



Synthesis and Glass Immobilization of Carbon and Nitrogen Doped TiO₂-SiO₂ and Its Effect on *E. coli* ATCC 25922 Bacteria

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJAST/2015/11049

Editor(s):

(1) Ahmed Fawzy Yousef, Deputy Minister of Egyptian Ministry of Agriculture and Land Reclamation for Studies and Water Associate Prof. of Water Resources in Desert Research Center, Egypt.

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Complete Peer review History: <http://www.sciencedomain.org/review-history.php?id=762&id=5&aid=6775>

Original Research Article

Received 25th April 2014
Accepted 7th August 2014
Published 5th November 2014

ABSTRACT

Aim: To evaluate the antimicrobial properties of carbon and nitrogen doped titanium dioxide (C-TiO₂ and N-TiO₂) immobilized on glass support by examining the inactivation of *E. coli* ATCC 25922 bacteria in water.

Study Design: Sol gel synthesis was used to prepare a series of visible light responsive photo-catalysts of titanium dioxide. The photo-catalysts were characterized via Fourier transform infrared spectroscopy (FT-IR), Scanning X-ray Photoelectron Spectroscopy (SXPS), X-ray Diffraction (XRD), Diffuse reflectance spectroscopy (DRS) and Energy Dispersive X-Ray Spectroscopy (EDS or EDX). Modified titanium dioxide photo-catalysts (TiO₂-SiO₂, C-TiO₂-SiO₂, and N-TiO₂-SiO₂) immobilized on glass supports were evaluated for their antimicrobial properties on the inactivation

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of *E. coli* ATCC 25922 in water.

Place and Duration of Study: Department of Chemistry (Water Research Group) and Department of Biochemistry and Microbiology, University of Fort Hare Alice Campus, between July 2013 and November 2013.

Methodology: A 0.5 McFarland standard solution containing approximately 1.5×10^8 organisms was prepared by adding *Escherichia coli* ATCC 25922 to a saline solution (0.85% NaCl). A battery of experiments was carried out to evaluate the antimicrobial properties of glass-immobilized and powder photo-catalysts. Every 30 minutes, swabs were taken from each reactor vessel and placed on the freshly prepared nutrient agar plates. Plates were incubated at 37°C for 24 hours.

Results: The number of active *E. coli* cells after treatment with TiO₂ was determined by colony counting after 24 hours of incubation. When *E. coli* ATCC 25922 was treated with powder TiO₂ (i), there was a small decrease in the number of colonies within the first 30 minutes, but after exposure for 60 minutes about 98% of the bacterial colonies had been destroyed.

Conclusion: Immobilized titanium dioxide photo-catalyst was shown to be less effective in the deactivation of *E. coli* bacteria. The three nano-composite photo-catalysts; TiO₂-SiO₂, C-TiO₂-SiO₂, and N-TiO₂-SiO₂ showed little cytotoxicity (the degree to which an agent possesses a specific destructive action on certain cells) towards the growth of *E. coli* ATCC 25922 while powder titanium dioxide proved to be very effective towards the inactivation of *E. coli* ATCC 25922 bacteria.

Keywords: Titanium dioxide/ silicon dioxide; nano-composites; glass; *E. coli* bacteria; photo-catalysis.

ABBREVIATIONS

N-TiO₂: nitrogen doped titanium dioxide, *C-TiO₂*: carbon doped titanium dioxide, *NaCl*: Sodium chloride, *BaCl₂*: Barium chloride, *H₂SO₄*: Sulphuric acid, *TEOS*: tetraethyl orthosilicate, *SiO₂*: silicon dioxide, *FT-IR*: Fourier Transform infrared spectroscopy, *XRD*: X-Ray diffraction, *SXPS*: Scanning X-ray Photoelectron spectroscopy, *DRS*: Diffuse reflectance spectroscopy, *EDX*: Energy dispersive X-Ray spectroscopy, *HUS*: haemolytic uremic syndrome

1. INTRODUCTION

Various microbes that may be found in contaminated water bodies pose a great risk to human health if consumed. The main microbes causing disease are bacteria, viruses, fungi and protozoa. *Escherichia coli* O157:H7 is a microorganism that causes diarrhoea ranging from mild, non-bloody stools to those that are virtually mainly blood [1]. *Escherichia coli* O157:H7 produces a toxin that damages the lining of the intestines resulting in haemorrhagic colitis [2]. This organism has a very low infectious dose and is thought to cause over 90% of all cases of diarrhoea-associated haemolytic uremic syndrome (HUS), a condition that causes acute renal failure, especially in young children. Waterborne transmission occurs from contaminated drinking water and from swimming in contaminated waters [3]. Shigellosis, leptospirosis, giardiasis, hepatitis A etc. are some of the waterborne diseases that pose risk to human health if contaminated water or fruit and vegetables irrigated with contaminated water are consumed.

The use of titanium dioxide (TiO₂) in the photo-mineralization of organic and inorganic pollutants in water is well established. The reactions are driven by UV light ($\lambda < 385$ nm), and lead to the generation of reactive oxygen species by TiO₂ photo-catalysts. The reactive oxygen species generated by the TiO₂ photo-catalytic reactions also cause varying degrees of damage to living organisms, which is not surprising since most living organisms usually contain an abundance of organic compounds. In 1985, Matsunaga and co-workers reported for the first time the microbe-destroying effect of TiO₂ photo-catalytic reactions [4].

In this study the focus was on the bactericidal action of TiO₂ thin films coated on glass substrate. Some TiO₂ coated tiles with self-cleaning and bactericidal functions have already been commercialized [5,6]. The self-cleaning function of TiO₂ coated substrates is explained simply by the photo-induced oxidative power of TiO₂ photo-catalysts. The bactericidal function, however, is not well understood, even though numerous reports have described photo-killing of

bacteria [7,8,9,10,11] viruses [12,13] and tumour cells [14,15]. Because these photo-killing reactions were carried out using TiO₂ powder, the possibility of cell de-activation by the co-aggregation of cells and loose TiO₂ particles cannot be excluded. In addition, the TiO₂ particles phagocytized by the cells may cause cellular injury [16]. In fact, Jacob and co-workers reported that TiO₂ particles ingested by phagocytosis caused rapid intracellular damage [11]. The fact that titanium dioxide is strictly ultraviolet responsive (UV only accounts for about 3-5% of the solar spectrum) necessitates its photo-sensitization towards visible light (a much wider spectrum). Photo-sensitization in visible light can be achieved by surface modification with transition metal complexes [17,18,19], as well as doping with metals (Pt, V, Au, Pd, etc.) [20,21,22] and non-metals (C, N, S, B, etc.), [23,24,25,26,27,28,29]. Mitoraj and co-workers [30], studied visible light inactivation of bacteria and fungi using carbon and platinum (IV) chloride modified titanium dioxide. They observed a biocidal effect in the case of *Escherichia coli* for the photo-catalysts they prepared [30]. In separate work by Jańczyk et al. [30] the platinum chloride modified titanium dioxide produced oxidative species under visible light irradiation which led to peroxidation of mouse melanoma cell membrane [17].

