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TiO_2 Photocatalytic Biocidal Activity on *Escherichia Coli* and On *Aspergillus Niger* under Different Methodological Conditions

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Abstract – Biological pollution is one major cause of the degradation of indoor air quality. It was shown that microbial communities from outdoor might impact significantly the communities detected indoor. In addition, microbial contamination of the surfaces of building materials and their release into the indoor air also significantly affect indoor air quality. Preventing the growth or at least reducing the amounts of microorganisms growing on indoor building materials is essential for reducing health risks for building occupiers. Photoactive TiO_2 has been widely studied as a photocatalyst that enable the inactivation of various bacterial strains. In this paper, we compare the antifungal activity of nanoparticles of TiO_2 on *Aspergillus niger* spores and its antibacterial activity on *Escherichia coli* under low light irradiation, near to common indoor values. The antimicrobial activity of TiO_2, expressed as log reduction, was assessed under UV irradiation in a sludge mixture of sterile water, suspension and nanoparticles of TiO_2. The results showed a strong bactericidal activity of TiO_2 on *E. coli* and a weak fungicidal activity against *A. niger*. Different parameters including concentration of TiO_2, intensity of light, and duration of contact between TiO_2 and microbial cells and spores, were investigated and significantly affected the antibacterial activity of TiO_2 while poorly affected its antifungal activity. Results of this study confirmed previous investigations on antibacterial activity of TiO_2 on *E. coli* and bring new insight on antifungal activity on the spores of *A. niger*. The effectiveness of the antimicrobial activity is enhanced by the duration of contact between suspension and TiO_2 nanoparticles through the stirring experiments for 2H, 4H and 24H.
Keywords **:** Bactericidal activity, Fungicidal activity, Indoor air, Aerosolization, Photocatalysis, TiO_2

1. Introduction

Indoor air pollution is an important cause of serious heallth problems for occupants including respiratory diseases, allergic symptoms, cancers and cardiovascular problems[1–9]. For several years now, there is an increased awareness to the effect of indoor air quality on human health and wellbeing[1,10]. Actually, people spend 80%-90% of their times indoors[11,12] so it's important to understand the main causes of indoor air pollution and find suitable solutions to ameliorate indoor air quality. The world health organization (WHO) reported in 2009 that biological pollution is one major cause for the degradation of indoor quality[13,14]. Indoor investigations have highlighted that microbial contamination of surfaces of building materials may have a significant impact on the microbial communities present indoors. However, quantitative evaluation of such impact is only little studied. When promoting conditions (humidity and nutrient content) are available, building materials are exposed to microbial growth[1,15]. Upon their growth on surfaces, microorganisms produce aerial particles such as spores, toxins, volatile organic compounds and other metabolites that can be inhaled by occupants[16–20]. To prevent or to reduce microbial contamination on surfaces, the antimicrobial activity of several chemical products has already been studied. They include semi-conductors products such as titanium dioxide (TiO_2), zinc oxide (ZnO), gallium arsenide, tungsten (VI) oxide (WO_3), gallium phosphide, and cadmium but also alternative products such as fatty acid and glycerol esters[21–23]. Titanium dioxide (TiO_2) is widely recognized as one of the most efficient photocatalysts used for air purification[21,22]. It has excellent optical and electronic properties, high photocatalytic activity, high chemical stability, non-toxicity, low cost, availability, and abundance[22]. Under "high" intensities of light, over >10 W/m², the photocatalysis of TiO_2 nanoparticles showed strong antimicrobial activity against a wide variety of microorganisms, including algae, viruses, bacteria and fungi [24–27]. Nevertheless, few studies have been carried out in the last five years investigating the effect of TiO_2 on various microorganisms at lower levels of intensity (≤5W/m²), closed to real-world conditions (30W/m² in sunny days and 5-10W/m² in cloudy days outdoors and 4-5 W/m² indoors)[21,28–30].

The objective of this study was to investigate the effeciency of TiO2 nanoparticles on Escherichia coli and Aspergillus niger through direct contact between microbial suspension and photocatalyst TiO2 under different experimental conditions

(concentration of TiO2 nanoparticles, light intensity and duration of contact). Experiments were carried in dark, and under light real-life irradiation using 8W black-light bulbs at 5W/m².

