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Synthesis and Characterization of N and N, S Co-Doped Nano-Titania Photocatalyst and its Antibacterial Study on Escherichia Coli

KEYWORDS: Sol-gel chemistry; Photocatalyst; Antibacterial; Nano-titania

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ABSTRACT *Photocatalytic antibacterial studies were carried out with Escherichia coli using N and S-N co-doped anatase TiO2 catalysts in Visible light irradiation. Characterization techniques such as XRD, UV-Vis.DRS and XPS demonstrated the good dispersion properties of N and S on TiO2 prepared by sol-gel precipitation method. Comparison of relative photo catalytic efficiencies demonstrated that S-N co-doped photocatalysts exhibited higher activity in visible. More than 90% of the E-coli were deactivated within one-hour irradiation with catalyst amount 5 mg.*

1. Introduction

Microbiological safety of water is very crucial not only for drinking water and wastewater, but also recreational activity in river and coastal area. Over one billion people each year are exposed to unsafe drinking water due to poor source water quality and lack of adequate water treatment. Therefore, water disinfection methods that are easily employed in developing countries are needed. Many chemical based disinfection methods such as use of alcohol, iodine, chlorine and phenol are common with great success. However, many of them are volatile and produce more toxic secondary bi-product during the process. Therefore, it is essential to develop a novel and safe strategies for water disinfection.

Honda and Fujishima have extensively studied titania as a photocatalyst since the discovery of its photosensitization effect in 1972 [1]. It has strong photo-oxidizing potential, high chemical stability, non-toxicity, and low cost of production and have been made into various forms. Thus, it has wide range of applications including degradation of organic pollutants, production and storage of renewal energy, environmental control, antimicrobial property, air and water cleaning, deodorization and disinfection [2,3]. One of the advantages of titania as photocatalyst is that it can perform its activity by absorption of solar energy therefore it is useful in areas where the electricity is insufficient.

However, its activity was limited to UV region of the solar source due to its wide band gap of 3.2 eV (anatase). The solar region contains around 5% of UV light and 40-45% Visible light. Therefore, in order to use solar energy effectively, it is essential to extend the absorption region of titania into visible region. Many attempts were reported by various research groups to bring the activity of titania into visible region such as doping with metals, non-metals, coupling with semiconductors, sensitizing titania with colorful inorganic or organic compounds. However, it was noted that the anionic dopant species were found to be better than others with respect to the stability of the doped materials, photocatalytic efficiency, and ease of the doping process [4]. Undeniably, the non-metal doped TiO_2 photocatalyst is a hot research topic, and it opens up a new possibility for the development of solar light induced photocatytic materials by co-doping with non-metals.

The antibacterial effect of heterogeneous titania semiconductor photocatalyst was first demonstrated by Matsunaga et al. in 1985. Compared with traditional disinfectants, the TiO_2 photocatalyst is safe, nontoxic, and does not produce hazardous by-products [5]. It is capable of repeated use; therefore, its costs can be minimized. Several groups have studied visible light active modified titania such as S, N, Ag/AgBr, AgI, C, Ag, Cu, Ag.C.S, N.Ag doped titania with different microorganism. Most of them gives better results with average of 2-3 hour radiation [3,6,7].

However, to our knowledge, there were rarely reports on the high percentage anti bactereial degradation of E-coli using anion co-doped titania via sol-gel method. Thus the present work is aimed to a simple route for synthesis of high visible light responsive N doped and S-N co-doped titania through sol-gel precipitation method. The catalysts are characterized by various techniques XRD, UV-Vis.DRS, TEM, and XPS. The photocatalytic ability of the prepared samples is evaluated by studying the antibacterial effect on E-coli in visible region and the results are compared with pure titania prepared in the same manner and one of the commercial anatase titania.

2. Materials and Methods

2.1 Catalyst Preparation:

For the preparation of pure titania, 0.96g of Titanium tetraiso-propoxide was dissolved in 7.85 g of isopropyl alcohol and to the mixture; 100 ml of distilled water was added drop wise with vigorous stirring at room tempera-

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ture. It was aged for one day, dried below 70^{0}C and calcined at 400^{0}C for 4 hours. The sample was denoted as L-TiO_2. In the case of nitrogen doped titania, a 100 ml solution of 10 %(w/v) urea and for S-N co-doping, 10 %(w/v) thiourea solution was used instead of 100 ml distilled water in the procedure adopted for pure titania. The samples were denoted as N-TiO_2 and N,S-TiO_2 respectively. The commercial titania was obtained from Sigma Aldrich having 100% anatase denoted as A-TiO_2.