2. MATERIALS AND METHODS

2.1 Photo-catalyst Preparation and Immobilization

Three photo-catalysts were prepared via sol gel synthesis using titanium (IV) chloride (99%, Merck). In situ carbon and nitrogen doping was carried out using glucose (MET-U-ED Chemical Co.) and urea (99% Kanto Chemical Co.) as carbon and nitrogen sources respectively. The sol was heated for 10 minutes at 100 °C and then pH adjusted 8.0 after cooling. The as prepared catalyst were dried in an oven at 60 °C, and then calcined in a muffle furnace at 600 °C to allow the transformation from amorphous to anatase titanium dioxide. Composites with silicon dioxide (SiO₂) were prepared using tetraethyl orthosilicate (TEOS) as the precursor. TEOS served as both a source of SiO₂ and binder of the composites to the glass support material. TiO₂ and TEOS were mixed in a 1:1 ratio in the preparation of the paste for coating the glass support material. Glass substrates, 6.5 cm², were treated with hydrofluoric acid (40%, Merck) to

introduce OH groups on their surfaces, and then dip coated with the pastes of the prepared nano-composites, namely TiO₂-SiO₂, C-TiO₂-SiO₂, and N-TiO₂-SiO₂. The coated plates were dried in an oven at 60 °C and then further treated in a muffle furnace at 600 °C for 3 hours.

2.2 Culture of Bacteria.

Escherichia coli ATCC 25922 bacteria were used in all the antimicrobial experiments carried out. *E. coli* ATCC 25922 bacteria were spread onto nutrient agar and allowed to grow for a period of 18 hours at an incubation temperature of 37 °C. A sterilized loop was then used to transfer bacterial cells into a freshly prepared saline medium (1% NaCl) to match a 0.5 McFarland standard (approximately 1.5×10⁸ organisms per mL). The standard was prepared by mixing 0.05 mL of a 1% solution (w/v) of BaCl₂ with 9.95 mL of a 1% solution (v/v) of H₂SO₄ [31,32]. The standard gave an absorbance of 0.08 abs units at a wavelength of 625 nm. The prepared standard was then used to estimate bacterial densities of *E. Coli* ATCC 25922 in saline solution by comparing absorbance.

2.3 Antimicrobial Evaluation

The prepared nano-composite thin films were evaluated for their antimicrobial properties on the inactivation of *E. coli* ATCC 25922 bacterial cells. The antimicrobial experiments were carried out under visible light irradiation. Fig. 1 shows the reactor setup that was used. Five experiments were carried out in triplicate using the four catalysts TiO₂-SiO₂, C-TiO₂-SiO₂, N-TiO₂-SiO₂, and TiO₂. The fifth setup was the control of the experiment, which only had the microbes in the saline medium and no catalyst added. The other four had the four different catalysts and 150 mL of the saline medium containing the *E. coli* ATCC 25922. In this experiment a comparison of the performance of powder catalyst and immobilized catalyst was carried out. Every 30 minutes swabs were taken from each reactor vessel and placed on the freshly prepared nutrient agar plates. The process was done for 3 hours. All the plates were labelled and then placed in a Cocono incubator operating at 37 °C for 24 hours.

3. RESULTS AND DISCUSSION

3.1 FT-IR, EDX and DRS Analyses

FT-IR analysis was used to identify the functional groups expected in TiO₂, TiO₂-SiO₂ composite and carbon doped TiO₂-SiO₂ composite. Fig. 2

shows the FT-IR spectra of the above mentioned photo-catalysts.

