrence of interstitial doping of Al on BiVO_4 was evident from XRD spectra, which displays additional Al peaks, along with characteristic monoclinic peaks of BiVO_4 . The increase in peak intensity with hydrothermal synthesis duration from 4 h to 8 h was attributed to the formation of well crystalline structures [15, 16].

The values of 2 θ , Muller's indices, full-width half maximum, calculated crystallite size were tabulated in Table 1.

Crystallite size of the particles was calculated using the following formula (Eq. (1)). d-spacing was calculated using Bragg's law.

$$n\lambda = 2d\sin\theta \quad (1)$$

Where,

D = crystallite size = wavelength of source ($\text{Cu} = 1.544$)

β = full-width half maximum (FWHM)

θ = Bragg's angle

n = order of the plane

d = d-space

hkl = Muller's indices

FTIR spectra (Fig. 2) of the synthesised $\text{Al}:\text{BiVO}_4$ samples show a band around $1,000\text{--}1,100\text{ cm}^{-1}$, which was attributed to Al-O, $730\text{--}1,000\text{ cm}^{-1}$ was attributed to the stretching mode of VO_4^{3-} and its branch at 613 cm^{-1} attributes to Bi-O [17]. The peak observed at $1,386\text{ cm}^{-1}$ was assigned to stretching mode of C-H group and the one at $1,760\text{ cm}^{-1}$ attributes to C=O stretch from SDS which was used as the surfactant.

FE-SEM micrographs (Fig. 3) show distinct morphological changes on Al doping and hydrothermal duration. Micrographs of pure BiVO_4 sample synthesised at 4 h period show irregular cheese-puff like particles (Fig. 3 (a0)). Whereas, upon addition of Al^{+3} at 4 h synthesis flake-like particles were formed (Fig. 3(a1), (b1), (c1)). With the increase in hydrothermal duration to 8 h, $\text{Al}:\text{BiVO}_4$ showed perfect rod like monoclinic crystals (Fig. 3(a2), (b2), (c2)) and FE-SEM images show the presence Al (or Al^{+3}) on the surface of BiVO_4 . Addition of Al to BiVO_4 showed a significant influence on its. Hence, we see the formation of flake like structures at 4 h synthesis. Significant growth of well-formed rod like crystals during the hydrothermal reaction has been observed, as the crystallinity increased with longer hydrothermal reaction time of 8 h.

Fig. 1. XRD spectra of $\text{Al}:\text{BiVO}_4$ (8 h and 4 h) and BiVO_4 .

Fig. 2. FTIR spectra of $\text{Al}:\text{BiVO}_4$ (8 h and 4 h) and BiVO_4 .

Fig. 3. (a0) EDS spectrum and FE-SEM image of pure BiVO_4 synthesized at 4 h. (a1 & b1 & c1) FE-SEM micrographs of flake like $\text{Al}:\text{BiVO}_4$ (4 h). (a2 & b2 & c2) FE-SEM micrographs of monoclinic like $\text{Al}:\text{BiVO}_4$ crystals (4 h).

Structural changes in the products are in agreement with XRD spectra obtained. The increase in peak intensity with upon longer duration of hydrothermal treatment resulted in well crystalline monoclinic structures. Atomic percentages of synthesised materials were determined by EDS spectra as shown in Fig. 3(a0) and 3(d). It shows a comparison between atomic percentages of pure BiVO_4 and changes upon Al doping. Percentage oxygen varied from 74.77% to 74.16%, vanadium 13.48% to 13.81% and bismuth 11.75% to 10.17%. Although 1% Al_2O_3 has been used for doping,

Table 1. Shows 2θ Values, $\{hkl\}$ Indices, Full-Width Half Maximum, Calculated Crystallite Size and Bandgap Energies and Morphology of the Synthesise Materials

Catalyst synthesized	2θ (hkl)	FWHM	d-spacing	Crystallite size (nm)	Morphology	Band gap (eV)
BiVO_4	28.6 {121}	0.3874	3.1244	30.68	Irregular	2.35
$\text{Al}:\text{BiVO}_4$ (4 h)	28.6 {121}	0.4190	3.1210	27.44	Flakes like	2.23
$\text{Al}:\text{BiVO}_4$ (8 h)	28.6 {121}	0.3569	3.1240	34.77	Rod like	2.21

because of the presence of 2Al^{+3} in the molecule, EDS spectrum shows 1.86% of Al has been doped.

