Synthesis and Glass Immobilization of Carbon and Nitrogen Doped TiO$_2$-SiO$_2$ 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.

ABSTRACT

Aim: To evaluate the antimicrobial properties of carbon and nitrogen doped titanium dioxide (C-TiO$_2$ and N-TiO$_2$) 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 photocatalysts 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 (TiO$_2$-SiO$_2$, C-TiO$_2$-SiO$_2$, and N-TiO$_2$-SiO$_2$) immobilized on glass supports were evaluated for their antimicrobial properties on the inactivation...
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 TiO_2 was determined by colony counting after 24 hours of incubation. When *E. coli* ATCC 25922 was treated with powder TiO_2 (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; TiO_2-SiO_2, C-TiO_2-SiO_2, and N-TiO_2-SiO_2 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-TiO_2: nitrogen doped titanium dioxide, C-TiO_2: carbon doped titanium dioxide, NaCl: Sodium chloride, BaCl_2: Barium chloride, H_2SO_4: Sulphuric acid, TEOS: tetraethyl orthosilicate, SiO_2: 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 (TiO_2) in the photo-mineralization of organic and inorganic pollutants in water is well established. The reactions are driven by UV light (λ < 385 nm), and lead to the generation of reactive oxygen species by TiO_2 photo-catalysts. The reactive oxygen species generated by the TiO_2 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 TiO_2 photo-catalytic reactions [4].

In this study the focus was on the bactericidal action of TiO_2 thin films coated on glass substrate. Some TiO_2 coated tiles with self-cleaning and bactericidal functions have already been commercialized [5,6]. The self-cleaning function of TiO_2 coated substrates is explained simply by the photo-induced oxidative power of TiO_2 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 TiO$_2$ powder, the possibility of cell de-activation by co-aggregation of cells and loose TiO$_2$ particles cannot be excluded. In addition, the TiO$_2$ particles phagocytized by the cells may cause cellular injury [16]. In fact, Jacob and co-workers reported that TiO$_2$ 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 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 Jańczyzk 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 (SiO$_2$) were prepared using tetraethyl orthosilicate (TEOS) as the precursor. TEOS served as both a source of SiO$_2$ and binder of the composites to the glass support material. TiO$_2$ 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 cm$^2$, 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 TiO$_2$-SiO$_2$, C-TiO$_2$-SiO$_2$, and N-TiO$_2$-SiO$_2$. 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 1.5×10$^8$ organisms per mL). The standard was prepared by mixing 0.05 mL of a 1% solution (w/v) of BaCl$_2$ with 9.95 mL of a 1% solution (v/v) of H$_2$SO$_4$ [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 TiO$_2$-SiO$_2$, C-TiO$_2$-SiO$_2$, N-TiO$_2$-SiO$_2$, and TiO$_2$. 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 TiO$_2$, TiO$_2$-SiO$_2$ composite and carbon doped TiO$_2$-SiO$_2$ composite. Fig. 2
shows the FT-IR spectra of the above mentioned photo-catalysts.

**Fig. 1. Schematic of immobilized TiO$_2$ photo-reactor**

**Fig. 2. FT-IR spectra of (i) C-TiO$_2$-SiO$_2$, (ii) TiO$_2$-SiO$_2$, (iii) N-TiO$_2$-SiO$_2$ and (iv) TiO$_2$**
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.

![Graph showing full scale counts and EDX spectra](image-url)
Fig. 3. EDX spectra of (i) TiO$_2$, (ii) C-TiO$_2$ and (iii) N-TiO$_2$
The SXPS spectrum of C-TiO$_2$ 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. iii. 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 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 photocatalysts prepared were found to be predominantly in the anatase phase. The phase composition is dependent 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) C-TiO$_2$-SiO$_2$, (ii) N-TiO$_2$-SiO$_2$ (iii) TiO$_2$-SiO$_2$ and (iv) TiO$_2$
When E. coli ATCC 25922 was treated with powder TiO$_2$ (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 TiO$_2$ 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 TiO$_2$ particles cannot be excluded since TiO$_2$ 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 TiO$_2$ 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; TiO$_2$-SiO$_2$, C-TiO$_2$-SiO$_2$, and N-TiO$_2$-SiO$_2$ 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 TiO$_2$ photo-catalyst, close to 98% of the E. coli
ATCC 25922 bacteria had been inactivated. Bacterial cells have got to come into contact with TiO$_2$ 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-TiO$_2$ and N-TiO$_2$ 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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