Biological agent inactivation in a flowing air stream by photocatalysis

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The first decontamination of a flowing air stream polluted by bacteria, *via* room temperature non-germicidal UV photocatalysis on titania, leads to a simple and 99.1–99.8% efficient process.

Heterogeneous photocatalysis has attracted great attention as an alternative method for air and water purification processes because of the strong oxidizing power of semiconductors under UV irradiation.[1–3] The TiO_2 semiconductor has up to now been the most efficient photocatalyst used industrially, illuminated TiO_2 being able to decompose and mineralize a large variety of organic compounds either by direct oxidation *via* photo-generated holes or *via* $OH^{\bullet}$ radicals, both acting as powerful oxidizing species. Such a strong oxidizing power damages microorganisms, which also consist of organic compounds. Since the cell killing power of illuminated TiO_2 suspensions was first reported in 1985, with the photoelectrochemical oxidation of *Escherichia coli* bacteria which caused a decrease in respiratory activity and cell death,[4] reports have described photokilling of bacteria and viruses using TiO_2 in aqueous media[5–10] and the tumor cell treatment.[11]

However, up to now, the bactericidal effects of illuminated TiO_2 have only focused on water disinfection using TiO_2 aqueous suspensions, or on the killing of cell suspensions deposited on substrates coated by TiO_2 thin films. The public concern over human health has created a strong incentive, stimulating and driving research in this area, due to recent worldwide damage caused by the pathogenic *Legionella pneumophila* and viruses such as SARS. However, no work has ever been reported on the photocatalytic decontamination of bacteria-containing flowing air, which is of great interest for public health reasons and deals with a large spectrum of applications for rooms or vehicles with high infection and contamination risks, such as hospitals, public buildings, domestic households, cars and aircraft.

Up to now, the most efficient decontamination processes for bacteria-polluted gaseous media consist of filtration, thermal treatments or disinfection using chemical agents.[12] Filtration generally implies high costs, due to the micrometric size of the biological species to be trapped and the recuperative nature of the process which requires post-degradation treatments to kill bacteria. Furthermore, the short lifespan of such filtration systems remains very restrictive and thermal or chemical treatments both require compulsory isolation of the contaminated zone during disinfection treatments.

Here, we report on the decontamination of a high-rate flowing bacteria-polluted air stream, assisted by room temperature photocatalysis, taking the non-pathogenic *Escherichia coli* as model bacteria. *Escherichia coli* and *Legionella pneumophila* bacteria being of similar size (0.5 × 2–3 μm), morphology and nature (both being Gram negative bacteria), *Escherichia coli* is a representative biological agent to evidence the decontamination efficiency and thus the practical feasibility and industrial applicability of the process for pathogenic bacteria.

A specially designed photocatalytic aero-decontamination device was built. A continuous bacteria-supplying system, consisting of an aerosol generator and a bacteria cultivation medium, coupled to a UV photoreactor with specific and patented geometry (Fig. 1) were assembled.[13] This equipment simulates a continuous and reproducible flow of bacteria-contaminated air in aerosol form, as can be the case in air-conditioning or ventilation systems in confined places like buildings, hospitals or factories. The bacteria-containing aerosol-like air flow was generated by injecting pressurized air at 1.5 10^{-5} Pa into an aqueous suspension of bacteria through a Laskin nozzle. Depending on the concentration of the bacteria in the aqueous suspension and on the total air flow, one can obtain different concentrations of bacteria in the gas phase, expressed as CFU (colony forming units) per m^3 of flowing air. This set-up allows the validation of the biological photocatalytic process under a large range of experimental conditions, with total flow rates of aerosol-contaminated air ranging from 1 L min^{-1} to 10 L min^{-1}, and gaseous concentrations of biological agents between 10^{-4} cm^{-3} and 10 cm^{-3}. At present, very few experiments have been performed on bioaerosols, these techniques only being used for determining the efficiency of filtration systems.[14] The photocatalytic reactor is a Vigreux-like Pyrex tubular reactor (length 300 mm, diameter 60 mm), allowing better contact between the solid photocatalyst and flowing bacteria-contaminated air to be obtained. Non-germicidal UV illumination centered around 380 nm is provided by four standard 8 W black light tubes surrounding the photoreactor and set 3 cm apart. 0.5 g of titania-based photocatalyst was evenly coated on the inside of the tube by evaporating a catalyst-containing aqueous slurry to dryness, resulting in a uniform TiO_2 film with a thickness of about 5 μm. As far as we know, no similar experimental device has been reported for the decontamination of bacteria-transporting air flow. A specific inner geometry, for optimizing the contact surface between the airborne biological agents and the active photocatalytic coating, is essential. No significant bacteria inactivation was observed when using a simple tubular reactor with similar external geometry, due to the very low contact surface between the tubular wall and the contaminated flow when using such high flow rates. No decontamination occurred under the sole influence of either the TiO_2 coating or the UV lamps. The photocatalytic coating is exclusively activated through UV light irradiation.

