

Synthesis and Glass immobilization of carbon and nitrogen doped TiO₂-SiO₂ and its effect on *E. coli* ATCC 25922 bacteria

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), 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 of *E. coli* ATCC25922 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]

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 ($\lambda < 385 \text{ nm}$), 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 the 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].

2. MATERIAL 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 $\text{TiO}_2\text{-SiO}_2$, C- $\text{TiO}_2\text{-SiO}_2$, and N- $\text{TiO}_2\text{-SiO}_2$. 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 up 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_2SO_4 [17, 18]. 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. Figure 1 shows the reactor setup that was used. Five experiments were carried out in triplicate, using the four catalysts $\text{TiO}_2\text{-SiO}_2$, C- $\text{TiO}_2\text{-SiO}_2$, N- $\text{TiO}_2\text{-SiO}_2$, 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 plated 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.

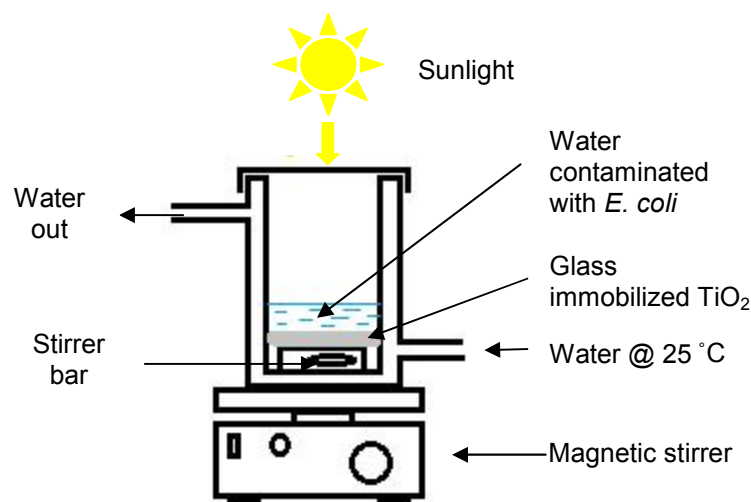


Fig. 1 Schematic of immobilized TiO₂ photo-reactor

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. Figure 2 shows the FT-IR spectra of the above mentioned photo-catalysts.