UV-Vis absorption spectroscopy (Fig. 4) was studied for catalysts that were synthesised at different hydrothermal durations. The obtained materials showed a characteristic absorption edge at around 550 nm, which correlates with n to π^* transition that corresponds to the excitation of an electron from one of the unshared pair to the π^* orbital [18]. It is noted that upon Al doping, absorption intensity was increased and further increased with increase in duration of synthesis. Band gap energies of the synthesised materials was determined from Tauc plot of $(\alpha h\nu)^2$ vs. photoenergy ($h\nu$)

Where, α = absorption coefficient.

Estimated E_g values are 2.35, 2.23, 2.21 eV for BiVO_4 , Al:BiVO_4 (4 h) and Al:BiVO_4 (8 h), respectively (Table 1 and Fig. 4). Thus, Al doping decreased the band gap energy of BiVO_4 significantly. The peaks below 400 nm are nothing but the maximum absorption intensity, usually observed when the initial impact of the photons on the material takes place.

Fig. 4. UV-Vis spectra and band gap energy determination.

Photooxidation inactivation of MRSA using the synthesized semiconductors was studied by the proposed 9 h experiment (Fig. 5). It was evident from the results obtained that all the synthesized samples showed a good activity against the growth of bacteria at all concentrations. It was also observed that due to insufficiency of direct interaction between the particles surface and the bacteria, the results were not spontaneous [19]. Thus, prolonged exposure to solar radiation facilitated the formation of more O-H radicals, which interact with the bacterial cell wall, causing its disruption. As proposed by Pablos et al., an electrostatic repulsion between the negatively charged bacterial surface and the photocatalyst surface would occur unless the intervention of an intermediate counter ion layer of cations takes place [20]. Thus, it was assumed that hydroxyl radical formation was hindered and hole transfer could be favoured under visible light [21].

Fig. 5. (a) Photocatalytic antibacterial activity of Al:BiVO_4 synthesized at 8 h of hydrothermal duration; (b) Photocatalytic antibacterial activity of Al:BiVO_4 synthesized at 4 h of hydrothermal duration.

As prepared photocatalysts showed good antibacterial activity in all concentrations. It is notable that 0.002 g/L of Al doped BiVO_4 particles showed higher antibacterial activity than the pure BiVO_4 and the reference material. Upon increasing the concentration, synthesized materials showed even higher antibacterial effect with highest being 94% by the particles synthesized at 8 h hydrothermal duration. The improved photocatalytic antibacterial performance of Al:BiVO_4 can be ascribed to the critical lessening in band-gap energies from 2.35 eV (unadulterated BiVO_4) to 2.23 eV (Al:BiVO_4 (4 h)) and further diminishment to 2.21 eV (Al:BiVO_4 (8 h)) with an increase in crystallinity of the material [18]. The presence of Al^{+3} (due to the usage Al_2O_3 as dopant) on the surface of the catalyst also improved charge transfer characteristics which resulted in significant increase in photocatalytic efficiency [19, 20].

MIC values of the nanoparticles against MRSA have been determined based in %T, calculated from OD values. MIC_{50} of both Al:BiVO_4 (4 h) and Al:BiVO_4 (8 h) has been determined as 5 $\mu\text{g/mL}$. Whereas, MIC_{90} of Al:BiVO_4 (4 h) is 50 $\mu\text{g/mL}$ and MIC_{90} of Al:BiVO_4 (8 h) is 10 $\mu\text{g/mL}$. This shows that there is a tremendous

Fig. 6. Microbial growth kinetic - (a) MRSA treated with Al:BiVO₄ (4 h); (b) MRSA treated with Al:BiVO₄ (8 h).

