Australian Journal of Basic and Applied Sciences, 7(7): 100-104, 2013
ISSN 1991-8178

Antibacterial performance of ZnO tetrapods prepared by thermal oxidation

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Abstract: The ZnO tetrapods were synthesized by a rapid thermal oxidation from mixing metallic zinc powder into hydrogen peroxide at 1,000 ^{o}C under atmospheric pressure. ZnO tetrapods structural morphology was observed and confirmed by field emission scanning electron microscope with average individual arm length and diameter about 10 and 2 μm, respectively. Crystal single structure was analyzed by Rietveld method using XRD showed the ZnO tetrapods had the pure wurtzite hexagonal structure with lattice parameters *a* and *c* are 0.3252 nm and 0.5211 nm, respectively. ZnO tetrapods were added into nutrient broth and diluting different concentration in rank of 0.10 to 2.00 mg/ml. Antibacterial test was carried out on *Bacillus thuringiensis israelensis* and *Escherichia coli*. Both strains were added into media and left in the incubator-shaker at 37 ^{o}C for 24 h. Inhibition rate was calculated from measuring optical density value using UV-Vis spectrophotometer at 600 nm. It was showed that inhibition rate increases as a function of increasing ZnO tetrapods concentration and both strains were absolute inhibited at higher tetrapods concentration of 0.75 mg/ml.

Key word: ZnO tetrapods, antibacterial performance, thermal oxidation

INTRODUCTION

Nowadays, various nanostructure materials have become an interest research field to be used for manufacturing industrial items such as semiconductors. Moreover, nanomaterials are widely being under investigation for revolutionizing biomedical sciences such as antibacterial agents (Hajipour *et al*., 2012; Ma *et al*., 2013) because biological systems are responses on the surface properties of nanomaterials. Several research for metal antibacterial agents have mentioned on the nanostructure development from metals such as copper, silver and gold (Ruparelia *et al*., 2008; Dreaden *et al*., 2012). However, silver and gold are high effective cost materials that unsuitable to be used for a large production scale such as industrial scale. Alternating metal oxide such as Al_2O_3, SiO_2, TiO_2, and ZnO have been several reports regarding the antimicrobial activity due to their unique advantages such as large surface-to-volume ratio (Jiang *et al*., 2009; Shi *et al*., 2013). Moreover, ZnO have been interested material in our research because of its vast properties; low effective cost, non-toxicity, chemical stability in physiological media, biologically safety, very useful against pathogenic bacteria and possibly produce a large scale (Zhao *et al*., 2013; Armelao *et al*., 2011; Nohynek *et al*., 2011; Hrenovic *et al*., 2012).

Therefore, in this work we have a mentioned to improving antibacterial performance by using ZnO tetrapods. A thermal oxidation technique was used to formed tetrapods structure. Antibacterial activity was investigated by a measurement of optical density (OD) value, *Bacillus thuringiensis israelensis* (*Bti*) and *Escherichia coli* (*E. coli*) were selected bacteria as gram-positive and gram-negative, respectively.

MATTERIALS AND METHODS

Zinc hydroxide ($Zn(OH)_2$) was formed by mixing 99.99% metallic zinc powder (Zn) into 30% hydrogen peroxide solution (H_2O_2) for 24 h by the Zn:H_2O_2 weight ratio is 10:1. After performing process, $Zn(OH)_2$ was transferred in to alumina crucible boat. ZnO tatrapods were synthesized from $Zn(OH)_2$ by a rapid thermal oxidation technique according to our previous work for the highest percent yield of the tetrapods by weight (Bhoomanee *et al*., 2011). An open air horizontal furnace was increasing a high temperature up to 1,000 ^{o}C, the increasing rate was about 5 ^{o}C/min under atmospheric pressure. The $Zn(OH)_2$ was rapidly placed into the furnace to synthesizing ZnO tetrapods for 1 min. After the tetrapods was synthesized, the crucible was suddenly pulled from the furnace and cooled down under atmospheric pressure at room temperature. Tetrapods structural mophology was observed by field emission scanning electron microscope (FESEM). The crystal structure of the samples was investigated by the powder X-ray diffraction technique using Cu K_{α} radiation (λ =

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1.5406 Å). The X-ray diffraction patterns were analyzed using Rietveld refinement method with Fullprof program, and the lattice parameters were determined.

