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## Performance evaluation of Ag/SnO<sub>2</sub> nanocomposite materials as coating material with high capability on antibacterial activity

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### ABSTRACT

The performance of silver/tin oxide (Ag/SnO<sub>2</sub>) nanocomposites as coating materials with high antibacterial capability is evaluated in this study. Scanning electron microscopy (SEM) and density functional theory (DFT) analyses were used to characterize the nanocomposite structures and confirm that Ag (1 1 1) and SnO<sub>2</sub> (1 1 0) form nanorods of Ag/SnO<sub>2</sub>. The antibacterial properties of these materials were investigated by using an Alamar Blue Assay (ABA) absorbance method. The results indicated that toxicity to *E. coli* and *S. aureus* increased with respect to the proportion of Ag within the composite, with the highest antibacterial activity being observed at a 4:1 ratio of Ag:SnO<sub>2</sub>. Additionally, the antimicrobial properties of the composites were enhanced when particle sizes were reduced from the micro- to nanometer range. Our study demonstrates that nano-Ag/SnO<sub>2</sub> composites are highly suitable for antibacterial coatings of glass-based materials.

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### 1. Introduction

Metal nanocomposites and metal oxide nanocomposites demonstrate excellent physical and chemical properties and are ideal candidates for novel functional materials for many applications. Metal oxide semiconductors such as SnO<sub>2</sub> and ZnO are known to have excellent gas-sensing sensitivities that are selective to greenhouse gases such as CO<sub>2</sub> and CO. Therefore, these materials can be utilized as filters to maintain good air quality [1–3]. In addition, several studies have also reported SnO<sub>2</sub> alongside with ZnO as having applications in the medical field as a material with antibacterial capabilities [4–7].

It has been shown in the literature that metal oxides can be used to eradicate bacteria via electrostatic interactions that change the integrity of bacterial membranes and the generation of free radicals which are toxic to bacteria. The effectiveness of this eradication depends on many factors such as the particle size of the nanomaterials, the liquid composition in the assay, the configuration of the composites, and the presence of light and UV exposure [8,9]. Ag has been indicated as an ideal candidate to combine with SnO<sub>2</sub> for antibacterial coatings. The flexibility of Ag/metal oxide nanomaterials and their ability to eradicate bacteria has led to a growing interest in forming Ag/SnO<sub>2</sub> composites among researchers [10–12].

Many methods to grow nanocomposite materials consisting of Ag/SnO<sub>2</sub> have been reported. Wang et al. [13] reported that Ag/SnO<sub>2</sub> can be successfully synthesized using a direct electroreduction method with HCl as a catalyst. Ag/SnO<sub>2</sub> composite nanotubes have also been produced by an electrospinning method [14]. Moreover, Liu et al. synthesized Ag/SnO<sub>2</sub> using a facile hydrothermal method and an in situ reduction method [15]. Other methods such as sol-gel [5], post annealing [16], precipitation

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combined with powder metallurgy [17], and chemical spray pyrolysis [18] have also been reported in the literature.

Although these methods can be used to synthesize Ag/SnO<sub>2</sub> nanocomposites with good properties, the synthesis itself often uses catalysts or additional materials that require higher safety standards due to associated environmental and health impacts. In this paper, a horizontal vapor phase growth (HVPG) technique was employed to synthesize Ag/SnO<sub>2</sub> nanocomposites. Previous studies show that HVPG can be used to synthesize various micro- and nano-materials such as SnO<sub>2</sub> [19], Ag/Ge [20], and Ag/TiO<sub>2</sub> [21–23]. Notably, the one-pot HVPG technique is environmentally friendly, does not require additional materials or steps in the synthetic process, and yields promising results.

Recently, nanotechnology applications for glass production have received a growing interest worldwide due to increased demand and use of larger glass areas on modern buildings and skyscrapers [24]. The use of large glass windows promotes the multiplication of bacteria and microorganisms, leaving people increasingly exposed to their harmful effects. Prolonged exposure to UVA and UVB radiation from sunlight especially during heat waves is also a big challenge for commercial architecture design [25–28].

Modified glass panes that can filter UVA and UVB light without going through the process of dyeing are essential for commercial architectural applications [29–32]. The elimination of bacteria and microbes on the surface of glass is also indicated for improved human health. This study combines both the biocidal and UV filter properties of Ag/SnO<sub>2</sub> nanoparticle composites to create optimal glass coatings. Here, the synthesis and fabrication of Ag/SnO<sub>2</sub> nanocomposites is reported, along with a density functional theory (DFT) analysis to computationally verify their respective geometries. DFT was used in this work due to the precision of this theory to calculate the chemical properties such as molecules and solids (energies, electronic structures, and binding lengths) [33].

## 2. Materials and methods

### 2.1. Preparation and synthesis of Ag/SnO<sub>2</sub> composites

Silver (Ag) powder of 99.9% purity containing <45 μm grain size was purchased from Sigma Aldrich. Tin oxide (SnO<sub>2</sub>) of 99% purity with <5 μm grain size was purchased from Merck. Ag:SnO<sub>2</sub> mass ratios of 0:5, 1:4, 2:3, 3:2, 4:1, and 5:0 formed the composite materials with each sample having a total mass of 35 mg. Ag, SnO<sub>2</sub> and Ag/SnO<sub>2</sub> materials were synthesized using an HVPG technique, which employs a thermal synthetic method that alters the materials' phases during processing, as seen in Fig. 1.

An ultrasonic machine was used to clean the quartz tube with a closed end. The tube containing Ag and SnO<sub>2</sub> was attached to the Thermionic High Vacuum System (THVS) and the pressure was maintained at 10<sup>-6</sup> torr. The dimensions of the quartz tube were d<sub>inner</sub> = 8.5 mm, d<sub>outer</sub> = 11 mm, and l = 220 mm. The Ag/SnO<sub>2</sub> mixtures were poured inside the quartz tube and placed horizontally inside the furnace. To create a thermal gradient within the furnace, the quartz tube was positioned one-third inside (zone 1), one-third near the opening of the furnace (zone 2), and one-third outside (zone 3) as shown in Fig. 2. The sealed quartz tube containing Ag/SnO<sub>2</sub> mixtures was heated for t = 6 hr at T = 800 °C with a ramp-up time of 80 min.

### 2.2. Characterization of Ag/SnO<sub>2</sub> composites

Surface analysis and elemental composition of both the Ag/SnO<sub>2</sub> composite materials with Ag and SnO<sub>2</sub> controls were performed on a JEOL SEM 5310 scanning electron microscope. Each of the three

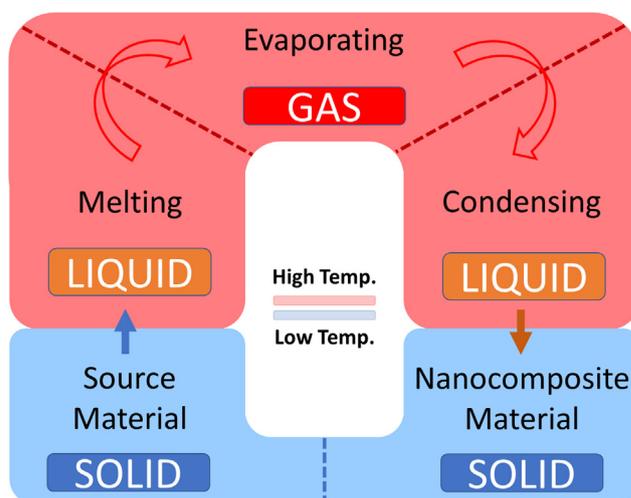


Fig. 1. HVPG induced phase conversion.

zones were measured for the characterization of all prepared nanoparticles.