2.2 Characterization of catalysts

The X-ray diffraction (XRD) patterns were recorded in the 2θ range of 10-70^0 using *Bruker* AXS D8 advance X ray Diffractometer with Ni filtered Cu Kα radiation(λ = 0.15406 nm). The crystallite size was determined from the broadening of the major peak using the Scherrer equation, D= K λ / β Cosθ. Where D is the crystallite size, K = 0.9 is a constant, λ is the X-ray wavelength, β is full width half maximum of the major peak and θ is the diffraction angle. The UV-Visible diffuse reflectance spectra (UV-Vis.DRS) were recorded in the range of 200-900 nm on a *Labomed* UVD–500 UV-Visible Double beam spectrophotometer equipped with an integrating sphere assembly, using $BaSO_4$ as reflectance standard. The transmission electron microscopy (TEM) images were recorded *Jeol* 3010 ultrahigh resolution analytical electron microscope. The X-ray photoelectron spectra (XPS) were recorded in an indigenously devel-oped electron spectrometer equipped with *Thermo* VG Clamp-2 Analyser and Mg Kα X-ray source (1253.6 eV, 30mA × 8 kV).

2.3 Photocatalytic Activity: The photocatalytic experiments carried out in an Oriel Uniform illuminator of 100 W Xe ozone free light sources with filter 420- 630 nm at room temperature. *Escherichia coli* were obtained from the Department of Marine Biology, Microbiology and Bio-chemistry, Cochin University of Science and Technology. A subculture was prepared using nutrient broth and incubated at 37^{0}C for 24 hr with 200 rpm in a rotary shaker. 10 ml of the culture was washed with a 0.9% saline solution by centrifugation at 12000 rpm for 15 minutes. The supernatant solution was discarded and the cells suspended in 10 ml normal saline. The cell suspension was serially diluted to get proper concentration that reported as number of colonies forming unit/ml (cfu/ml).

A fixed amount of the photocatalyst was added to 9 ml 0.9% saline solution in a reagent bottle. It was sterilized by autoclaving at 120 ^{0}C and 15 lbs for 30 minutes and mixed with 1ml of the prepared cell suspension after cooling. The reaction mixture was stirred with a magnetic stirrer to prevent settling of the photocatalyst and irradiated with light source. A bacterial suspension without photocatalyst was irradiated as a control, and the reaction mixture with no visible light irradiation was used as a dark control. After irradiation, 1 ml of the sample spread on petridish containing nutrient agar and incubated for 48 hours at 37 ^{0}C and then it taken out for counting. A triplicate of each experiment was conducted and its average taken. Antibacterial efficiency (in percent) was reported using the equation, {Co – C} X 100 / Co, where Co and C are the number of colonies formed before and after irradiation.

3. Results and Discussion

3.1. XRD spectra

All the samples showed XRD spectra with a major peak at 2θ of 25.3^0, which is the characteristic of (101) plane of anatase phase **(Fig.1).** The absence of other major peaks confirmed the presence of anatase phase only. The average crystallite size calculated from the broadening of (101) XRD peaks of anatase using the Scherrer equation reported in **table 1.** The broadening of the peaks in the prepared samples indicated that the particle size has decreased compared to commercial sample [8]. Peaks due to the dopants were absent. This revealed that the structure of titania is not affected by the incorporation of the dopants.

3.2 UV-Visible diffuse reflectance spectra

UV Visible Diffuse reflectance spectra of the samples are shown in **Fig.2** The absorption edges obtained as the wavelength of the onset of the spectrum is converted to band gap using one of the forms of Kubelka-Munk function (Eg = 1239.8 / λ) as shown in **Table 1.** The doped samples showed a red shift in absorption maximum and possessed two absorption edge compared to pure titania. The absorption edges were related to original structure and doped structure of titania [9]. The red shift was due to the impurities incorporated into titania framework which resulted in narrowing the bandgap by mixing their p states with O 2p orbitals.