The bactericidal photocatalytic efficiency of commercially available TiO_2 P25 photocatalyst from Degussa, with an anatase

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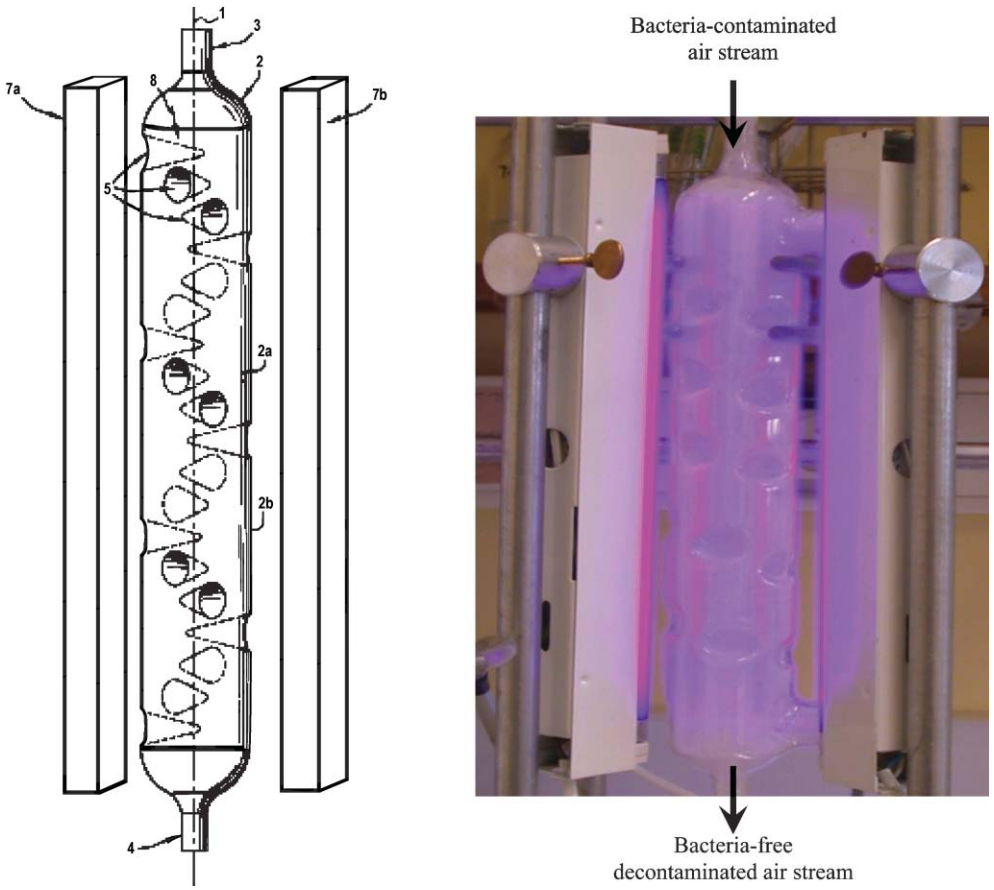

Fig. 1 *Left*: Schematic view of the patented photocatalytic reactor. 1: reactor central axis; 2a/b: lateral photocatalyst coating; 3: reactor inlet; 4: reactor outlet; 5: internal Pyrex spikes; 7a/b: UV black light tubes; 8: photocatalytic coating on internal spikes. *Right*: A working UV-illuminated photocatalytic reactor during bacteria inactivation in a flowing contaminated air stream.

: rutile ratio of about 80 : 20 and a specific surface area of about 50 $m^2\ g^{-1}$, is reported in Table 1 for different flow rates and various bacteria concentrations in the aqueous suspension, leading to various bacteria concentrations in the flowing air stream. The number of bacteria was expressed as CFU. The numeration of the number of CFU bacteria by counting the breathing bacteria before and after the photocatalytic reactor was performed using three complementary techniques depending on the bacteria concentration. Low and medium concentrations of bacteria-contaminated inlet flows (using 10^7 and 10^8 CFU mL^{-1} in the initial bacteria-containing aqueous suspension) allow the incubation of bacteria collected on a sampling membrane. Medium concentrations (10^8 CFU mL^{-1}) allow the use of the epifluorescence microscopy method. Using 5-cyano-2,3-ditolyl tetrazolium as fluorescent indicator, it distinguishes between breathing and dead bacteria. Very high concentrations of bacteria were obtained