### 2.3. Computational assessment of Ag/SnO<sub>2</sub> composites via DFT

The surface structures were modelled according to the generalized gradient approximation (GGA) Functional (BLYP). BLYP functional was used in this study based on the previous research that done by Sensato et al. and Yamaguchi et al. where the DFT calculation on SnO<sub>2</sub> was applied. The similar model is used in this study to computationally determine the atomic geometry and electronic properties of the Ag/SnO<sub>2</sub> composites with the possibility to study in different theoretical descriptions which the same correlation formalism was used [34–36]. The reciprocal lattice vector for each k-point was calculated by Monkhorst Pack [37]. The k-point mesh for the bulk SnO<sub>2</sub> was set to 4x4x6 points while the bulk Ag was fixed to 5x5x5 points that gives satisfying results during the relaxation protocol. Computational calculations of the crystal structures and binding energies were performed using quantum chemical software DMol3. Comparisons between their structural crystalline and amorphous properties were made. DMol3 method was used due to the efficiency in numerical local orbital basis sets and it used standard molecular calculations by using arithmetic operations which can be lowered by using reciprocal space method [38]. The parameters that used in this study are listed as follow: Energy cut-off: 400 eV, Convergence criteria: 10E-5, and maximum SCF: 500.

### 2.4. Antibacterial testing

Two-part antibacterial tests were implemented to determine the effectiveness of the nanomaterials against bacteria. The first test involved the absorbance measurement of the turbidity of the bacterial solutions exposed to different nanomaterial mixtures. Using the Perkin Elmer UV/Vis Spectrophotometer Lambda 25, absorbance at λ = 600 nm was measured for solutions of *Escherichia coli* and *Staphylococcus aureus* [39,40]. High turbidity indicates an increase in the colony forming unit (cfu) of bacteria and results in higher absorbance wavelength values as light is scattered by the presence of microorganisms. Composites of Ag and SnO<sub>2</sub> with different mass ratios were collected from the three zones of the quartz tubes and added to test tubes containing a solution of nutrient broth and bacteria. Powder Ag and SnO<sub>2</sub> were also added to test the antibacterial effectiveness between bulk and nanomaterials.

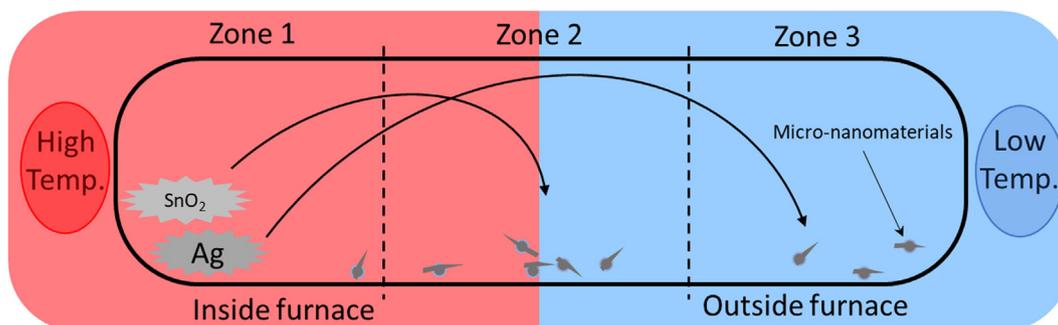


Fig. 2. Cross-sectional diagram of the furnace during the heating of the quartz tube sectioned into three equal segments: zones 1, 2 and 3 [34].

The second test used an Alamar Blue Assay (ABA) technique for quantitative analysis. The proliferation from cell lines of human, animal, bacteria and fungi samples were studied. ABA methods incorporate a fluorometric or calometric indicator to detect metabolic activity. Fluorescence and color changes occur in response to reduction-oxidation reactions within mediums containing cell growth. The active ingredient of ABA, Resazurin, is reduced from its blue, non-fluorescent form into red, fluorescent Toresorufin after successfully permeating a viable cell. ABA techniques have several advantages over existing testing methods, such as non-toxicity, ease of application, and non-interference with cell metabolism. In our set-up, 500  $\mu\text{L}$  of Ag/SnO<sub>2</sub> composites and powder Ag + SnO<sub>2</sub> solutions were transferred to sterilized test tubes, followed by the addition of 50  $\mu\text{L}$  ABA to form a 10:1 ratio. The test tubes containing the solution-reagent mixtures were incubated at 37 °C and monitored for color changes 12 and 24 hr after incubation. Color changes from the original blue color of ABA were used

as indicators of the presence of viable cfu bacteria within the mixture.

### 3. Results and discussion

#### 3.1. SEM imaging

A summary of the surface morphologies of Ag, SnO<sub>2</sub>, and Ag/SnO<sub>2</sub> composite materials and bulk SnO<sub>2</sub> and Ag powders is shown in Fig. 3. Three different zones across the temperature gradient of the quartz tubes are examined. The SEM micrographs depict the presence of randomly oriented nanowires, nanofibers, nanospheres and nanocotton-like structures. The representative EDX analysis from Ag/SnO<sub>2</sub> nanocomposite material where the ratio between Ag:SnO<sub>2</sub> is 1:4 can be seen in Fig. 4. The results show that since the synthesis of nanocomposites process occurred in the vacuum

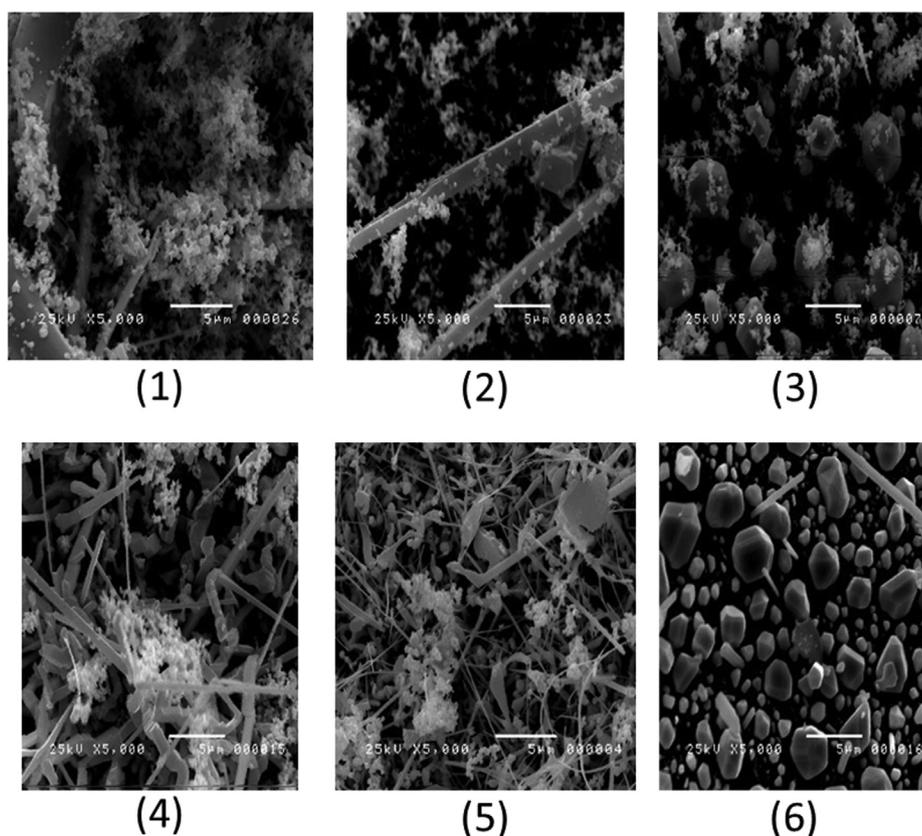


Fig. 3. Representative SEM images of Ag/SnO<sub>2</sub> in Ag:SnO<sub>2</sub> mass ratios of (1) 0:5, (2) 1:4, (3) 2:3, (4) 3:2, (5) 4:1, (6) 5:0.