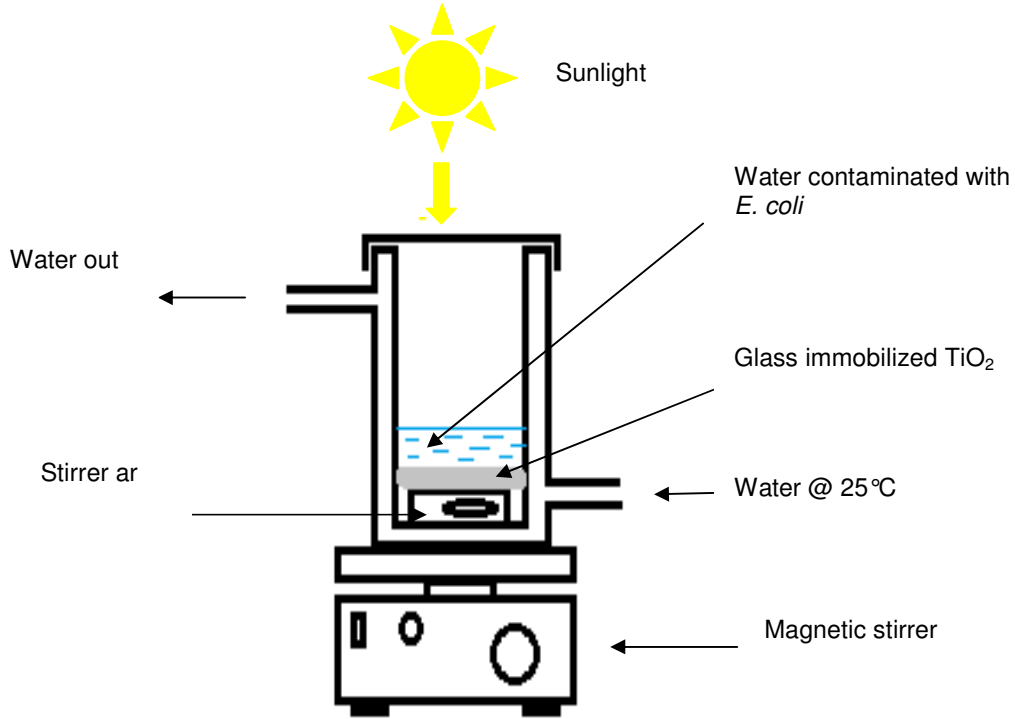


Fig. 1. Schematic of immobilized TiO₂ photo-reactor

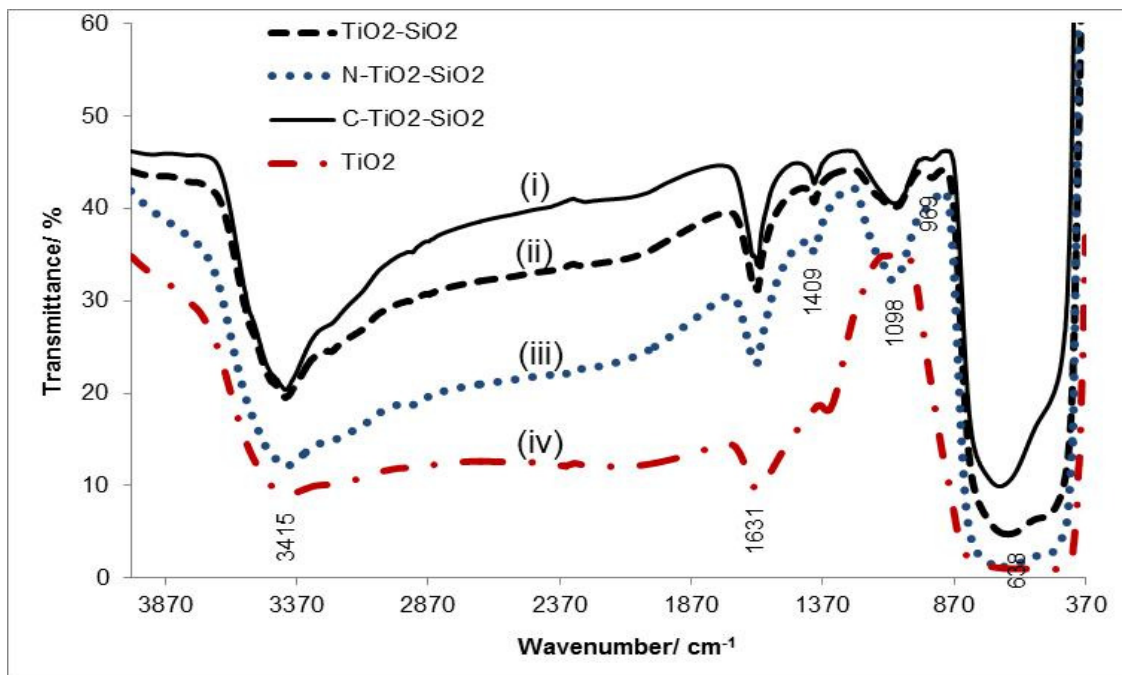


Fig. 2. FT-IR spectra of (i) C-TiO₂-SiO₂, (ii) TiO₂-SiO₂, (iii) N-TiO₂-SiO₂ and (iv) TiO₂

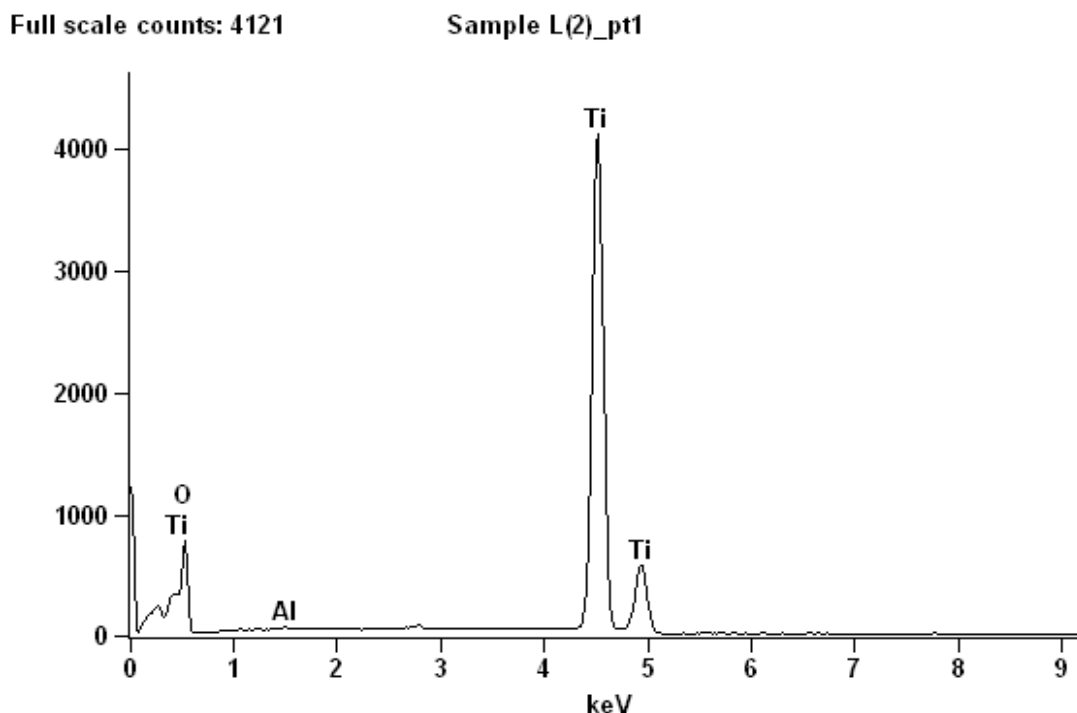
The labelled peaks can be attributed to OH stretching vibration (around 3415 cm^{-1}), Si-O-Si band asymmetric stretching vibrations (1098 cm^{-1}), and Ti-O-Si linkage stretching band (969 cm^{-1}). The existence of Si-O-Ti bond in FT-IR analysis may be ascribed to the existence of covalent bonding between amorphous SiO_2 and crystalline TiO_2 [33,34]. The peak around 1631 cm^{-1} is assigned to the bending vibration of OH bond, which is due to the chemisorbed water, and the peak around 3415 cm^{-1} is ascribed to the stretching mode of OH bond and related to free water. The FT-IR spectra of anatase and rutile titanium dioxide are similar to each other, with both having a broad peak between 800 and 470 cm^{-1} but with the anatase form exhibiting a valley in the peak centered around 600 cm^{-1} [35]. FT-IR confirmed successful preparation of the photocatalyst.