Antibacterial activity of ZnO tetrapods against *Bti* and *E. coli* were experimented by growth bacteria in media solution. Starter cultures of the both strains were grown in nutrient agar (NA) at 37 ^{o}C for 24 h (Li *et al*., 2009). Nutrient broth (NB) was used as medium for antibacterial test (Snega *et al*., 2013). The media was prepared by mixing desired among of ZnO tetrapods into NB and diluted by distilled water for ZnO tetrapods concentration in rank of 0.10 to 2.00 mg/ml to forming solution media. All of media were transferred into test tubes and sterilized under pressure of 15 pounds and temperature of 121 ^{o}C for 15 min (Hashemi *et al*., 2008). After sterilization of media, *Bti* and *E. coli* were inoculated with 20 μl in test tubes and were kept in incubation at 37 ^{o}C for 24 h (Lellouche *et al*., 2012). Antibacterial activity was investigated from observing bacterial growth in test tubes by measurement of optical density (OD) using UV-Vis spectrophotometer at 600 nm (Snega *et al*., 2013). Inhibition rate was calculated from OD value following as (Wang and Zhang, 2012),

$$Inhibition\, rate\,(\%) = \left(1 - \frac{OD_{sample}}{OD_{control}}\right) \times 100\% \qquad (1)$$

Where $OD_{control}$ is the optical density of a blank control sample (without ZnO tetrapods) and OD_{sample} is optical density of samples with various ZnO tetrapods concentration.

RESULTS AND DISCUSSION

ZnO tetrapods have been successfully synthesized under atmospheric pressure at 1,000 ^{o}C for 1 min. We have used this annealing temperature for our system to be sure that thermally oxidized ZnO tetrapods occurs according to our previous work (Bhoomanee *et al*., 2011; Hongsith *et al*., 2009), which was confirmed by FESEM show in Fig.1. An average individual arm length and diameter are about 10 μm and 2 μm, respectively. The reaction between metallic Zn powder and H_2O_2 solution forming as ZnO has been described in equation (2) and (3) (Ji *et al*., 2005).

$$Zn + H_2O_2 \rightarrow Zn(OH)_2 \downarrow \qquad (2)$$

$$Zn(OH)_2 \xrightarrow{\Delta} ZnO + H_2O \qquad (3)$$

$Zn(OH)_2$ particles are formed by a thermodynamically stable material at an initial stages from a chemical reactions during mixing of metallic Zn powder into H_2O_2 solution under atmospheric pressure at room temperature. Dehydration of $Zn(OH)_2$ gives rise to a ZnO during $Zn(OH)_2$ was placed in a furnace with high temperature. Loosed bounding of $Zn(OH)_2$ particles were occurs over a short time period during $Zn(OH)_2$ absorbed heat energy until internal temperature is up to around 100 ^{o}C. Raising internal thermal energy of $Zn(OH)_2$ leads to the controlled release of free zinc ions (Zn^{2+}) and hydroxide ions (OH^-) [19] and form as ZnO and H_2O. After water evaporation at high temperature, only ZnO is in the crucible. When the ZnO formed reaches supersaturation, due to continuously absorption of thermal energy, it would condense and ZnO nuclei were extended their arm out of the grain boundary to develop tetrapods structure (Fan *et al*., 2011).