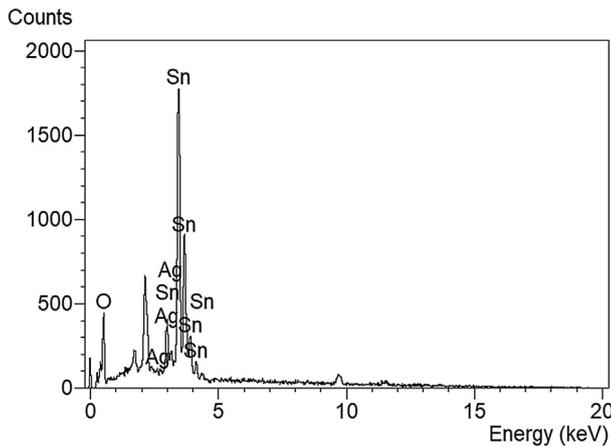


Fig. 4. EDX spectrum of Ag/SnO<sub>2</sub> nanocomposite material with ratio 1:4.

condition, the result from EDX show both Ag, Sn, and O are the element that have high intensity. The absence of the other element are the prove that HVPG technique are capable to synthesis nanomaterial with high purity.

Most nanomaterials are observed in zone 3 while zone 1 and zone 2 display structures ranging from nanometer to micrometer size. Two different types of growth mechanisms can potentially explain the deposition of nanomaterials found in each zone. In the first growth mechanism, the source material is initially found in zone 1. It receives heat from its surroundings, acting as an agent of heat transfer and undergoing a phase transition from solid to gas for effective spreading. The convective motion of gases inside the quartz tube influences the existing longitudinal thermal gradient of tube, making any material accumulation or deposition impossible. The source powder is likely vaporized from zone 1 and 3, but a possible phase transition from vapor to liquid can occur as vapor traverses from zone 1 to 2. Thus, nanostructures are formed by the process of creep flow and cooling after the dwell time of 6 hr.

The second type of growth mechanism involves the formation of SnO<sub>2</sub> nanowires as explained by Bhardwaj et al. [41]. Because Sn has a low melting point, the deposited Sn nanoparticles are

likely converted into liquid nanodroplets which induce self-catalytic growth of SnO<sub>2</sub> nanowires. As the Ag content of the source material increases, Ag nanoparticles form and dissolve Sn, thereby creating Ag-Sn eutectic nanodroplets. Functioning as a catalyst, the Ag-Sn eutectic nanodroplets dissolve incoming Sn, O and Ag atoms. Due to the temperature-gradient-driven supersaturation, precipitation of Ag, Sn and O follows the formation of SnO<sub>2</sub> nanowires. A further increase in Ag concentration results in the creation of Ag<sub>3</sub>Sn alloy nanodroplets which dissolve any inbound Sn, O and Ag atoms from the vapor phase, inducing the formation of SnO<sub>2</sub> nanowires. The initial growth of SnO<sub>2</sub> nanowires produces a deposition site for Sn and Ag nanoparticles in the vapor phase, where Ag<sub>3</sub>Sn alloy nanodroplets can grow as secondary nucleation centers for SnO<sub>2</sub> nanowire formation in a self-catalytic cycle. These secondary nucleation centers then create bridged interconnections between discrete SnO<sub>2</sub> nanowires.

### 3.2. DFT analysis

#### 3.2.1. SnO<sub>2</sub> surface structure

SnO<sub>2</sub>(1 1 0) is considered a thermodynamically and electrostatically stable material with a predominantly crystal face surface since the [1 0 0] plane has the least surface energy relative to other orientations [42,43]. This plane consists of O and Sn atoms separated by planes containing only O atoms which can be written as O–Sn<sub>2</sub>O<sub>2</sub>–O, with ionic charges of 2-, 4+ and 2-, respectively [43]. At the surface, the two outermost layers of O are called “bridging” oxygen atoms such that removing one O creates a “bridging vacancy.” The lattice constants are calculated to be  $a = 4.84 \text{ \AA}$ ,  $b = 3.28 \text{ \AA}$  and  $c = 18.0 \text{ \AA}$  for both single- and double-unit cells, which is in close agreement with a study carried out by Habgood and Harrison [44]. Fig. 5. illustrates the SnO<sub>2</sub>(1 1 0) crystal and band structures and the density of states for a double-unit cell, with computed band gaps of 3.73 eV (332.63 nm). Based on the calculated band gap energies, the SnO<sub>2</sub>(1 1 0) surface structure is a good candidate for UVB and UVC ray absorption.

#### 3.2.2. SnO<sub>2</sub>(1 1 0) on Ag(1 1 1) surface structure

SnO<sub>2</sub>(1 1 0) on Ag(1 1 1) was analyzed according to a bottom-top termination along the surface in order to determine the

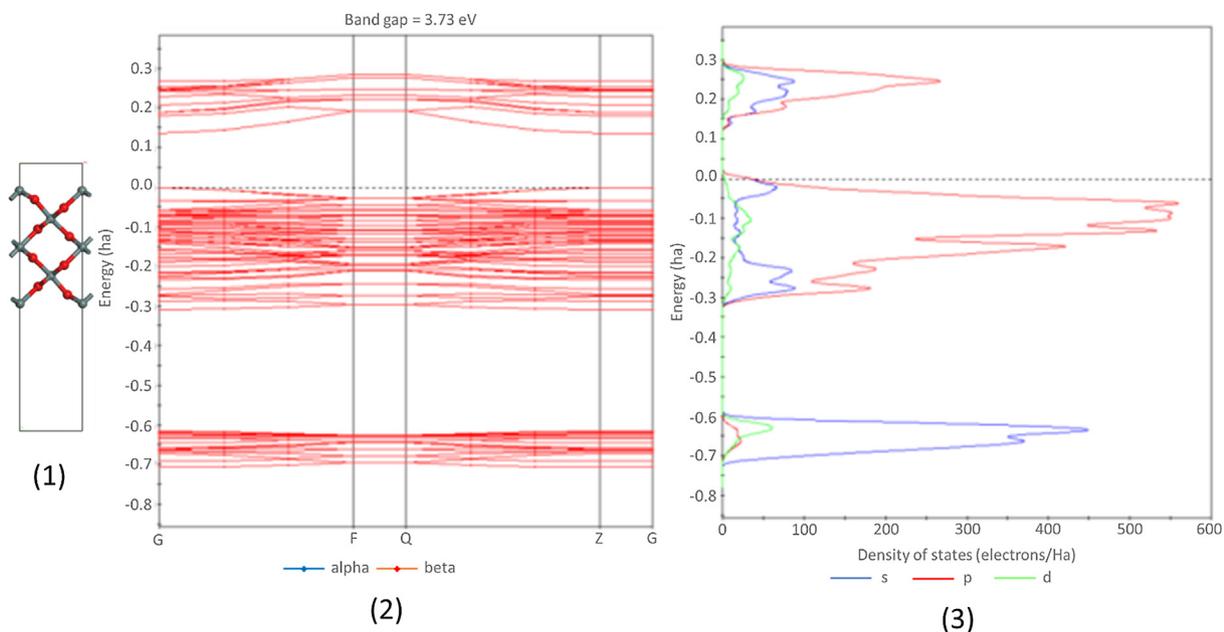


Fig. 5. SnO<sub>2</sub>(1 1 0) (1) Crystal Structure, (2) Band Structure, and (3) Density of States.