Energy dispersive X-ray analysis (EDX) was used for elemental analysis to determine if carbon or nitrogen dopant had successfully been incorporated into the titanium dioxide lattice. Figs. 3 (i), (ii) and (iii) shows the EDX spectra

obtained for TiO_2 , C- TiO_2 and N- TiO_2 respectively.

EDX analysis revealed that carbon and nitrogen dopants were successfully incorporated into the titanium dioxide lattice. The EDX spectrum for nitrogen doped titanium dioxide also showed the presence of carbon. This can be explained from the fact that urea was used as the nitrogen source, and urea also contains carbon which resulted in the co-doping of titanium dioxide. The EDX spectrum of undoped TiO_2 does not show presence of either carbon or nitrogen. Thus presence of carbon or nitrogen in the two doped samples was solely due to the doping process carried out. Doping with N and C allowed access to visible light. This was found effective and reported by Kisch et al [19,25,27] who also used other elements including transition metal ions as successful dopants.

Scanning X-ray photoelectron spectroscopy (SXPS) was carried out on C- TiO_2 and N- TiO_2 to identify the elements present from their unique binding energies as shown in Figs. 4 i, ii, iii, and iv respectively.



(i)

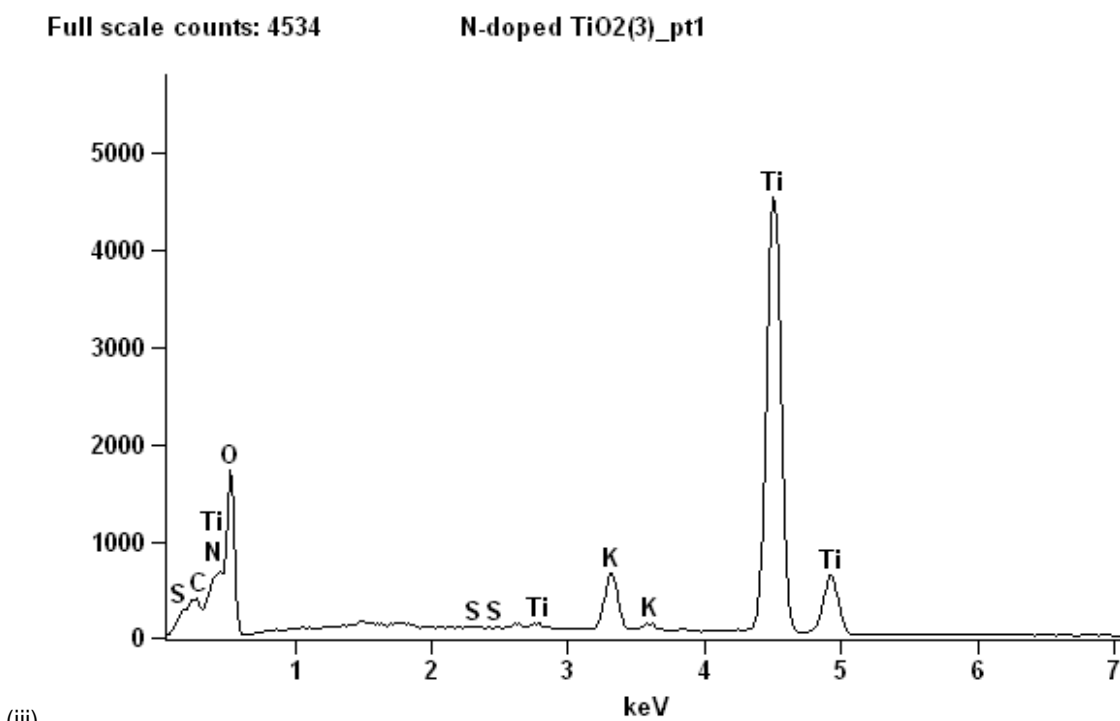
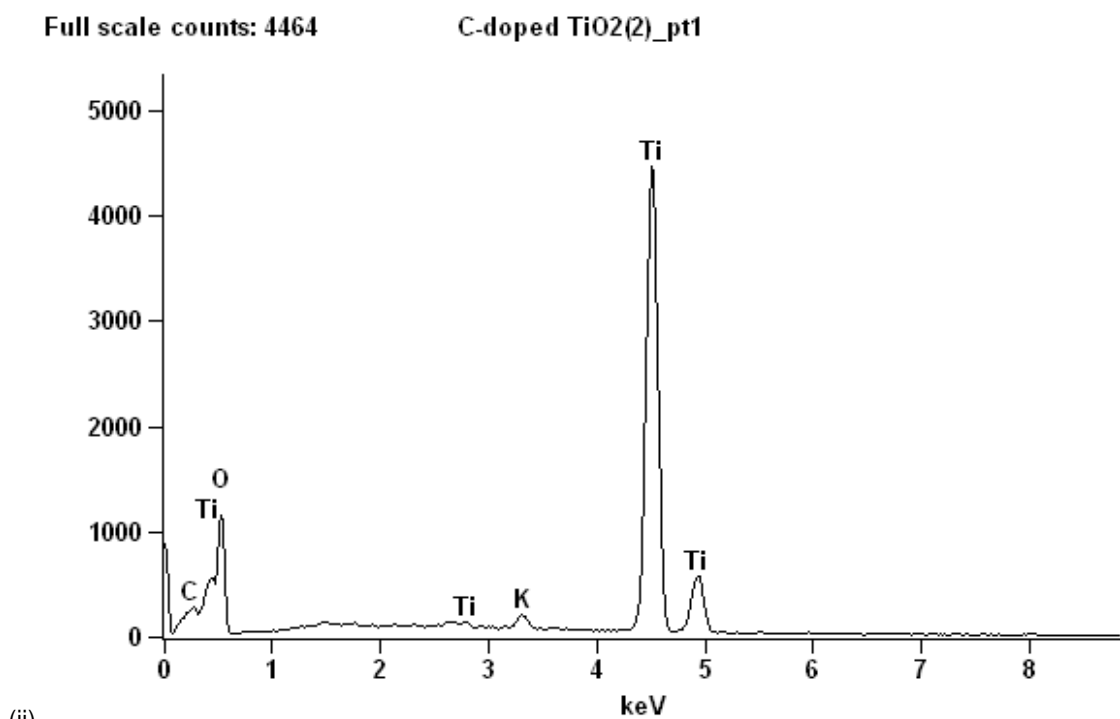
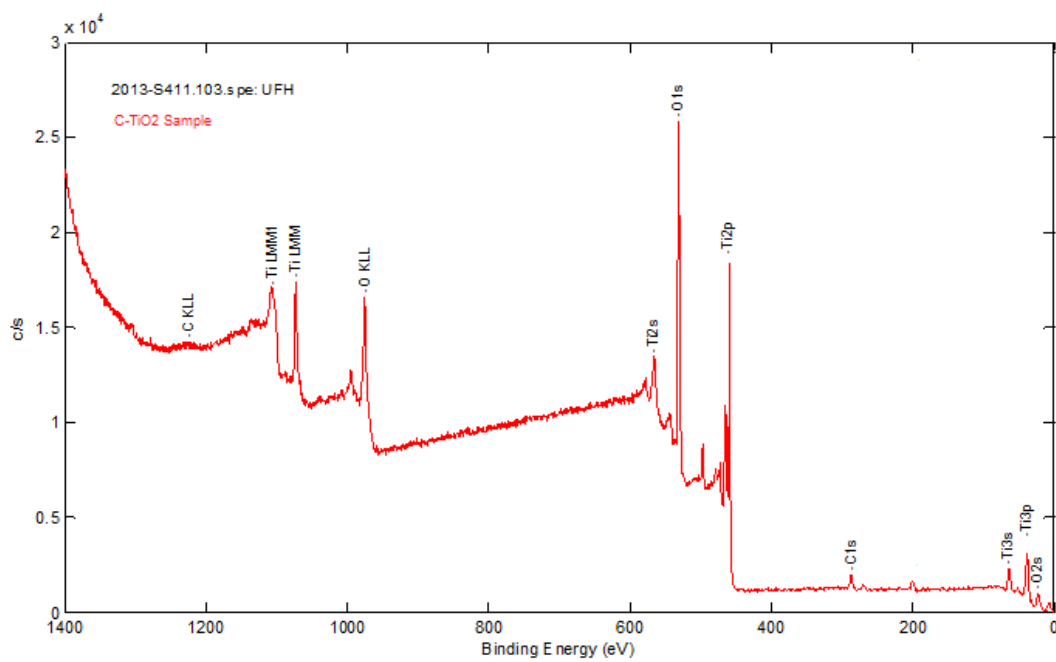
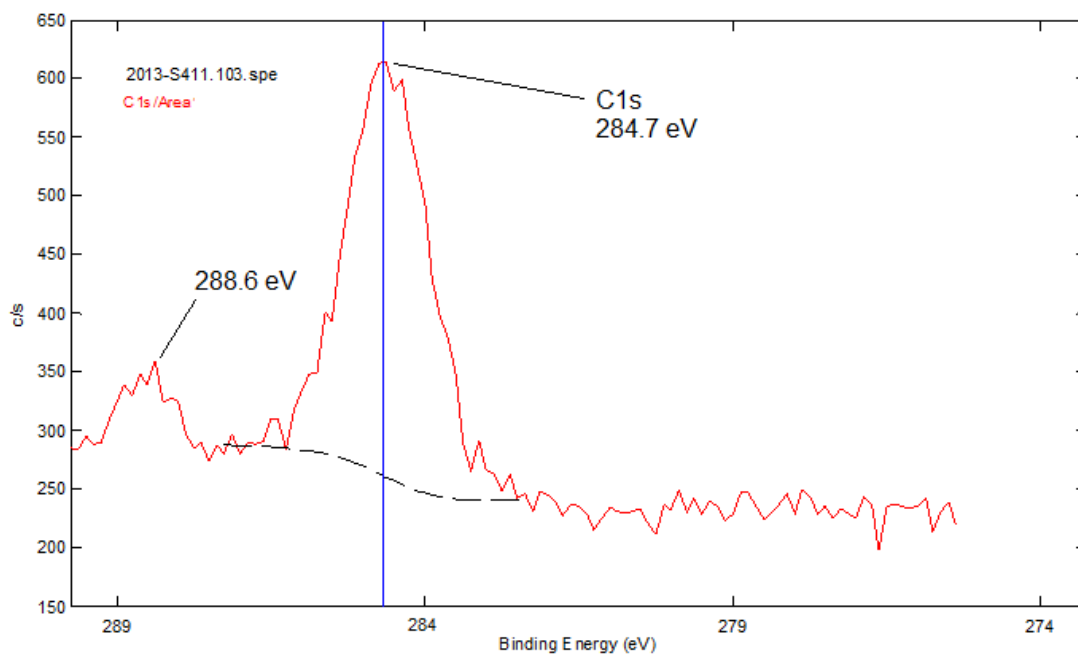