3.3 X-ray Photoelectron Spectra studies

The XPS spectra are presented in **Fig.3. (a-g)** and atomic concentration values derived from the XPS data are presented in **Table 2.** The Ti 2p XPS spectra showed two peaks, one at 458.2 and other at 464.7 eV in the case N doping and 458.4 and 464.1 eV in the case of N,S co-doping **[Fig.3. (a-b)]**. These were assigned to the Ti $2p_{3/2}$ and Ti $2p_{1/2}$ states. These doublet peaks were due to the spin-orbit splitting of Ti 2p [10]. The above values corresponded to the (IV) oxidation state of Ti. Thus in the prepared samples titanium is in (IV) oxidation state with stable Ti-O bond.

The O1s XPS spectrum **[Fig.3.(c-d)]** showed a strong peak at 529.3 eV corresponding to bulk oxygen bonded to titanium where as a weak shoulder at 531 eV was due to oxygen attached to N. In the case N,S co-doping weak shoulder at higher binding energy of 532.2 eV which may be due to the incorporation of S as SO_4^{2-}.

The N 1s XPS spectrum **[Fig.3 (e-f)]** showed a peak at 399.5 eV corresponding to the N doping and peak at 400.2 eV corresponding to the N,S co-doping catalysts. The results indicates a peak at 399.5 eV of N doping catalyst which can be assigned to the interstitial N bound to one lattice oxygen as Ti-O-N. The peak at 400.2 eV of N, S co-doping system was due to the presence of incorporated S atom in the lattice. The shift of binding energy to higher values indicated a decrease in the electron density of N atom. We propose that the positive shift of binding energy of N 1s in N,S co-doping system could be due to the presence of S incorporated as S (VI) assigned to the SO_4^{2-} adsorbed on the surface.

The S 2p XPS spectrum **[Fig.3 (g)]** showed a peak at 168.5 eV corresponding S $2p_{3/2}$ state. Ohno and Wang reported that the peak of S 2p at 168.0 – 170.0 eV corresponds to the S atom incorporated as cation in the form of S (VI) in titania network and peak at 160.0 – 163.5 eV corresponds to S atom as S^{2-} by anionic replacement of O from titania lattice [6]. Thus the result indicated S in S (VI) state (as SO_4^{2-}). These sulphate ions can form S=O and S-O-S bonds on the TiO_2 surface, creating unbalanced charge on Ti and vacancies/defects in the titania network

3.4 Photocatalytic activity

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Due to the shift of absorption edge into the visible region, the modified catalyst should be photocatalytically active under visible light irradiation. The photocatalytic ability of the prepared samples were evaluated by the its antibacterial effects on *Escherichia coli* bacteria.

In this experiment, about 5.0 mg of catalyst was taken, that was optimized in earlier studies with visible light irradiation of one hour. In this section, we have discussed the results obtained by conducting the experiments in dark and controlled conditions with modified titania, pure titania prepared in the laboratory and commercial titania.

Table3 shows a comparison study of the antibacterial efficiency of the modified catalyst against pure titania with a controlled and dark conditions. The interaction between bacteria and the catalyst in the dark revealed that there was a very little antibacterial efficiency. This indicates that the toxicity of the applied photocatalyst to bacteria is not appreciable under the experimental condition of this study. However, a controlled experiment without catalyst shows a little significant antibacterial activity, which is attributed to the effect of light intensity that causes the damage of cell wall under irradiation. It is interesting to note that the modified catalyst resulted in more than 95% antibacterial efficiency in visible light compared to pure samples that has a maximum of 54% antibacterial efficiency **(Fig.4 a-e)**. During the photocatalytic process, there is a possibility for the damage of cell walls of the bacterial through photo oxidation by the photoactive reaction intermediates, which results in the dead of bacterial cell. The visible light activity in non-metal doped titania may be caused by band-gap narrowing from mixing the N 2p or S 2p states with O 2p states.