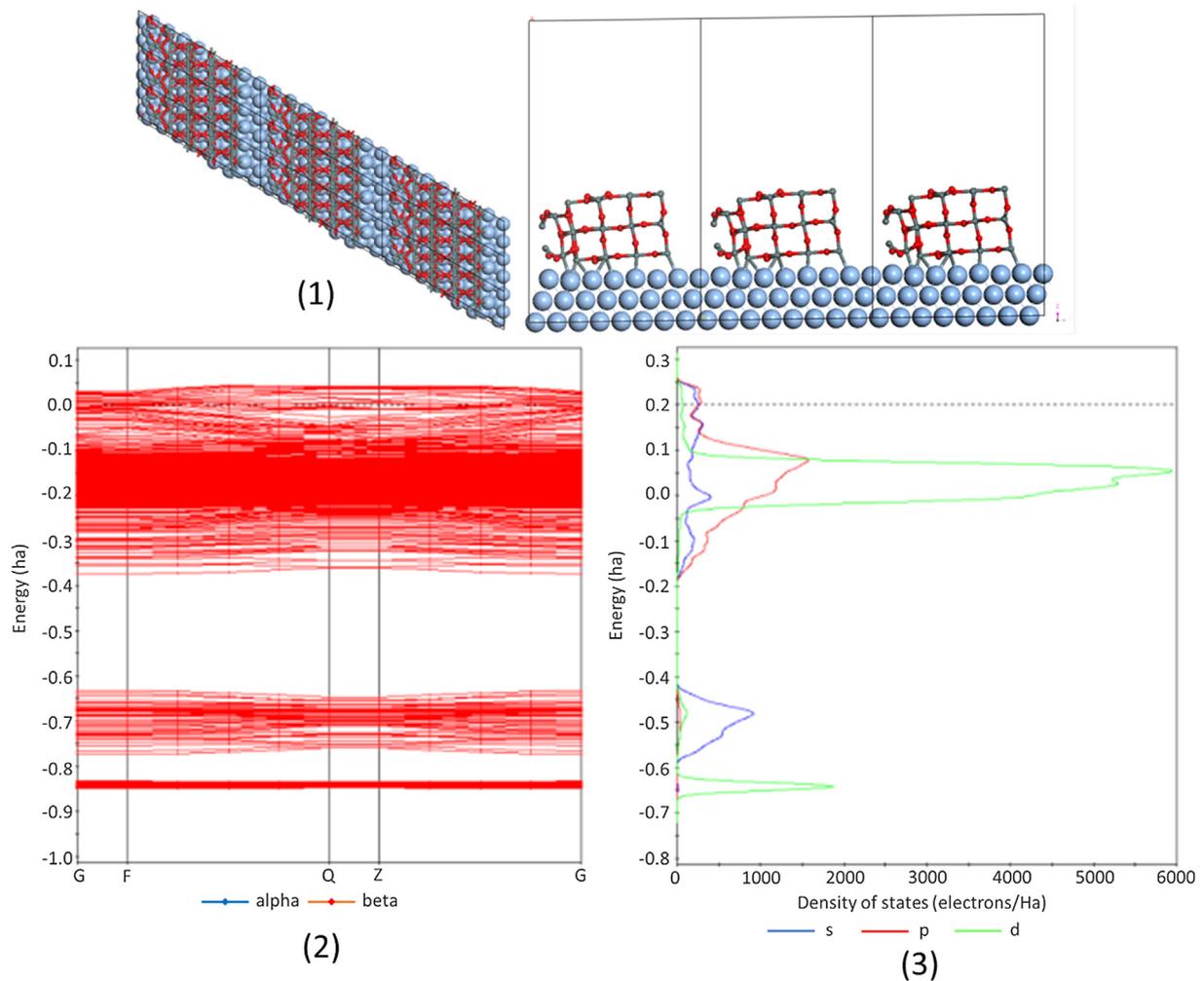


Fig. 6. (1)  $\text{SnO}_2(1\ 1\ 0)$  on  $\text{Ag}(1\ 1\ 1)$  Crystal Structure, (2) Band Structure, and (3) Density of States for larger unit cells on  $\text{SnO}_2$  Nanorods over a silver surface.

geometric and electronic structure of the  $\text{Ag}/\text{SnO}_2$  composite. The unit cell,  $\text{SnO}_2$  atoms, and first layer of  $\text{Ag}(1\ 1\ 1)$  are relaxed, whereas the bottom layer of  $\text{Ag}(1\ 1\ 1)$  is fixed. The computed lattice constants are  $a = 5.45\ \text{\AA}$ ,  $b = 6.31\ \text{\AA}$ , and  $c = 18\ \text{\AA}$ . Fig. 5. shows the crystal structures of  $\text{SnO}_2(1\ 1\ 0)$  on  $\text{Ag}(1\ 1\ 1)$  for possible atomic termination combinations. The lowest band gap is found at the Sn–Sn termination while the highest band gaps are calculated at the O–Sn and O–O terminations.

The geometric crystal structure for  $\text{SnO}_2$  nanorods using Ag surfaces is shown in Fig. 6, along with the corresponding band structure and density of states. These computational results are experimentally supported by the SEM images where  $\text{SnO}_2$  nanorods were attached to the Ag nanoparticles. A direct experimental comparison for this computational model can be made with a report done by Kim et al. [45] where the  $[1\ 0\ 0]$  facets were seen to be prominent in  $\text{SnO}_2$  nanorods synthesized at 1200 K by thermal evaporation. The results suggest the direct increase of the relative intensity of  $[1\ 0\ 0]$  facets as O partial pressure increases simultaneously.

### 3.3. Antibacterial properties of $\text{Ag}/\text{SnO}_2$ nanoparticles

#### 3.3.1. Antibacterial effect on *E. Coli*

The change or retention of color within ABA solutions indicates the proliferation or abatement of *E. coli* after incubation, respectively. Fig. 7. shows the biocidal effect of nanomaterial mixtures

with *E. coli* 12 hr after incubation. Samples that indicated color changes are *E. coli* (+control), *E. coli* with  $\text{SnO}_2$  bulk powder, and *E. coli* with Ag bulk powder, revealing that *E. coli* remains viable when exposed to the larger, powdered forms of pure Ag and  $\text{SnO}_2$ . As *E. coli* continues to grow, the innate metabolic activity of the bacteria results in the chemical reduction of AB (alamarBlue), causing the redox indicator to change from the oxidized (non-fluorescent blue) form to the reduced (fluorescent red) form. When AB is added to cell cultures, the oxidized AB enters the cytosol and is converted to its reduced form via accepting electrons from mitochondrial enzymes, including cytochromes, NADPH, FADH, FMNH and NADH. This redox reaction is accompanied by a shift of the culture medium from indigo blue to fluorescent pink [46–48].

After 24 hr of incubation, a shift from pink to orange is observed on both the Ag and  $\text{SnO}_2$  powders with *E. coli* as shown in Fig. 8. A halt to the spread of *E. coli* cells may have caused the change of color due to either a high cell population or extended incubation time. However, there was no remarkable difference in the color of *E. coli* solutions with the  $\text{Ag}/\text{SnO}_2$  composite and bulk nanomaterials. Based on these observations, the effectiveness of the composite against *E. coli* is proportional to the reduction of the  $\text{Ag}/\text{SnO}_2$  composite particle size. The absorbance values presented in Table 1 show that the intensity decreased after 12 hr and further reduced after 24 hr, thereby indicating the eradication of *E. coli* due to prolonged exposure to the nanomaterial

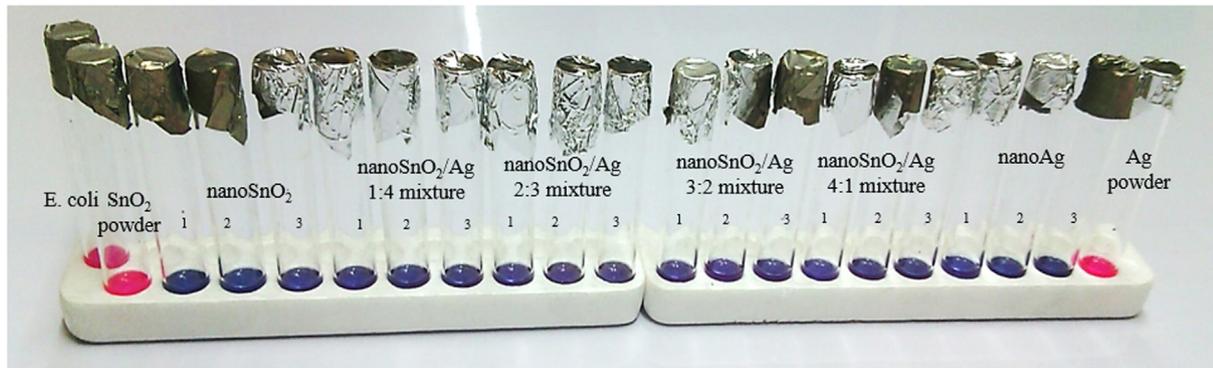


Fig. 7. ABA/*E. coli* after 12 hr of incubation.