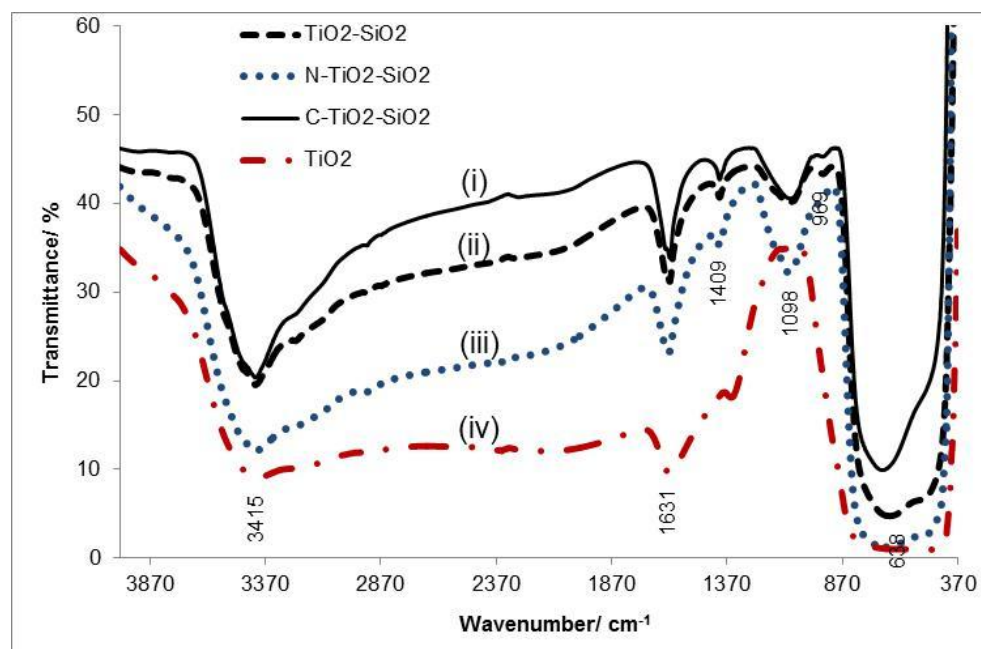


Fig. 2. FT-IR spectra of (i) C-TiO₂-SiO₂, (ii) TiO₂-SiO₂, (iii) N-TiO₂-SiO₂ and (iv) TiO₂

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 SiO₂ and crystalline TiO₂ [19, 20]. 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 [21]. FT-IR confirmed successful preparation of the photo-catalyst.

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. Figure 3 (i) and (ii) shows the EDX spectra obtained for C-TiO₂ and N-TiO₂ respectively.

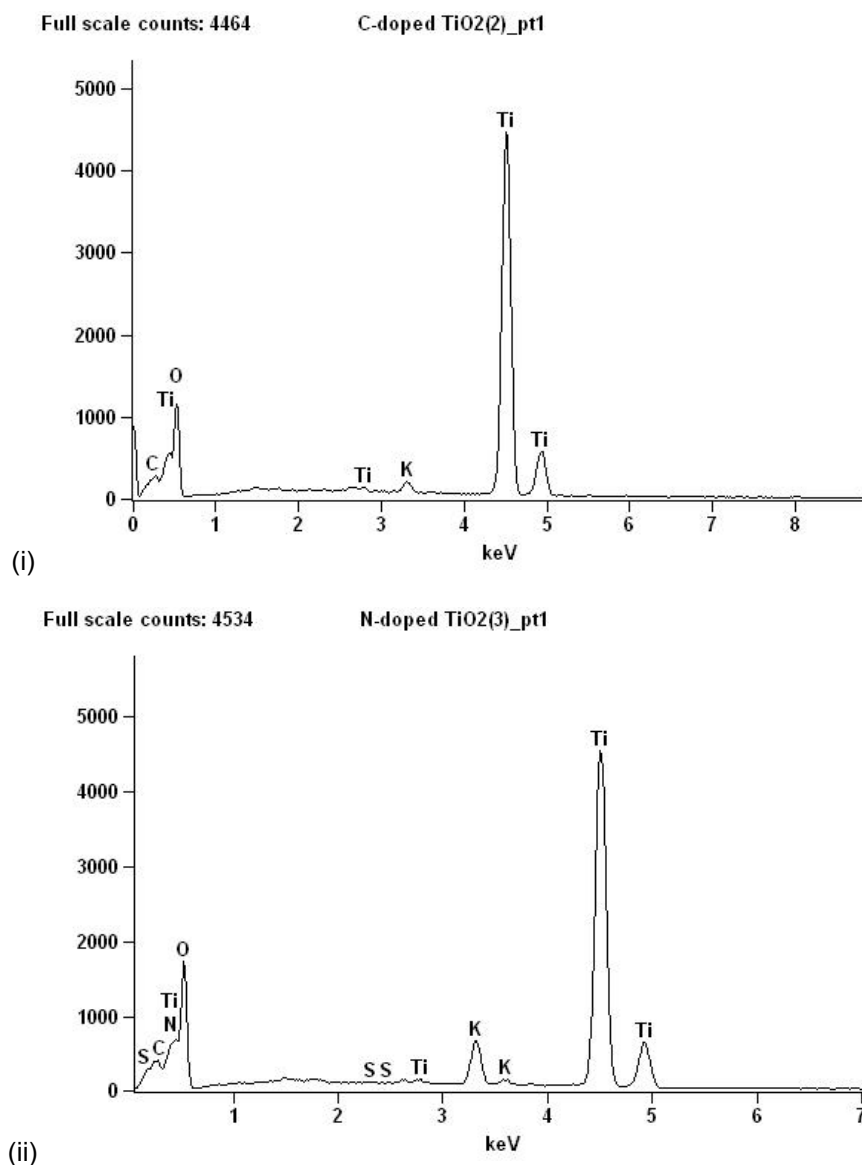


Fig. 3. EDX spectra of (i) C-TiO₂ and (ii) N-TiO₂

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.