Conclusion

We were successful to synthesize visible light responsive anatase N and N,S co-doped nano crystalline titania. The XRD characterization showed that particles were crystalline, with 101-anatase phase and size (9–11 nm). The presence of dopant N and S were confirmed by XPS. The XPS showed that the chemical nature of dopant N existed as Ti-O-N and S as S (VI) cation. UV Visible DRS spectrum showed that the absorption edges of doping catalyst shifted to visible region, which lead to narrowing of band gap. The photo-catalytic activities of the doped samples were evaluated by the antibacterial efficiency against *Escherichia coli* bacteria in visible light. More than 95% of the *E-coli* were deactivated within one-hour irradiation of visible light with catalyst amount of 5 mg. The higher activity is due to the presence impurity (such as N and S) in their lattice, which are responsible for consistent reduction of band gap.

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Figures

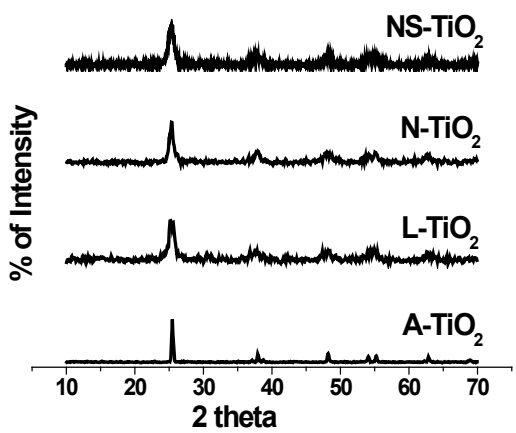

Fig. 1. XRD spectrum of NS-TiO2, N-TiO2, L-TiO2 and A-TiO2,

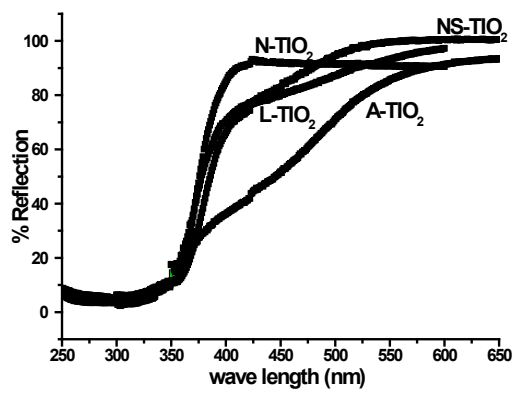

Fig.2 UV-Visible DRS spectrum of NS-TiO2, N-TiO2, L-TiO2 and A-TiO2

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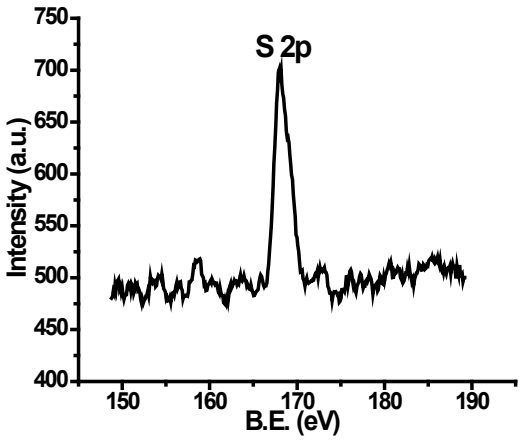
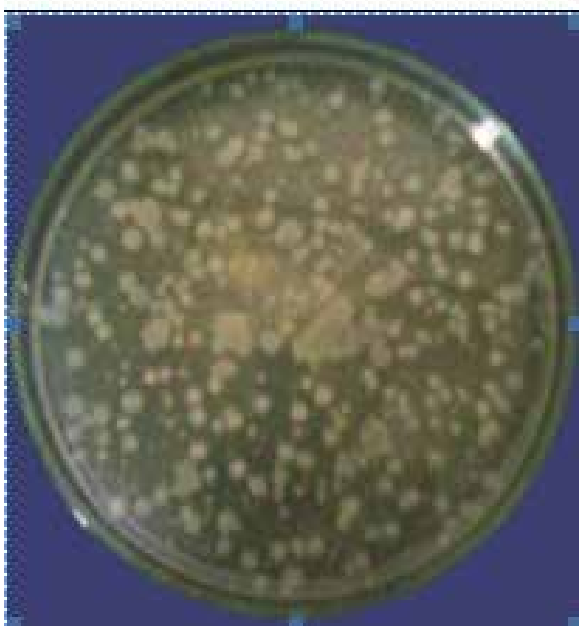