2. Materials And Methods

2.1 Microbial cultivation and preparation of suspensions

Most frequently detected microorganisms in the indoor environment (on surfaces of building materials and airborne) are (i) Gram negative bacteria and mycobacteria[15,31], (ii) fungal genera such as Aspergillus, Penicillium, Cladosporium and Stachybotrys[31–34]. In this study, Escherichia coli and Aspergillus niger were chosen to evaluate TiO2 nanoparticles antimicrobial activity.

E. coli CIP 53126 was obtained from the collection of the Pasteur Institute (CIP), Paris, France. Strains were stored at −80 °C in his Eugon medium (Biomérieux, Craponne, France) supplemented with 10% glycerol. Bacterial cells were pre-cultured on trypticase soy agar (TSA) before each experiment (incubation 36 °C ± 1 °C for 16-24 h). A new subculture was performed (36 °C ± 1 °C for 16-20 hours) prior to testing. For testing, bacterial cells were dispersed in 1/500 broth (NB)[21,31,35] and the bacterial cell content of the inoculum suspension was determined using a spectrophotometer (640 nm) at approximately 10^8 cells/mL. The cell suspension was then diluted 10-fold and 1 mL of each dilution was plated incorporated in TSA to determine the number of CFU/mL.

For fungal suspension, A. niger strain CBS 733.88 was cultured in a flask, on Sabouraud agar medium (SAB) (Biomérieux) at 22.5 °C for 10 to 14 days to prepare the spore suspension[36]. Ten mL of sterilized distilled water + tween 80 with sterile glass balls were inserted into the flask of strain and the flask was shaken gently for at least 2 minutes. The suspension was then collected and filtered through sterile frit 080557-2 (40-100 µm) into a sterile pot containing sterile glass beads to prevent clustering of spores. The concentration of suspension was obtained by counting spores on Malassez cell through optical microscope. One mL of suspension adjusted to 1*107 cfu/mL was inoculated into SAB Petri dishes and then incubated at 22°C for 48 h before CFU counts.

2.2 Stirring experiment: Evaluation of antimicrobial activity of TiO_2

During each experiment, 1mL was taken from each beaker at t_0=0 min and every 30 minutes for 2 or 4 hours and ten-fold diluted in sterile distilled water for CFU numerations (as for the suspensions). Petri dishes from *E. coli* experiments and *A. niger* experiments were incubated at 36°C ±1°C for 48 hours and at 30°C ±1°C for 48 hours respectively. The antimicrobial activity (log reduction) was then calculated using Equation 1.

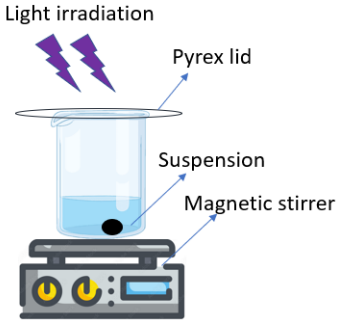

Figure 1: A schema illustrating the stirring experiment: Magnetic stirrer at 300 rpm; Beaker containing suspension: (*E. coli* or *A. niger*), TiO_2 in test tubes or distilled water in control tubes; Light irradiation of 5 W/m² in light conditions; Pyrex lid.

$$R = Log(\text{Csusp}) - Log(Ctest) = Log(\frac{Csusp}{Ctest}) \quad \text{Equation 1}$$

Where R: Log_{10} Reduction of TiO_2 (referred as antimicrobial activity in the text), C_{susp}: Average concentration of suspension in control tube without TiO_2, C_{test}: Average concentration of suspension in test tube with TiO_2 in CFU/mL.

Each experiment was repeated at least twice and results present the average obtained from each series of experiments with corresponding standard deviations (SD).