Fig. 3. EDX spectra of (i) TiO₂, (ii) C-TiO₂ and (iii) N-TiO₂



(i)



(ii)

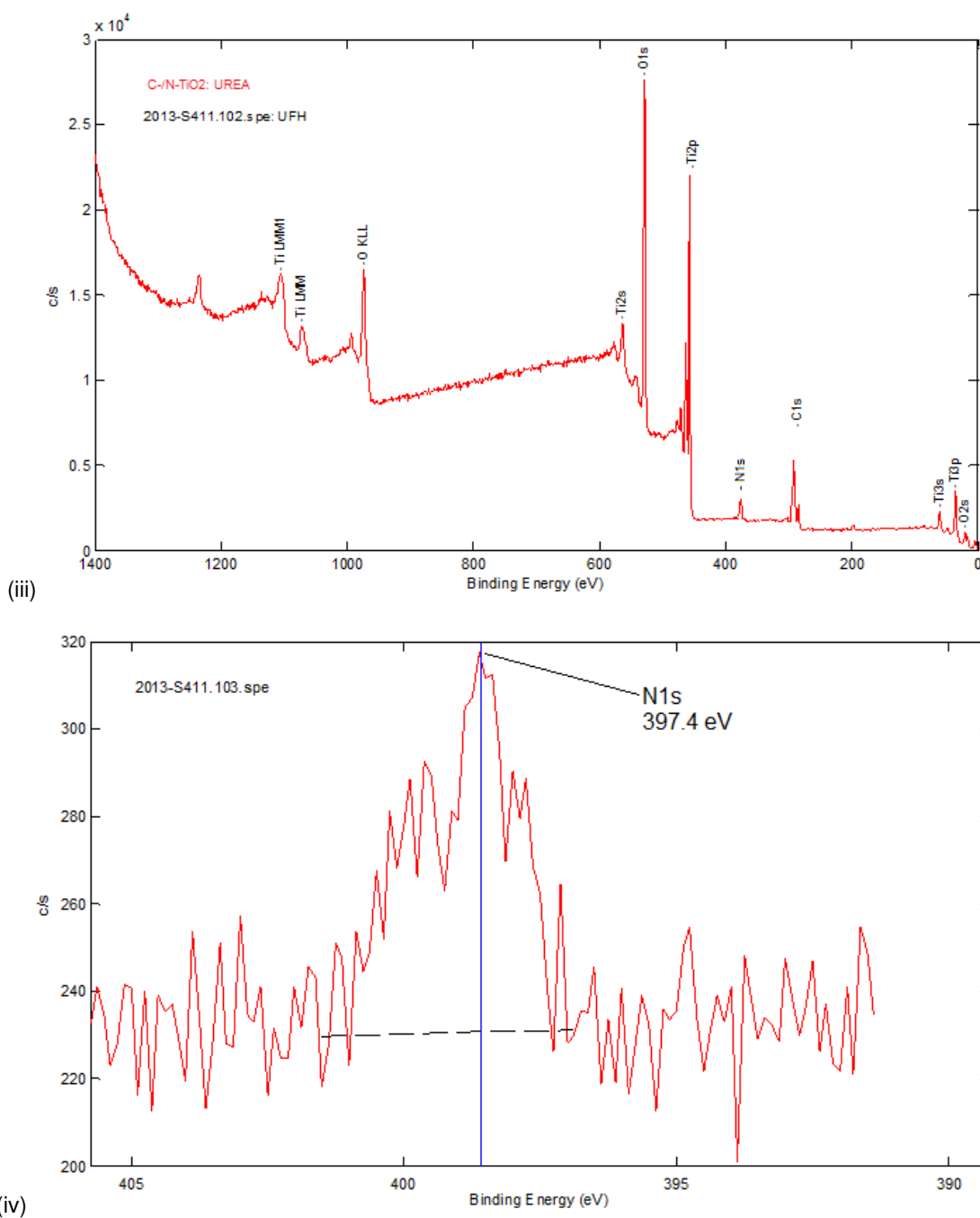


Fig. 4. SXPS spectra of (i & ii) C-TiO₂ and (ii & iii) N-TiO₂

The SXPS spectrum of C-TiO₂ revealed that the sample was solely composed of titanium, oxygen and carbon with binding energies of 458.5 eV, 529.6 eV and 284.7 eV respectively. The C1s core level shows two peaks with binding energies of 288.6 eV and 284.7 eV. The peak at 284.7 eV can be ascribed to presence of adventitious elemental carbon and the one at 288.6 eV

indicates existence of C-O bonds. The data obtained suggest that carbon may substitute some lattice oxygen and form O-Ti-C structures [36, 37]. Sakthivel and Kisch, 2003 also prepared carbon doped titanium dioxide. They assigned the peak at 285.6 eV to adventitious elemental and the ones at 287.5 & 288.5 eV to the presence of carbonate species in their XPS

studies. Their XPS studies were also supported by FT-IR studies in which spectra exhibited low-intensity peaks at 1738, 1096, and 798 cm^{-1} indicative of the presence carbonate ion [37].

The SXPS spectrum of N-TiO₂ revealed the presence of titanium, oxygen, nitrogen as well as carbon. This observation may suggest nitrogen and carbon co-doped titanium dioxide as indicated by EDX analysis Fig. 3. lii. However the binding energy obtained for the C1s peak (284.9 eV) suggests adventitious elemental carbon [36,37,38]. The peak at 397.4 eV can be attributed to the nitrogen that replace lattice oxygen in titanium dioxide to form the N-Ti-N bonds [26,39,40]. SXPS analysis confirmed successful preparation of N-TiO₂. Yang et al. [39] prepared a series of nitrogen doped titanium dioxide photo-catalysts which had enhanced absorption in the visible light region, and exhibited high photo-catalytic activity on the degradation of methylene blue and methyl orange.

X-ray diffraction (XRD) analysis was carried out to identify the phase composition of TiO₂, C-TiO₂, and N-TiO₂ prepared via sol gel synthesis. Fig. 5 shows the X-ray diffractograms of the three photo-catalysts respectively. The three photo-catalysts prepared were found to be predominantly in the anatase phase. The phase composition is dependant on the calcination temperature. The anatase phase of titanium dioxide has the most photo-catalytic activity [41,42], so the prepared catalysts exhibited the desired phase for the purpose of photo-catalytic activity. However, some noticeable differences exist among the x-ray diffractograms of TiO₂ compared to the ones for carbon and nitrogen doped titanium dioxide. The peaks at 2 theta angles of 28.3°, 40.5°, 49.9°, 58.4°, and 66.3° in the diffractogram of titanium dioxide (TiO₂) do not appear in the diffractograms of carbon and nitrogen doped titanium dioxide. The disappearance of these peaks can be attributed to the changes brought about by the carbon and nitrogen dopants introduced in the lattice structure of titanium dioxide. Nevertheless the major peaks remain unchanged in the diffractograms of pristine titanium dioxide and the carbon and nitrogen doped titanium dioxide. The phase composition of TiO₂, C-TiO₂ and N-TiO₂ were successfully determined via XRD analysis.

Diffuse reflectance spectroscopy (DRS) was used to analyse the shift in absorption band edge of titanium dioxide after doping with carbon and nitrogen. Fig. 6 shows the diffuse reflectance spectra obtained for C-TiO₂-SiO₂, N-TiO₂-SiO₂, TiO₂-SiO₂, and TiO₂. Doping titanium dioxide with carbon and nitrogen allowed the reduction of its band gap resulting in a shift in its absorption band edge from UV into the visible region. The observed shifts in absorption band are 403 nm (C-TiO₂-SiO₂), 400 nm (N-TiO₂-SiO₂), 397 nm (TiO₂-SiO₂), and 366 nm (TiO₂). Unmodified anatase TiO₂ is a strong UV light absorber as evidenced from the DRS results obtained.