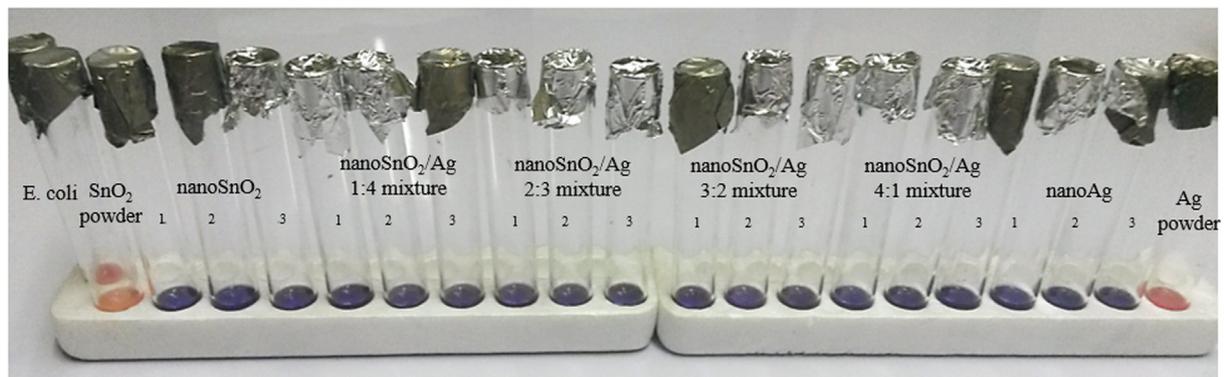


Fig. 8. ABA/*E. coli* after 24 hr of incubation.

**Table 1**  
Absorbance values of *E. coli* at 600 nm.

Wavelength	600 nm					
	Samples	0 h	12 h	$A_{12h} - A_{0h}$	24 h	$A_{24h} - A_{12h}$
<i>E. coli</i> , no material		0.559	0.629	0.07	0.626	-0.003
SnO <sub>2</sub> powder		2.071	2.125	0.054	2.12	-0.005
SnO <sub>2</sub> nanomaterial zone 1		1.921	1.915	-0.006	1.893	-0.022
SnO <sub>2</sub> nanomaterial zone 2		1.896	1.889	-0.007	1.863	-0.026
SnO <sub>2</sub> nanomaterial zone 3		2.081	2.078	-0.003	2.047	-0.031
Ag/SnO <sub>2</sub> 1:4 zone 1		1.356	1.341	-0.015	1.304	-0.037
Ag/SnO <sub>2</sub> 1:4 zone 2		1.392	1.38	-0.012	1.337	-0.043
Ag/SnO <sub>2</sub> 1:4 zone 3		1.426	1.407	-0.019	1.354	-0.053
Ag/SnO <sub>2</sub> 2:3 zone 1		1.262	1.244	-0.018	1.191	-0.053
Ag/SnO <sub>2</sub> 2:3 zone 2		1.388	1.364	-0.024	1.31	-0.054
Ag/SnO <sub>2</sub> 2:3 zone 3		1.348	1.319	-0.029	1.26	-0.059
Ag/SnO <sub>2</sub> 3:2 zone 1		1.199	1.168	-0.031	1.111	-0.057
Ag/SnO <sub>2</sub> 3:2 zone 2		1.264	1.236	-0.028	1.178	-0.058
Ag/SnO <sub>2</sub> 3:2 zone 3		1.216	1.174	-0.042	1.111	-0.063
Ag/SnO <sub>2</sub> 4:1 zone 1		0.962	0.904	-0.058	0.823	-0.081
Ag/SnO <sub>2</sub> 4:1 zone 2		1.144	1.083	-0.061	0.998	-0.085
Ag/SnO <sub>2</sub> 4:1 zone 3		1.138	1.068	-0.07	0.954	-0.114
Ag nanomaterial zone 1		0.645	0.589	-0.056	0.512	-0.077
Ag nanomaterial zone 2		0.687	0.625	-0.062	0.542	-0.083
Ag nanomaterial zone 3		0.996	0.932	-0.064	0.839	-0.093
Ag powder		0.628	0.668	0.04	0.657	-0.011

mixtures. Furthermore, the amount of Ag in the mixture is inversely proportional to its absorbance, confirming its effectiveness as an antibacterial agent. Since Ag is a natural biocidal material, the toxicity of a solution of *E. coli* is improved with an increased presence of Ag within Ag/SnO<sub>2</sub> mixtures [49,50].

A possible explanation for the elimination of the *E. coli* within Ag/SnO<sub>2</sub> solutions, determined from absorbance measurements,

is due to the nanomaterial's breaking of the bacterial cell wall. Since the subsequently leaked cytoplasmic material is composed of soluble proteins, salts, and small molecules, these components do not contribute to light scattering and in contrast, would result in a decrease of the optical or absorbance intensity of the sample. Moreover, reformed cells would be smaller and scatter less light which results in a lower absorbance as shown in Table 1.

### 3.3.2. Antibacterial effect on *S. Aureus*

The biocidal effect of SnO<sub>2</sub> and Ag bulk, powder and composite nanomaterials were further tested on the bacteria *Staphylococcus aureus* (*S. aureus*). After 12 hr of incubation, only two of the samples, *S. aureus* (+control) and SnO<sub>2</sub> powder with *S. aureus*, demonstrated a color change, indicating no effect on the elimination of *S. aureus*, depicted in Fig. 9. Further incubation to 24 hr revealed that SnO<sub>2</sub> nanoparticles and Zone 1-harvested Ag nanomaterials are also ineffective as antibacterial agents against *S. aureus* as illustrated in Fig. 10. Both the powdered and nanoparticle forms of

SnO<sub>2</sub> are viable agents against the *S. aureus* solution. However, nanocomposites of Ag/SnO<sub>2</sub> prove to be a consistently effective antibacterial material on both *E. coli* and *S. aureus*. Thus, nano-sized materials have a higher toxicity towards these bacteria compared to their powdered forms, which is consistent with the results found by previous studies [50,51].

The absorbance intensity decreased for all solutions after a 12 hr incubation period except for the *S. aureus* control, as presented in Table 2. For the case of *S. aureus*, the inhibitory action of the Ag/SnO<sub>2</sub> composite can be tied to the respiration of the bac-

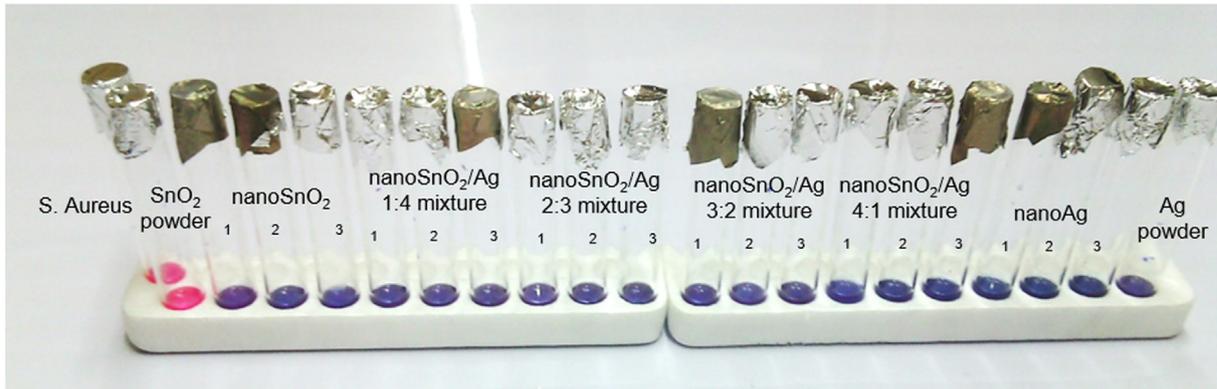


Fig. 9. ABA/*S. Aureus* after 12 hr of incubation.