3. Results And Discussion

3.1 Effect of TiO_2 in the dark

In the dark, after two hours of contact between 10g/L TiO_2 and cell/spore suspension, the average log_{10} reduction was 0.04±0.01 for *A. niger* suspension and 2.27± 0.08 for *E. coli* (Figure 2). These results are in agreement with previous findings regarding TiO_2 efficacy[37]. The strong antibacterial activity may be explained by the ability of well-dispersed nano-particles of TiO_2 to interact with bacterial cells absorbing them to their surfaces and leading to a remarkable decrease in their concentration[38]. Regarding antifungal activity, the resistance of fungal spores to TiO_2[37,39] implies no interaction between nanoparticles of TiO_2 and fungal spores of *A. niger* and thus a negligible fungicidal activity was observed after 2 hours of contact in the dark.

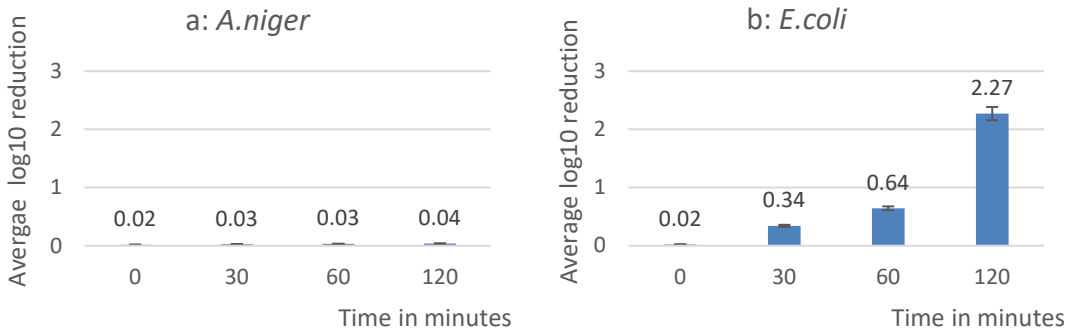

Figure 2: Average ±SD of log reduction of 10g/L TiO_2 in dark conditions on *A. niger* suspension (a) vs on *E. coli (b)*.

3.2 Effect of TiO_2 under light

Under a light intensity of 5W/m^2, both antifungal and antibacterial activity of 10g/L TiO_2 were evaluated. After 2 hours of contact between suspension and TiO_2, the average log_{10} reduction 'R' was 0.85±0.03 for *A. niger* spore suspension and 2.58±0.08 for *E. coli*. TiO_2 shows a weaker activity on *A. niger* spores compared to its activity on *E. coli* cells (Figure 3). Previous findings showed a higher antifungal activity of TiO_2 mixed with Ag nanoparticles on *Aspergillus niger* but these results were obtained using a very high light intensity of 40W/m^2[40] or by continuous UV irradiation for 20 days[39]. The ability of TiO_2 to damage the cell membrane of *E. coli* explains its high antibacterial activity[22,41]. The differences between fungal cell membranes and bacterial ones may also contribute to the difference of TiO_2 effects observed in our study[42,43].

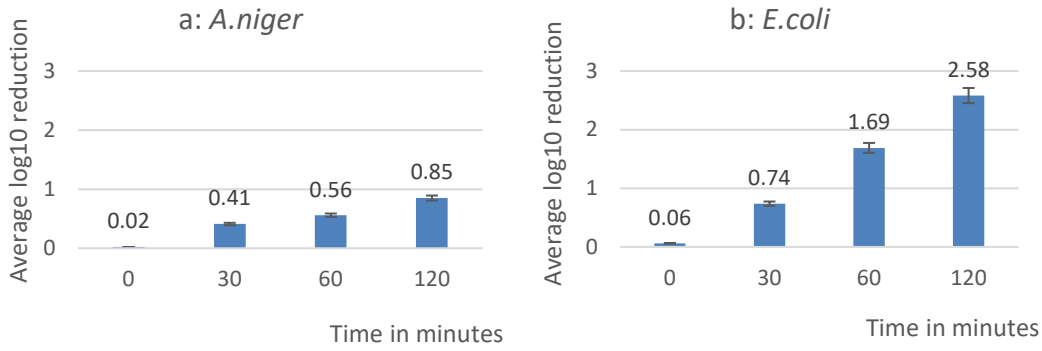

Figure 3: Average ± SD of log reduction of 10g/L TiO_2 in light conditions on *A. niger* suspension (a) vs on *E. coli (b)*.