A shift into the visible region allows the utilization of visible light in the activation of the titanium dioxide photo-catalyst. The visible region has an advantage in that it is a much wider spectrum compared with the ultraviolet region; hence it improves the performance of the titanium dioxide photo-catalyst. Other researchers have reported similar results after carrying out nitrogen doping on titanium dioxide [43].

3.2 Effect of TiO₂ Immobilization on *E. coli* Inactivation

The number of active *E. coli* cells after treatment with TiO₂ was determined by colony counting after 24 hours of incubation. The results obtained are shown in Fig. 7. Within the first 30 minutes of exposure, no effect was observed; all the *E. coli* cells remained viable. There was overgrowth on the plates such that colony count could not be executed. From 60 minutes onwards, a very small gradual decrease in the number of *E. coli* was observed for those vials that were exposed to immobilized photo-catalysts; N-TiO₂-SiO₂ (ii), C-TiO₂-SiO₂ (iii), and TiO₂-SiO₂ (iv). This showed that immobilized TiO₂ composites with silicon dioxide had little effect on the *E. coli* cells.

The control of the experiment (v) showed uninhibited growth of *E. coli* cells over the 3 hour period. The result obtained in these tests is clear evidence that TiO₂ photo-catalyst is less effective towards bacterial cell inactivation when immobilized on glass substrate. Another test was carried out using powder titanium dioxide to check the difference in the mode of action in powdered and immobilized titanium dioxide.

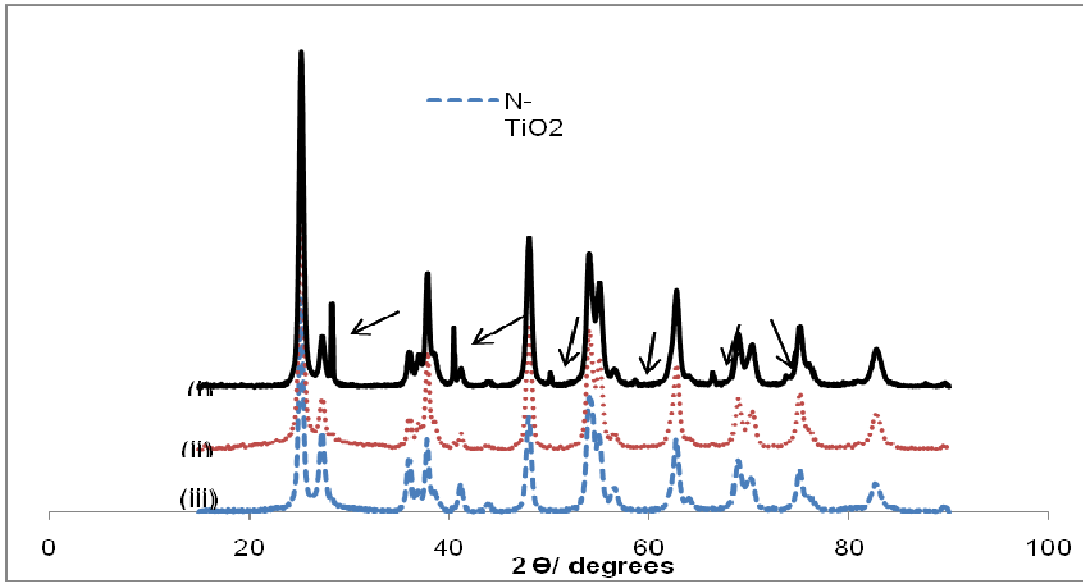


Fig. 5. XRD diffractograms of (i) TiO_2 , (ii) C-TiO_2 and (iii) N-TiO_2

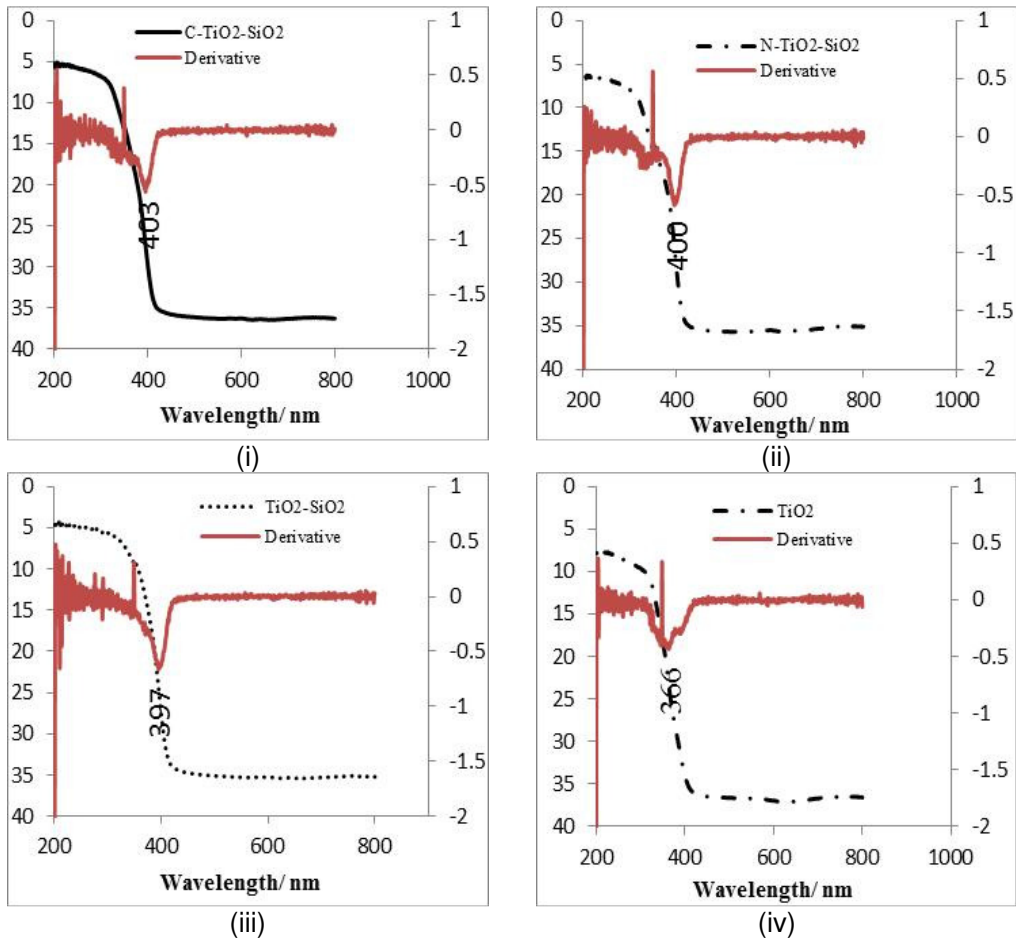


Fig. 6. DRS spectra of (i) $\text{C-TiO}_2\text{-SiO}_2$, (ii) $\text{N-TiO}_2\text{-SiO}_2$ (iii) $\text{TiO}_2\text{-SiO}_2$ and (iv) TiO_2