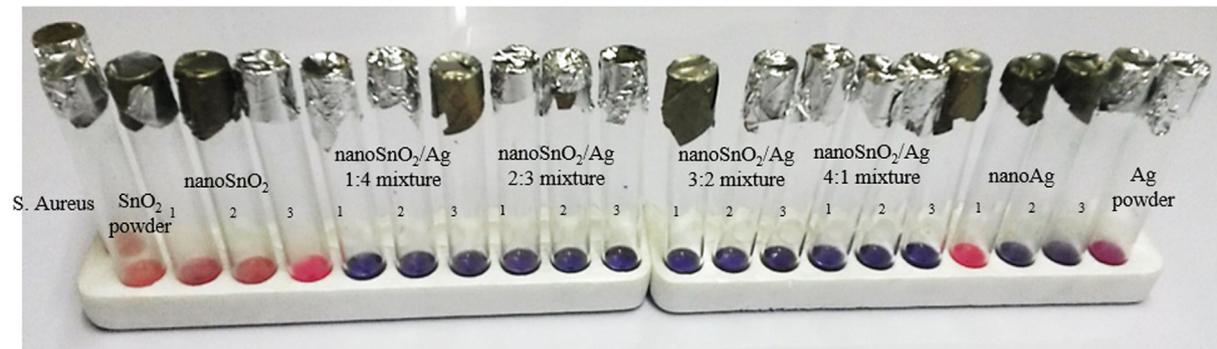


Fig. 10. ABA /*S. Aureus* after 24 hr of incubation.

Table 2

Absorbance values of *S. aureus* at 600 nm.

Wavelength Samples	600 nm 0 h	12 h	$A_{12h} - A_{0h}$	24 h	$A_{24h} - A_{12h}$
<i>S. aureus</i> , no material	0.208	0.316	0.108	0.425	0.109
SnO <sub>2</sub> powder	1.968	2.069	0.101	2.176	0.107
SnO <sub>2</sub> nanomaterial zone 1	1.741	1.812	0.071	1.871	0.059
SnO <sub>2</sub> nanomaterial zone 2	1.67	1.697	0.027	1.769	0.072
SnO <sub>2</sub> nanomaterial zone 3	1.418	1.452	0.034	1.554	0.102
Ag/SnO <sub>2</sub> 1:4 zone 1	1.581	1.575	-0.006	1.539	-0.036
Ag/SnO <sub>2</sub> 1:4 zone 2	1.595	1.42	-0.175	1.354	-0.066
Ag/SnO <sub>2</sub> 1:4 zone 3	1.567	1.403	-0.164	1.327	-0.076
Ag/SnO <sub>2</sub> 2:3 zone 1	1.444	1.229	-0.215	1.144	-0.085
Ag/SnO <sub>2</sub> 2:3 zone 2	1.414	1.388	-0.026	1.334	-0.054
Ag/SnO <sub>2</sub> 2:3 zone 3	1.473	1.225	-0.248	1.129	-0.096
Ag/SnO <sub>2</sub> 3:2 zone 1	1.331	1.117	-0.214	1.035	-0.082
Ag/SnO <sub>2</sub> 3:2 zone 2	1.366	1.261	-0.105	1.169	-0.092
Ag/SnO <sub>2</sub> 3:2 zone 3	1.411	1.192	-0.219	1.092	-0.100
Ag/SnO <sub>2</sub> 4:1 zone 1	1.292	1.087	-0.205	0.995	-0.092
Ag/SnO <sub>2</sub> 4:1 zone 2	1.309	1.146	-0.163	1.013	-0.133
Ag/SnO <sub>2</sub> 4:1 zone 3	1.403	1.157	-0.246	1.008	-0.149
Ag nanomaterial zone 1	0.635	0.617	-0.018	0.727	0.110
Ag nanomaterial zone 2	0.776	0.59	-0.186	0.492	-0.098
Ag nanomaterial zone 3	1.069	0.896	-0.173	0.786	-0.110
Ag powder	0.687	0.734	0.047	0.802	0.048

teria and the corresponding change in turbidity, as stated by Avitor et al. [52]. Furthermore, previous studies show that nanoparticles' toxicity mechanism depends on the composition, surface modification, intrinsic properties and type of bacteria species.

Nanoparticles can either penetrate the bacterial cell wall and bind to the phospholipid layer of the cytoplasmic membrane, bind to the DNA of the bacteria and disrupt its replication, or impair the ability of the ribosome to transcribe RNA or bind to the sulfhydryl group of the cytochrome [53]. Although the exact mechanism remains unknown, our experimental results recapitulate that reducing the size of the Ag/SnO<sub>2</sub> composites to the nanoparticle range proves to be effectively toxic to both *E. coli* and *S. aureus* bacteria.

#### 4. Conclusion

Ag/SnO<sub>2</sub> nanocomposite materials were successfully synthesized using a simple HVPG technique which resulted in high purity results, an eco-friendly synthesis, and a low cost of production. The SEM images indicated that the morphology of Ag/SnO<sub>2</sub> composites changed with respect to the ratio of starting materials, which is reflected in the absorbance data and subsequent antibacterial capabilities. This study shows various nanomaterials were successfully synthesized including wires, rods, spheres and cotton-like composites from the three zone areas of the quartz tube. DFT analysis using the quantum chemical software DMol3 illustrates that the computed band gap energy of Ag (1 1 1) matches the SnO<sub>2</sub> (1 1 0) surfaces, supporting the experimental findings.

The ABA technique demonstrates that Ag/SnO<sub>2</sub> composites are toxic to both *E. coli* and *S. aureus* bacteria. The ABA indicator induced color changes when exposed to powered macromaterials of Ag and SnO<sub>2</sub>, revealing the proliferation of bacteria within these mediums. However, color shifts were not seen within the Ag/SnO<sub>2</sub> composites that were both nanosized and contained high ratios of Ag:SnO<sub>2</sub>, suggesting that these materials are highly capable as antibacterial agents. These results confirm that antibacterial properties are enhanced in nanoparticles and suggest that Ag/SnO<sub>2</sub> nanocomposite materials are suitable for coating applications with a high performance in antibacterial activity. Based on the results, this material can be applied in residential and commercial glass windows along with glass-based materials that are exposed to UV light. Additionally, medical applications have yet to be explored but the present results are promising for the production of materials with highly antimicrobial properties against both gram-positive and gram-negative bacteria.

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#### References

[1] Ge S, Zheng H, Sun Y, Jin Z, Shan J. Ag/SnO<sub>2</sub>/graphene ternary nanocomposites and their sensing properties to volatile organic compounds. *J Alloy Compd* 2016;659:127–31. doi: <https://doi.org/10.1016/j.jallcom.2015.11.046>.  
 [2] Maldonado A, Olvera MDL. Gas-sensing characteristics of undoped-SnO<sub>2</sub> thin films and Ag/SnO<sub>2</sub> and SnO<sub>2</sub>/Ag structures in a propane atmosphere. *Mater Charact* 2007;58:740–4. doi: <https://doi.org/10.1016/j.matchar.2006.11.016>.