3.3 Influence of the concentration of TiO_2 on its antibacterial activity

The antibacterial activity of TiO_2 was tested at 10g/L where it showed R > 2.00 in dark and light conditions. Another series of experiments was carried at 1g/L concentration of TiO_2 to investigate its activity on *E. coli* at a lower concentration tested previously by Verdier[21]. In the dark, after 4 hours of contact, 1g/L of TiO_2 showed a clearly weaker activity compared to that observed after 2 hours of contact to 10g/L TiO_2 with only 0.61±0.01 log reduction (Figure 4). By contrast, under light, 4 hours of contact to 1g/L TiO_2 showed approximately similar antibacterial activity as that of 10g/L TiO_2 after 2 hours of contact. The maximum log reduction was 2.49±0.07 after 4 hours of contact for 1g/L and 2.58±0.08 after 2 hours of contact for 10g/L of TiO_2 (Figure 5). As said before, high concentration of TiO_2 (10g/L) was capable to inhibit the growth and/or kill the cells of *E. coli* either by damaging their cell membranes under light[22,41], or by absorbing them onto their surfaces in the dark[38]. At low concentration (1g/L), the number of well dispersed nano-particles of TiO_2 might not be sufficient to interact directly with bacterial cells and reduce their concentrations in the dark, whereas under the light, the antibacterial activity of 1g/L of TiO_2 may be explained by its capability to damage cell membrane of *E.coli* when photocatalyzed (Figure 4). The reduced concentration of TiO_2 requires therefore a longer application time to be active. As an illustration, concentrations of TiO_2 as low as 0.1g/L have shown a strong antibacterial activity but after 24 hours of contact using a light intensity range of 14- 55 W/m²[44].

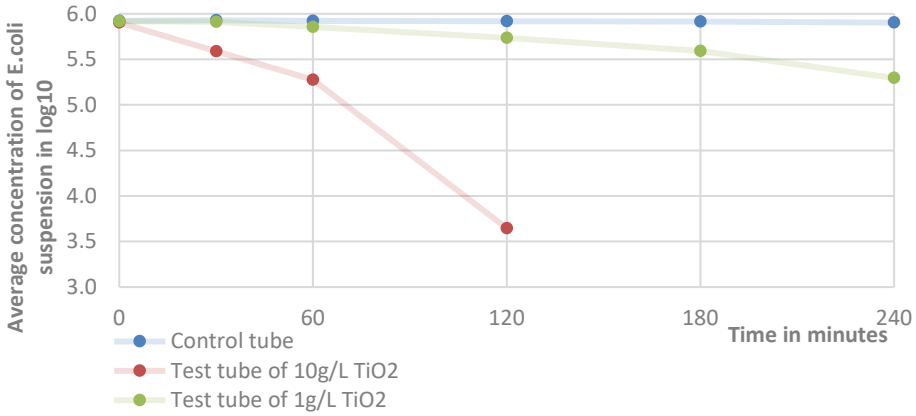

Figure 4: Variation with time of average concentration of *E. coli* suspension (log CFU) in the dark in: control tube; test tube containing 10g/L of TiO_2; test tube containing 1g/L of TiO_2.