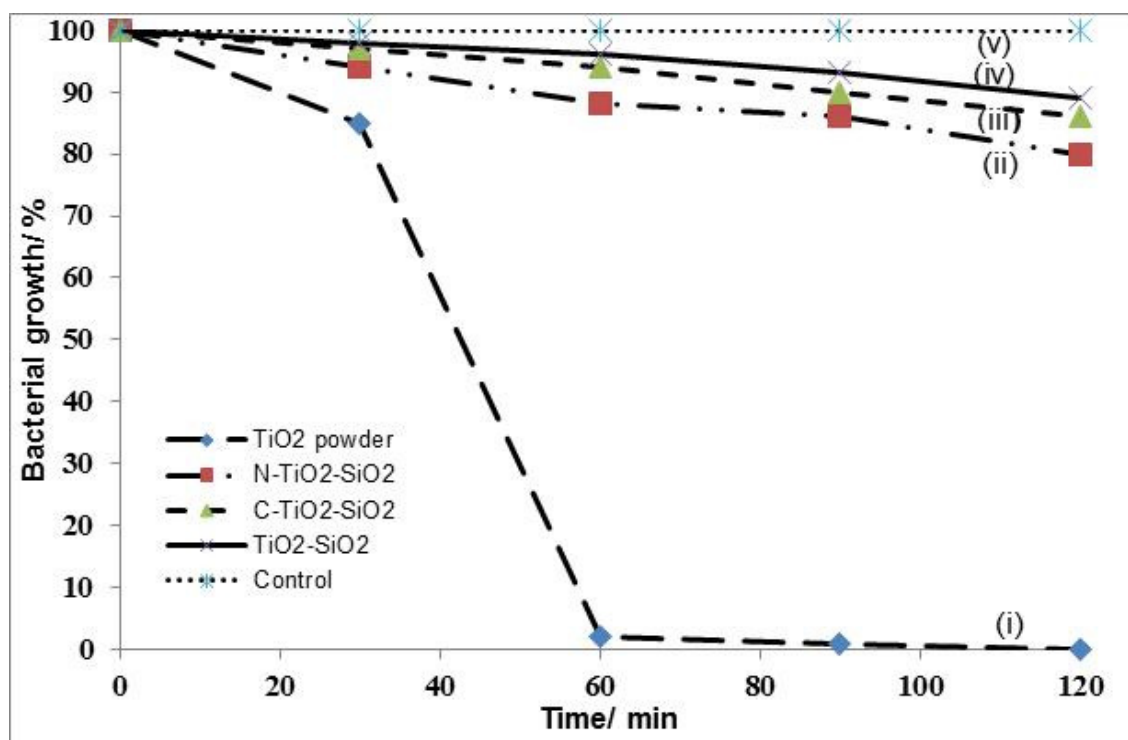


Fig. 7. Bacterial growth profiles in the presents of TiO₂, N-TiO₂-SiO₂, C-TiO₂-SiO₂, & TiO₂-SiO₂ photo-catalyst

When *E. coli* ATCC 25922 was treated with powder TiO₂ (i), there was a small decrease in the number of colonies within the first 30 minutes, but after exposure for 60 minutes about 98% of the bacterial colonies had been destroyed. This proved that TiO₂ photo-catalyst is more effective in its powder form. The bactericidal function, however, is not well understood, even though numerous reports have described photo-killing of bacteria [7,8,9,10,11]. Here the possibility of cell inactivation by the co-aggregation of cells and TiO₂ particles cannot be excluded since TiO₂ powder was used in the photo-killing reaction. Titanium dioxide particles ingested by phagocytosis are known to cause rapid intracellular damage [11,16]. The fact that immobilized titanium dioxide was less effective in the inactivation of *E. coli* than the powdered one is a clear indication that some modes of action of TiO₂ do not come into play when the photo-catalyst is immobilized. It is possible that one side of the bacteria was shielded by glass thus reducing the complete ingestion through phagocytosis. However, the effectiveness of a photo-catalyst towards microbial inactivation depends on various factors including the strains in question. Mitoraj and co-workers, 2007

observed microbial photo-inactivation using carbon doped titanium dioxide [30]. The order of decrease of inactivation observed under visible light irradiation was: *E. coli* > *S. aureus* ≈ *E. faecalis* >> *C. albicans* ≈ *A. niger*. They attributed this phenomenon to differences in cell wall or cell membrane structures among these microorganisms and not catalase production [30].

4. CONCLUSION

Carbon and nitrogen doped titanium dioxide nano-particles were successfully prepared and immobilized on glass support material. Immobilized titanium dioxide photo-catalyst was shown to be less effective in the deactivation of *E. coli* bacteria. The three nano-composite photo-catalysts; TiO₂-SiO₂, C-TiO₂-SiO₂, and N-TiO₂-SiO₂ showed little cytotoxicity (the degree to which an agent possesses a specific destructive action on certain cells) towards the growth of *E. coli* ATCC 25922 while powder titanium dioxide proved to be very effective towards the inactivation of *E. coli* ATCC 25922 bacteria. Within 60 minutes of exposure to the powder TiO₂ photo-catalyst, close to 98% of the *E. coli*

ATCC 25922 bacteria had been inactivated. Bacterial cells have got to come into contact with TiO₂ for it to show cytotoxicity towards them. The control of the experiments carried out showed uninhibited growth of the *E. coli* ATCC 25922 bacteria since no photo-catalyst was added to the vial. However, improved spectral response of C-TiO₂ and N-TiO₂ indicate they are potentially useful antimicrobial agents if immobilized on material that does not hinder the mode of action of these photo-catalytic materials even though the doping does inhibit radical generation to some extent. This is expected to be possible due to the substitution of O by C/N or the coverage by carbonate groups over the surface of the substrate. Further work will be focused on identifying the most suitable support material.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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