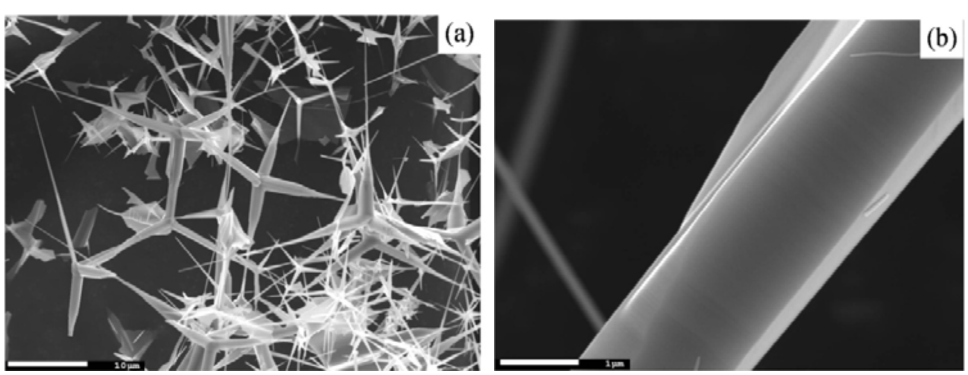

Fig. 1: FESEM image of synthesized ZnO tetrapods by a thermal oxidation technique.

The measured and calculated XRD spectra of ZnO tetrapods sample with designated Miller indices are shown in Fig. 2. To determine the crystal structure of the samples, the XRD spectrum was refined by the Fullprof program. It is verified that predominantly single phase ZnO tatrapods with wurtzite structure is formed with P_{63}mc symmetry. The lattice parameters *a* = 0.3252 nm and *c* = 0.5211 nm, and unit cell volume V = 55.109 $Å^3$ are obtained from the refinement process.

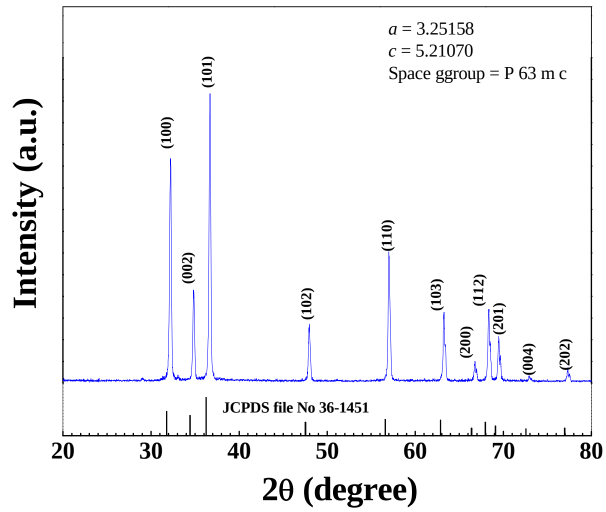

Fig. 2: XRD diffraction pattern of ZnO tetrapods prepared by a thermal oxidation technique.