Fig. 7. Schematic representation of charge transfer mechanism and bacterial inactivation.

increase in antibacterial property of Al:BiVO₄ with increase in crystallinity. MIC₉₉ of Al:BiVO₄ (8 h) is 50 µg/mL, where 100% growth inhibition occurred.

Monoclinic BiVO₄ and Al/Al³⁺ could be excited to generate electrons and holes upon adequate visible light illumination, as shown in Fig. 7. These electrons further interact with O₂ and produce O₂^{·-}, which splits H₂O to form OH⁻ and ·OH. It also reacts with proton (H⁺) to form hydroperoxyl radical (HO₂[·]). These reactive oxygen species (ROS) in turn react with dyes, effluents, organic material present in water to release majorly CO₂ and H₂O, thus assisting in water treatment [21].

Oxidative stress shoulders a major role in antibacterial activity causing cytotoxic effects. This marks for a superior property of BiVO₄ in inhibiting the growth of pathogenic bacteria, which can be harnessed in real time applications after few toxicological studies.

4. Conclusions

Al modified BiVO₄ nanocrystals were synthesised successfully using hydrothermal treatment for the first time to enhanced photocatalytic properties of the material. It was evident that Al doping had a significant influence on the morphology of synthesised products. Upon Al³⁺ doping (valency of Al in Al₂O₃ is Al³⁺) on the surface of BiVO₄ matrix, irregular cheese-puff like morphology

of BiVO₄ changed to flake-like structures, which gradually formed rod like monoclinic structures upon increasing hydrothermal duration. This modification projected in a distinct enhancement of photocatalytic antibacterial property of the as-prepared materials. The highest antibacterial rate has been performed by Al:BiVO₄ which was synthesised at 8 h of hydrothermal treatment. The enhanced photocatalytic activity of Al:BiVO₄ can be attributed to the significant decrease in band-gap energy from 2.35 eV (pure BiVO₄) to 2.23 eV (Al:BiVO₄ (4 h)) and further reduction to 2.21 eV (Al:BiVO₄ (8 h)) with an increase in crystallinity of the material. The presence of Al/Al³⁺ on the surface of the catalyst also improved charge transfer characteristics which resulted in significant increase in photocatalytic efficiency. This research shows promising results for real time usage of Al:BiVO₄ for waste water treatment, after conducting few toxicological analyses.

Acknowledgement

The authors gratefully acknowledge the financial support given by University with Potential for Excellence project grants from UGC, India. The authors recognise the help received from the research scholars of our group, Mr Kashinath Lellela and Mr Abdo Hezam.

References

- Linic S, Christopher P, Ingram DB. Plasmonic-metal nanostructures for efficient conversion of solar to chemical energy. *Nat. Mater.* 2011;10:911-921.
- Dunlop PSM, Byrne JA, Manga N, Eggins BR. The photocatalytic removal of bacterial pollutants from drinking water. *J. Photochem. Photobiol.* 2002;148:355-363.
- Gkika E, Kormali P, Antonaraki S, Dimoticali D, Papaconstantinou E, Hiskia A. Polyoxometallates as effective photocatalysts in water purification from pesticides. *Int. J. Photoenerg.* 2004;6:227-231.
- Belver C, Adán C, García-Rodríguez S, Fernández-García M. Photocatalytic behavior of silver vanadates: Microemulsion synthesis and post-reaction characterization. *Chem. Eng. J.* 2013;224:24-31.
- Kubacka A, Fernández-García M, Colón G. Advanced nanoarchitectures for solar photocatalytic applications. *Chem. Rev.* 2012;112:1555-1614.
- Yin WZ, Wang WZ, Zhou L, Sun SM, Zhang L. CTAB-assisted synthesis of monoclinic BiVO₄ photocatalyst and its highly efficient degradation of organic dye under visible-light irradiation. *J. Hazard. Mater.* 2010;173:194-199.
- Zhang X, Du L, Wang H, et al. Highly ordered mesoporous BiVO₄: Controllable ordering degree and super photocatalytic ability under visible light. *Micropor. Mesopor. Mater.* 2013;173: 175-180.
- Yu JQ, Kudo A. Effects of structural variation on the photocatalytic performance of hydrothermally synthesized BiVO₄. *Adv. Funct. Mater.* 2006;16:2163-2169.
- Asmaa F. Methicillin-resistant *Staphylococcus aureus* (MRSA)