Fig.3. XPS spectra - (a) Ti 2p of N-TiO2, (b) Ti 2p of NS-TiO2 , (c) O 1s of N-TiO2, (d) O 1s of NS-TiO2, (e) N 1s of N-TiO2, (f) N 1s of NS-TiO2 and (g) S 2p of NS-TiO2



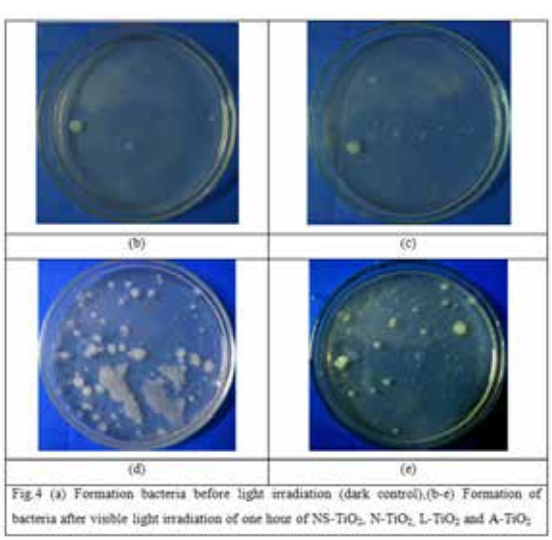


Fig.4 (a) Formation bacteria before light irradiation (dark control),(b-e) Formation of bacteria after visible light irradiation of one hour of NS-TiO_2, N-TiO_2, L-TiO_2 and A-TiO_2

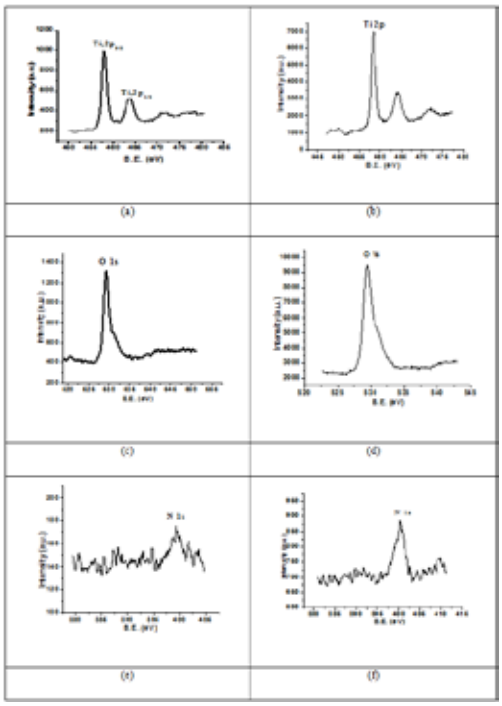


Table.1. Crystallite size and Band gap of the catalysts

Catalyst	Crystallite size[a] (nm)	Band gap[b] (eV)
NS-TiO_2	10.02	2.3
N-TiO_2	9.61	2.3
L-TiO_2	11.6	3.1
A-TiO_2	36.14	3.1

aCrystalline size calculated from XRD, bBand gap calculated from UV-Visible DRS spectrum

Table. 2. Atomic ratios derived from XPS data

Catalyst	Ti 2p3/2 peak (5.22)[a]			O 1s peak (2.48)[a]			N 1s peak (1.74)[a]			S 2p peak (1.25)[a]			Ti:O:N / Ti:O:N:S
	B.E. (eV)	FWHM (eV)	Peak Area	B.E. (eV)	FWHM (eV)	Peak Area	B.E. (eV)	FWHM (eV)	Peak Area	B.E. (eV)	FWHM (eV)	Peak Area	
N-TiO_2	458.2	1.87	977.6	529.2	1.87	3612.9	399.5	3.0	89.3	-	-	-	1:2.36: 0.27
NS-TiO_2	458.4	1.13	7194.8	529.4	1.85	7869.2	400.3	2.41	485.9	168.3	1.78	435	1:3.86:0.09:0.18

a- sensitivity factor

Table.3. Antibacterial efficiency
(Catalyst amount: 5.0 mg; Irradiation time: 1hour)

Catalyst	Anti bacterial efficiency (%)
NS-TiO_2	98.6
N-TiO_2	95.1
L-TiO_2	53.1
A-TiO_2	27.1
Without irradiation	6.5
Without Catalyst	10.0

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