ZnO tetrapods were added into NB with different tetrapods concentration for antibacterial test. Inhibition rate was showed in Fig. 3, it has been observed that *E. coli* is susceptibility to damage by ZnO tetrapods than *Bti* at low tetrapods concentration in rank of 0.10 to 0.50 mg/ml. For low tetrapods concentration, 0.10 to 0.50 mg/ml, it is showed a significant differences of antibacterial activities for *Bti* and *E. coli*. The antibacterial activity for *Bti* strain was a linear-like increasing as a function of tetrapods concentration while *E. coli* shown a strongly responses to be killed up to about 100% at tetrapods concentration of 0.25 mg/ml. These results shown higher inhibition rate for *E. coli* than *Bti* at low tetrapods concentration due to different strains of bacteria may have different sensitivity to antibacterial agents. Moreover, since the tetrapods concentration are 0.75 mg/ml and more, all samples were observed clear like a blank control, the inhibition rate is up to 100% and there are not difference between the both strains in a high tetrapods concentration. Both strains cannot survival in a high tetrapods concentration more than 0.75 mg/ml. The exact mechanism of antibacterial activity of nanomaterials against is still unknown completely. Generally, antibacterial activity of metal oxide is due to a production of reactive oxygen species (ROS) such as hydroxyl group (OH^-), superoxide anions (O_2^-), and hydrogen peroxide (H_2O_2), generation of this ROS is dysfunction to bacterial cell membrane and inhibits the growth of bacterial cell because they are highly reactive and powerful oxidizing agents (Snega *et al*., 2013). The most of ROS induction for ZnO is H_2O_2 (Li *et al*., 2008) which is a strong oxidizing agent harmful to bacterial cell membrane. Thus it can be said that the generation of H_2O_2 in ZnO slurries is a high probability of primary ROS for antibacterial activity in this work. Therefore, little among of generated ROS at low tetrapods concentration, lower than 0.50 mg/ml, have a tendency to slow down the bacterial growth. On the other hand, both strains were be absolute killed where ZnO tetrapods were dissolved with higher tetrapods concentration of 0.75 mg/ml. Disruption of bacterial cell membrane may be explained as resulting a saturated reaction rate of ROS and changed the cell membrane components (Liu *et al*., 2009). Moreover, depressing the bacterial growth is due to the structural size of metal oxide, smaller size was more toxic to bacteria than bigger size (Jones *et al*., 2008). The antibacterial activity shows an absolute inhibition of bacterial growth at the tetrapods concentration of 0.75 mg/ml for both strains. Thus at this concentration can be called as a minimum inhibitory concentration (MIC) (Gokulakrishnan *et al*., 2012), the lowest concentration of the ZnO tetrapods synthetic compounds in media solution that did not observed any bacterial growth.

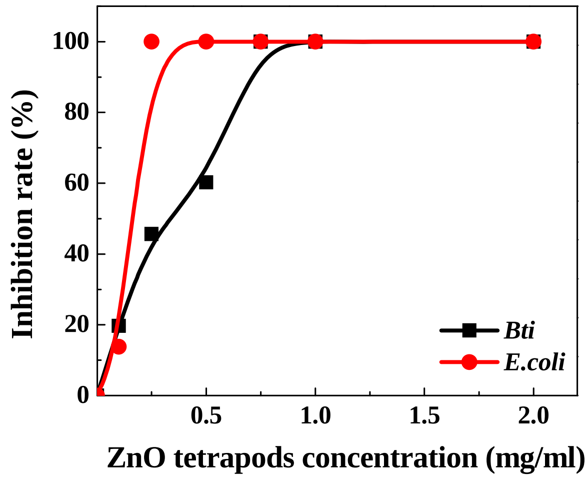

Fig. 3: Dependence of inhibition rate as a function of ZnO tetrapods concentration.

Conclusion:

We have been successfully synthesized ZnO tetrapods from a mixed metallic Zn powder and H_2O_2 solution by a rapid thermal oxidation technique in open furnace under atmospheric pressure at 1,000 ^{o}C. Tetrapods morphology was observed and confirmed by FESEM with average individual arm length and diameter are about 10 μm and 2 μm, respectively. ZnO crystallinity was characterized by XRD diffraction pattern and wurtzite hexagonal structure was confirmed with lattice parameters *a* and *c* are 0.3252 nm and 0.5211 nm, respectively. Antibacterial activities showed a significant difference for *Bti* and *E. coli* at low tetrapods concentration and *E. coli* shows more responsibility than *Bti*. However, there is no difference in antibacterial activities for both strains at higher 0.75 mg/ml tetrapods concentration. Finally, we have successfully demonstrated a performance of ZnO tetrapods for use as antibacterial agents.

ACKNOWLEDGMENTS

This work was supported by Kasetsart University Research and Development Institute (KURDI), Kasetsart University. Chawalit Bhoomanee would like to thanks Thailand Center of Excellence in Physics (ThEP) for financial support.

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