[3] Shyplenko A, Pshyk AV, Grzeszkowiak B, Medjanik K, Peplinska B, Oyoshi K, et al. Effect of ion implantation on the physical and mechanical properties of Ti-Si-N multifunctional coatings for biomedical applications. *Mater Des* 2016;110:821–9. doi: <https://doi.org/10.1016/j.matdes.2016.08.050>.  
 [4] Paraguay-delgado F, Orrantia-borunda E, Ch A, Luna-velasco A. Chemosphere Size effect of SnO<sub>2</sub> nanoparticles on bacteria toxicity and their membrane damage 2016;165:33–40. doi: <https://doi.org/10.1016/j.chemosphere.2016.09.003>.  
 [5] Kumar K, Kumar P, Kumar V, Harris RA, Kroon RE, Viljoen B, et al. Synthesis and evaluation of optical and antimicrobial properties of Ag-SnO<sub>2</sub> 2 nanocomposites. *Phys B Phys Condensed Matter* 2018;535:338–43. doi: <https://doi.org/10.1016/j.physb.2017.08.028>.  
 [6] Turlybekuly A, Pogrebnyak AD, Sukhodub LF, Sukhodub LB, Kistaubayeva AS, Savitskaya IS, et al. Synthesis, characterization, in vitro biocompatibility and antibacterial properties study of nanocomposite materials based on hydroxyapatite-biphasic ZnO micro- and nanoparticles embedded in Alginate matrix. *Mater Sci Eng C* 2019;104:109965. doi: <https://doi.org/10.1016/j.msec.2019.109965>.  
 [7] Pogrebnyak A, Sukhodub L, Sukhodub L, Bondar O, Kumeda M, Shaimardanova B, et al. Composite material with nanoscale architecture based on bioapatite, sodium alginate and ZnO microparticles. *Ceram Int* 2019;45:7504–14. doi: <https://doi.org/10.1016/j.ceramint.2019.01.043>.  
 [8] John N, Somaraj M, Tharayil NJ. Science direct synthesis, characterization and anti - bacterial activities of pure and Ag - doped SnO<sub>2</sub> Nanoparticles. *Mater Today: Proc* 2017;4:4351–7. doi: <https://doi.org/10.1016/j.matpr.2017.04.005>.  
 [9] Duan Z, Deng L, Shi Z, Zhang H, Zeng H, Crittenden J. In situ growth of Ag-SnO<sub>2</sub> quantum dots on silver phosphate for photocatalytic degradation of carbamazepine : performance, mechanism and intermediates toxicity assessment. *J. Coll. Interf. Sci.* 2019;534:270–8. doi: <https://doi.org/10.1016/j.jcis.2018.09.039>.  
 [10] Wang J, Wang C, Kang Y. The effects of annealing treatment on microstructure and contact resistance properties of cold sprayed Ag-SnO<sub>2</sub> coating. *J Alloy Compd* 2017;714:698–703. doi: <https://doi.org/10.1016/j.jallcom.2017.04.227>.  
 [11] Li H, He Y, Jin P, Cao Y, Fan M, Zou X, et al. Highly selective detection of trace hydrogen against CO and CH<sub>4</sub> by Ag / Ag<sub>2</sub>O - SnO<sub>2</sub> composite microstructures. *Sens Actuata. B* 2016;228:515–22. doi: <https://doi.org/10.1016/j.snb.2016.01.078>.  
 [12] Muflikhun MA, Chua AY, Santos GNC. Statistical design analysis of silver-titanium dioxide nanocomposite materials synthesized via horizontal vapor phase growth (HVPG). *Key Eng Mater* 2017;735:210–4. doi:10.4028/www.scientific.net/KEM.735.210.  
 [13] Wang X, Xiao W, Zhang J, Wang Z, Jin X. Electrochemistry communications nanoporous Ag-Sn derived from codeposited AgCl-SnO<sub>2</sub> for the electrocatalytic reduction of CO<sub>2</sub> with high formate selectivity. *Electrochem Commun* 2019;102:52–6. doi: <https://doi.org/10.1016/j.elecom.2019.03.017>.  
 [14] Miao Y, He S, Zhong Y, Yang Z, Wee W, Liu T. Electrochimica Acta a novel hydrogen peroxide sensor based on Ag/SnO<sub>2</sub> composite nanotubes by electrospinning. *Electrochimica Acta* 2013;99:117–23. doi: <https://doi.org/10.1016/j.electacta.2013.03.063>.  
 [15] Liu D, Pan J, Tang J, Liu W, Bai S, Luo R. Journal of physics and chemistry of solids Ag decorated SnO<sub>2</sub> nanoparticles to enhance formaldehyde sensing properties. *J. Phys. Chem. Solids* 2019;124:36–43. doi: <https://doi.org/10.1016/j.jpcs.2018.08.028>.  
 [16] Zhao J, Deng R, Qin J, Song J, Jiang D, Yao B, et al. Photoresponse enhancement in SnO<sub>2</sub>-based ultraviolet photodetectors via coupling with surface plasmons of Ag particles. *J Alloy Compd* 2018;748:398–403. doi: <https://doi.org/10.1016/j.jallcom.2018.03.180>.  
 [17] Li G, Cui H, Chen J, Fang X, Feng W, Liu J. Formation and effects of CuO nanoparticles on Ag/SnO<sub>2</sub> electrical contact materials. *J Alloy Compd* 2017;696:1228–34. doi: <https://doi.org/10.1016/j.jallcom.2016.12.092>.  
 [18] Kolhe PS, Koinkar PM, Maiti N, Sonawane KM. Synthesis of Ag doped SnO<sub>2</sub> thin films for the evaluation of H<sub>2</sub>S gas sensing properties. *Physica B: Phys Condensed Matter* 2017;524:90–6. doi: <https://doi.org/10.1016/j.physb.2017.07.056>.  
 [19] Briones JC, Castillon G, Delmo MP, Santos GNC. Magnetic-field-enhanced morphology of tin oxide nanomaterials for gas sensing 2017 Applications. *J. Nanomater.* 2017. doi: <https://doi.org/10.1155/2017/4396723>.  
 [20] Muflikhun MA, Castillon GB, Santos GNC, Chua AY. Micro and Nano Silver-Graphene Composite Manufacturing via Horizontal Vapor Phase Growth (HVPG) Technique. *Mater. Sci. Forum* 2017;901:3–7. doi:10.4028/www.scientific.net/msf.901.3.  
 [21] Muflikhun MA, Frommelt MC, Farman M, Chua AY, Santos GNC. Structures, mechanical properties and antibacterial activity of Ag/TiO<sub>2</sub> nanocomposite materials synthesized via HVPG technique for coating application. *Heliyon* 2019;5: 1–21. doi:10.1016/j.heliyon.2019.e01475.  
 [22] Muflikhun MA, Santos GN, Chua AY. Synthesis and characterization of silver-titanium nanocomposite via horizontal vapor phase growth (HVPG) technique. *DLSU Res Congress* 2015:1–5.  
 [23] Muflikhun MA, Chua AY, Santos GNC. Structures, morphological control, and antibacterial performance of Ag/TiO<sub>2</sub> nanocomposite materials. *Adv Mater Sci Eng* 2019. doi: <https://doi.org/10.1155/2019/9821535>.  
 [24] Jelle BP, Gustavsen A, Nilsen TN, Jacobsen T. Solar material protection factor (SMPF) and solar skin protection factor (SSPF) for window panes and other glass structures in buildings. *Sol Energy Mater Sol Cells* 2007;91:342–54. doi: <https://doi.org/10.1016/j.solmat.2006.10.017>.  
 [25] Skarstad K, Steen HB, Boye E. Cell cycle parameters of slowly growing *Escherichia coli* B/r studied by flow cytometry. *J Bacteriol* 1983;154: 656–62.