Diffuse reflectance spectroscopy (DRS) was used to analyse the shift in absorption band edge of titanium dioxide after doping with carbon and nitrogen. Figure 4 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.

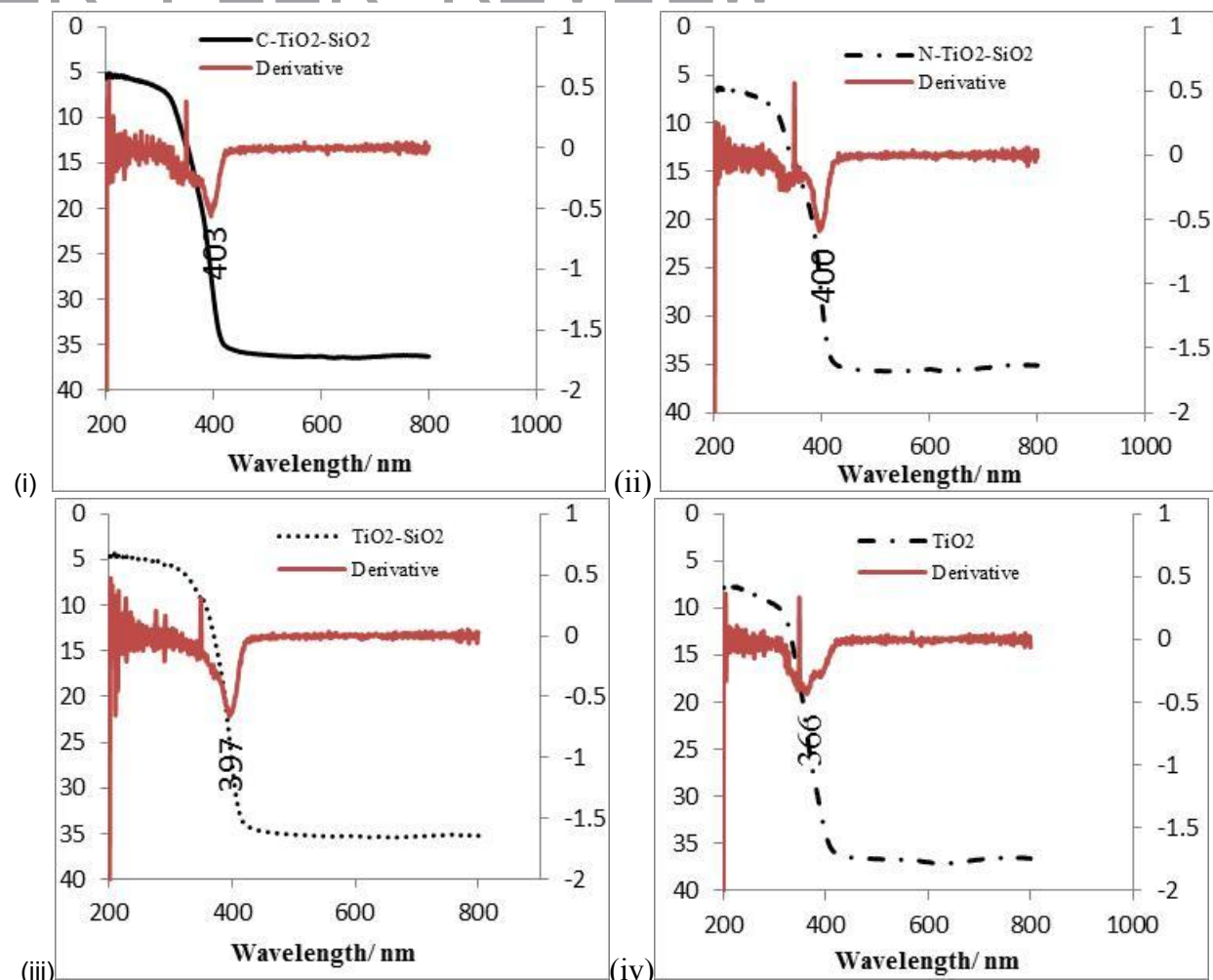


Fig. 4. DRS spectra of (i) C-TiO₂-SiO₂, (ii) N-TiO₂-SiO₂ (iii) TiO₂-SiO₂ and (iv) TiO₂

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 [22].

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 figure 3. 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.

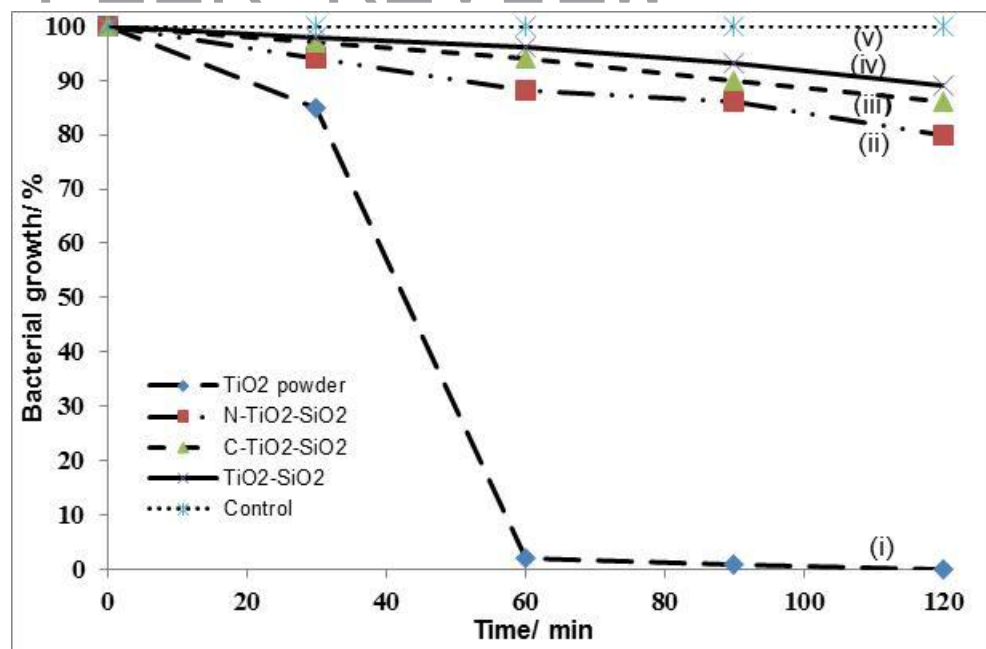


Fig. 5 Bacterial growth profiles in the presents of TiO₂, N-TiO₂-SiO₂, C-TiO₂-SiO₂, & TiO₂-SiO₂ photo-catalyst.

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.

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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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225 DEFINITIONS, ACRONYMS, ABBREVIATIONS

- 228 **N-TiO₂**: nitrogen doped titanium dioxide
- 229 **C-TiO₂**: carbon doped titanium dioxide
- 230 **NaCl**: Sodium chloride

231 **BaCl₂**: Barium chloride
232 **H₂SO₄**: Sulphuric acid
233 **TEOS**: tetraethyl orthosilicate
234 **SiO₂**: silicon dioxide
235 **FT-IR**: Fourier Transform infrared spectroscopy
236 **DRS**: Diffuse reflectance spectroscopy
237 **EDX**: Energy dispersive X-Ray spectroscopy
238 **HUS**: haemolytic uremic syndrome

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