- screening of hospital dental clinic surfaces. *Saudi J. Biol. Sci.* 2018; <https://doi.org/10.1016/j.sjbs.2018.03.006>.
10. Harada D, Nakaminami H, Miyajima E, et al. Change in genotype of methicillin-resistant *Staphylococcus aureus* (MRSA) affects the antibiogram of hospital-acquired MRSA. *J. Infect. Chemother.* 2018;24:563-569.
 11. Grmek-Kosnik I, Dermota U, Ribic H, Storman A, Petrovic Z, Zohar-Cretnik T. Evaluation of single vs pooled swab cultures for detecting MRSA colonization. *J. Hosp. Infect.* 2018;98:149-154.
 12. Guo Y, Yang X, Ma F, et al. Additive-free controllable fabrication of bismuth vanadates and their photocatalytic activity toward dye degradation. *Appl. Surf. Sci.* 2010;256:2215-2222.
 13. Byrappa K, Adschiri T. Hydrothermal technology for nanotechnology. *Prog. Cryst. Growth Charact. Mater.* 2007;53: 117-166.
 14. Obregón S, Caballero A, Colón G. Hydrothermal synthesis of BiVO₄: Structural and morphological influence on the photocatalytic activity. *Appl. Catal. B. Environ.* 2012;117-118:59-66.
 15. Wang D, Jiang H, Zong X, et al. Crystal facet dependence of water oxidation on BiVO₄ sheets under visible light irradiation. *Chem. Eur. J.* 2011;17:1275-1282.
 16. Zhao Y, Xie Y, Zhu X, Yan S, Wang S. Surfactant-free synthesis of hyperbranched monoclinic bismuth vanadate and its applications in photocatalysis, gas sensing, and lithium-ion batteries. *Chem. Eur. J.* 2008;14:1601-1606.
 17. Garcia-Perez UM, Sepulveda-Guzman S, Martinez- de la Cruz A, Peral J. Selective synthesis of monoclinic bismuth vanadate powders by surfactant-assisted co-precipitation method: Study of their electrochemical and photocatalytic properties. *Int. J. Electrochem. Sci.* 2012;7:9622-9632.
 18. Stanculescu F, Stanculescu A. Polycarbonate-based blends for optical non-linear applications. *Nanoscale Res. Lett.* 2016;11:87.
 19. Wang W, Yu Y, An T, et al. Visible-light-driven photocatalytic inactivation of E. coli K-12 by bismuth vanadate nanotubes: Bactericidal performance and mechanism. *Environ. Sci. Technol.* 2012;46:4599-4606.
 20. Pablos C, Rafael van G, Javier M, Indranil C, Sharon LW. Study of bacterial adhesion onto immobilized TiO₂: Effect on the photocatalytic activity for disinfection applications. *Catal. Today* 2013;209:140-146.
 21. Vicas CS, Namratha K, Nayan MB, Byrappa K. Synthesis of manganese doped BiVO₄ with enhanced photocatalytic activity via hydrothermal method. *Mater. Focus* 2017;6:636-640.