- [26] Leadbetter ER, Poindexter JS. *Methods and special applications in bacterial ecology*. Springer Science & Business Media; 1986.
- [27] Damiani E, Rosati L, Castagna R, Carloni P, Greci L. Changes in ultraviolet absorbance and hence in protective efficacy against lipid peroxidation of organic sunscreens after UVA irradiation. *J Photochem Photobiol B* 2006;82. doi: <https://doi.org/10.1016/j.jphotobiol.2005.03.011>.
- [28] Matsumura Y, Ananthaswamy HN. Toxic effects of ultraviolet radiation on the skin. *Toxicol Appl Pharmacol* 2004;195:298–308.
- [29] deRichter R, Caillol S. Fighting global warming: The potential of photocatalysis against CO<sub>2</sub>, CH<sub>4</sub>, N<sub>2</sub>O, CFCs, tropospheric O<sub>3</sub>, BC and other major contributors to climate change. *J Photochem Photobiol C* 2011;12:1–19. doi: <https://doi.org/10.1016/j.jphotochemrev.2011.05.002>.
- [30] Babu B, Cho M, Byon C, Shim J. One pot synthesis of Ag-SnO<sub>2</sub> quantum dots for highly enhanced sunlight-driven photocatalytic activity. *J Alloy Compd* 2018;731:162–71. doi: <https://doi.org/10.1016/j.jallcom.2017.10.011>.
- [31] Natarajan TS, Natarajan K, Bajaj HC, Tayade RJ. Study on identification of leather industry wastewater constituents and its photocatalytic treatment. *Int J Environ Sci Technol* 2013;10:855–64. doi: <https://doi.org/10.1007/s13762-013-0200-9>.
- [32] Tuchinda C, Srivannaboon S, Lim HW. Photoprotection by window glass, automobile glass, and sunglasses. *J Am Acad Dermatol* 2006;54:845–54. doi: <https://doi.org/10.1016/j.jaad.2005.11.1082>.
- [33] Parr R. [Robert\_G.\_Parr\_Yang\_Weitao]\_Density-functional\_t(z-lib.org).pdf. Oxford University Press; 1989.
- [34] Lenglet M. Iono-covalent character of the metal-oxygen bonds in oxides: A comparison of experimental and theoretical data. *Acta Physica Electronica* 2004;27:1–60. doi: <https://doi.org/10.1080/0882751031000116142>.
- [35] Sensato FR, Custódio R, Calatayud M, Beltrán A, Andrés J, Sambrano JR, et al. Periodic study on the structural and electronic properties of bulk, oxidized and reduced SnO<sub>2</sub>(1 1 0) surfaces and the interaction with O<sub>2</sub>. *Surf Sci* 2002;511:408–20. doi: [https://doi.org/10.1016/S0039-6028\(02\)01542-X](https://doi.org/10.1016/S0039-6028(02)01542-X).
- [36] Yamaguchi Y, Nagasawa Y, Shimomura S, Tabata K, Suzuki E. A density functional theory study of the interaction of oxygen with a reduced SnO<sub>2</sub> (110) surface. *Chem Phys Lett* 2000;316:477–82. doi: [https://doi.org/10.1016/S0009-2614\(99\)01365-2](https://doi.org/10.1016/S0009-2614(99)01365-2).
- [37] Pack JD, Monkhorst HJ. Special points for Brillouin-zone integrations"-a reply. *Phys Rev B* 1977;16:1748–9. doi: <https://doi.org/10.1103/PhysRevB.16.1748>.
- [38] Delley B. Time dependent density functional theory with DMol3. *J Phys: Condens Matter* 2010;22. doi: <https://doi.org/10.1088/0953-8984/22/38/384208>.
- [39] El-Shanshoury AE-RR, ElSilk SE, Ebeid ME. Extracellular biosynthesis of silver nanoparticles using escherichia coli ATCC 8739, bacillus subtilis ATCC 6633, and streptococcus thermophilus ESh1 and their antimicrobial activities. *ISRN Nanotechnology* 2011;2011:1–7. doi: <https://doi.org/10.5402/2011/385480>.
- [40] McBirney SE, Trinh K, Wong-Beringer A, Armani AM. Wavelength-normalized spectroscopic analysis of Staphylococcus aureus and Pseudomonas aeruginosa growth rates. *Biomed Opt Exp* 2016;7:4034. doi: <https://doi.org/10.1364/boe.7.004034>.
- [41] Bhardwaj N, Kuriakose S, Mohapatra S. Structural and optical properties of SnO<sub>2</sub> nanotowers and interconnected nanowires prepared by carbothermal reduction method. *J Alloy Compd* 2014;592:238–43.
- [42] Batzill M, Diebold U. The surface and materials science of tin oxide. *Prog Surf Sci* 2005;79:47–154. doi: <https://doi.org/10.1016/j.progsurf.2005.09.002>.
- [43] Floriano EA, Scalvi LV, Sambrano JR, Geraldo V. Evaluation of bulk and surfaces absorption edge energy of sol-gel-dip-coating SnO<sub>2</sub> thin films. *Mater Res* 2010;13:437–43.
- [44] Habgood M, Harrison N. An ab initio study of oxygen adsorption on tin dioxide. *Surf Sci* 2008;602:1072–9.
- [45] Kim HW, Lee JW, Shim SH, Lee C. Controlled growth of SnO<sub>2</sub> nanorods by thermal evaporation of Sn powders. *J Korean Phys Soc* 2007;51:198–203.
- [46] Al-Nasiry S, Geusens N, Hanssens M, Luyten C, Pijnenborg R. The use of Alamar Blue assay for quantitative analysis of viability, migration and invasion of choriocarcinoma cells. *Hum Reprod* 2007;22:1304–9.
- [47] Räs B, Iten M, Grether-Bühler Y, Kaminsky R, Brun R. The Alamar Blue® assay to determine drug sensitivity of African trypanosomes (Tb rhodesiense and Tb gambiense) in vitro. *Acta Tropica* 1997;68:139–47.
- [48] Rampersad SN. Multiple applications of alamar blue as an indicator of metabolic function and cellular health in cell viability bioassays. *Sensors* 2012;12:347–60. doi: <https://doi.org/10.3390/s120912347>.
- [49] Fu F, Gu J, Cao J, Shen R, Liu H, Zhang Y, et al. Reduction of silver ions using an alkaline cellulose dope: straightforward access to Ag/ZnO decorated cellulose nanocomposite film with enhanced antibacterial activities. *ACS Sustain Chem Eng* 2017;6:738–48.
- [50] Sinha T, Ahmaruzzaman M, Adhikari PP, Bora R. Green and environmentally sustainable fabrication of Ag-SnO<sub>2</sub> nanocomposite and its multifunctional efficacy as photocatalyst and antibacterial and antioxidant agent. *ACS Sustain Chem Eng* 2017;5:4645–55.
- [51] Yalcinkaya F, Lubasova D. Quantitative evaluation of antibacterial activities of nanoparticles (ZnO, TiO<sub>2</sub>, ZnO/TiO<sub>2</sub>, SnO<sub>2</sub>, CuO, ZrO<sub>2</sub>, and AgNO<sub>3</sub>) incorporated into polyvinyl butyral nanofibers. *Polym Adv Technol* 2017;28:137–40.
- [52] Avi-Dor Y, Kuczynski M, Schatzberg G, Mager J. Turbidity changes in bacterial suspensions: kinetics and relation to metabolic state. *Microbiology* 1956;14:76–83.
- [53] Hajipour MJ, Fromm KM, Ashkarran AA, de Aberasturi DJ, de Larramendi IR, Rojo T, et al. Antibacterial properties of nanoparticles. *Trends Biotechnol* 2012;30:499–511. doi: <https://doi.org/10.1016/j.tibtech.2012.06.004>.



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