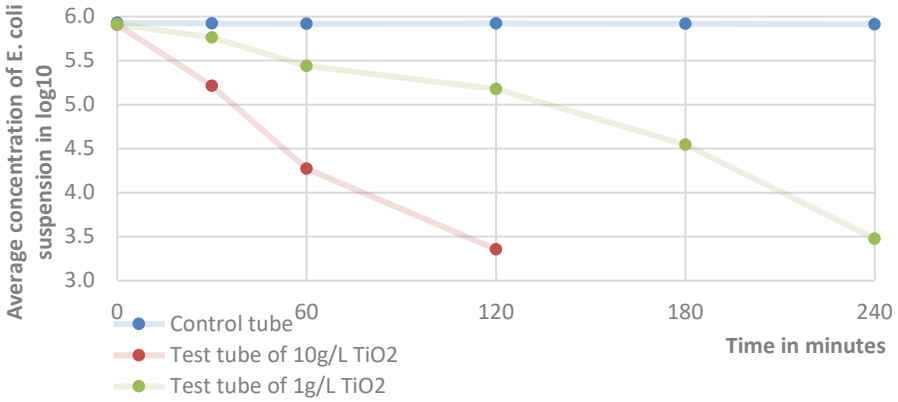

Figure 5: Variation with time of average concentration of E. coli cell suspension (log CFU) under light conditions in: control tube; test tube containing 10g/L of TiO2; test tube containing 1g/L of TiO2.

3.4 Resistance of *Aspergillus niger* to TiO_2 photocatalyst

To investigate the resistance of *A. niger*, another series of stirring experiments was carried out for 24 hours of contact between *A. niger* and TiO_2, in light conditions. After 24 hours of contact, the resistance of *A. niger* against the photocatalyst TiO_2 was confirmed as the maximum reduction reached was only 0.86±0.02 (Figure 6). No study has previously highlighted the resistance of *A. niger* to the photocatalyst TiO_2 within real-life conditions. One investigation has shown the ability of TiO_2 to inhibit the growth of *A. niger* on woods but these results were obtained at very high light intensities[39]. By contrast, our results are in agreement with the resistance of *A. niger* against TiO_2 observed after 24 hours of contact in the dark[37].

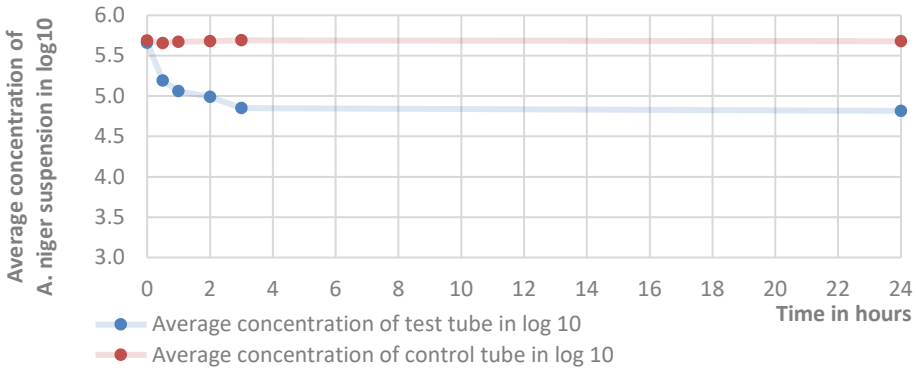

Figure 6: Variation with time of average concentration of *A. niger* spore suspension (log CFU) in control tube and in test tube during contact with TiO_2 nanoparticles for 24 hours in light conditions

4. Conclusion

The main objective of this study was to investigate the difference between the antibacterial effect from one side and antifungal effect on other side of TiO_2 nanoparticles through stirring experiments allowing a direct contact between TiO_2 and cells at low light intensity close to real-world conditions. The obtained results confirmed previous findings on the antibacterial activity of TiO_2 on *E. coli* and highlighted the variation of its efficiency as a function of its concentration, duration of contact, light/dark conditions and especially as a function of microorganisms (Spores of *A. niger* vs *E. coli* in current study*)*. TiO_2 is an efficient photocatalyst, non-toxic and a low-cost product that is employed in most air and water purification systems using high light intensities[22]. In addition, this substance is already used in coatings, paints, and in cementitious materials[31,45]. Indeed, as the antibacterial activity of TiO_2 nanoparticles is important, it is also essential to highlight the resistance of other microorganisms such as some fungal spores to this product. The inclusion of particles such as fungal spores may affect negatively the efficiency of TiO_2 on vegetative cells suggesting that the application of TiO_2 nanoparticles in paint[21] is perhaps not the best way to use it. These findings suggest the limitation of photocatalytic products interest in the destruction of microorganisms under common indoor light irradiation. On the basis of new research, their applications should be aimed toward prevention through growth inhibition.

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