Table 1 *Escherichia coli* inactivation in an aerosol-like flowing air stream as a function of total flow rate and bacteria concentration, with 10^8 CFU mL^{-1} (bottom) and 10^7 CFU mL^{-1} (top) in the initial bacteria-containing aqueous suspension used for the aerosol air stream generation. The bacteria were collected on the sampling membrane for 5 min. Great care was taken with the collection conditions to prevent the bacteria from withering and dying on the membranes

Aerosol flow rate		90 L h^{-1} (1.5 L min^{-1})	240 L h^{-1} (4 L min^{-1})	350 L h^{-1} (6 L min^{-1})
Bacteria concentration (CFU m^{-3} of flowing air)		15000	12000	7500
Bacteria content (CFU) collected on sampling membranes	Contaminated-inlet air stream (before reactor)	110	160	220
	Purified-outlet air stream (after reactor)	0	0	0
Bacteria removal efficiency (%)		>99.1	>99.4	>99.5
Bacteria concentration (CFU m^{-3} of flowing air)		26000	15500	13500
Bacteria content (CFU) collected on sampling membranes	Contaminated-inlet air stream (before reactor)	200	310	410
	Purified-outlet air stream (after reactor)	0	1	1
Bacteria removal efficiency (%)		>99.5	99.7	99.8

using 10^9 CFU mL^{-1} in the initial bacteria-containing aqueous suspension and in this case the aerosol was collected in flasks containing 10 mL of sterile water supplemented with 9 g L^{-1} of NaCl, before and after the reactor. The enumeration of viable bacteria (CFU) in the resulting suspension was performed by spreading 100 µl of the pure or the diluted suspension on solid Luria-Bertani (LB) medium. Emphasis must be placed on the highly efficient process leading to 99.1–99.8% of bacteria removal from the contaminated air stream flow under the whole range of experimental conditions for 10^7–10^8 CFU concentrations and for bacteria concentrations in flowing air of around 15000 CFU m^{-3} (Table 1). Using such bacteria concentrations and air flow rates, less than 1 CFU was observed in the purified outlet air stream after the photocatalytic reactor. Those air flow rates and bacteria concentrations used are well-suited to practical *on-line* applications in contaminated areas. It should be noted that 90% of bacteria abatement was achieved in 4 L min^{-1} flowing air with a very high bacteria concentration in flowing air of 50×10^6 CFU m^{-3}, obtained using 10^9 CFU mL^{-1} in the aqueous suspension.

This first decontamination of a bacteria-containing air stream by room temperature photocatalysis opens new routes for inactivating biological species in flowing air by a soft, practical, non-restrictive and easy to use technique. The scope and the extent of the application spectrum is of great interest for human health; the laboratory is currently studying the design of photocatalysts, for treating flowing air contaminated by very large volumes of pathogenic bacteria such as *Legionella pneumophila* and viruses such as SARS.

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Notes and references

1 D. Bahnemann, in *Environmental Photochemistry*, ed. P. Boule, Springer, Berlin, 1999, vol. 2, pp. 285–351.
2 P. Pichat, in *Chemical Degradation Methods for Wastes and Pollutants*, ed. M. A. Tarr, Marcel Dekker Inc., New York, 2003, pp. 77–119.
3 A. Fujishima, K. Hashimoto and T. Watanabe, *TiO_2 Photocatalysis – Fundamentals and Applications*, BKC, Inc., Tokyo, Japan, 1999.
4 Since the precursor paper in 1985 by (*a*) T. Matsunaga, R. Tomoda, T. Nakajima and H. Wake, *FEMS Microbiol. Lett.*, 1985, **29**, 211, all literature discussing biological agent inactivation deals with liquid phase decontamination; (*b*) D. M. Blake, P. C. Maness, Z. Huang, E. J. Wolfrum, J. Huang and W. A. Jacoby, *Sep. Purif. Methods*, 1999, **28**, 1.
5 K. Sunada, Y. Kikuchi, K. Hashimoto and A. Fujishima, *Environ. Sci. Technol.*, 1998, **32**, 5, 726 and related papers by A. Fujishima's group.
6 A. G. Rincon and C. Pulgarin, *Appl. Catal., B*, 2004, **49**, 99 and related papers by the authors.
7 K. P. Kühn, I. F. Chaberny, K. Massholder, M. Stickler, V. W. Benz, H. G. Sonntag and L. Erdinger, *Chemosphere*, 2003, **53**, 71.
8 K. Sunada, T. Watanabe and K. Hashimoto, *J. Photochem. Photobiol., A*, 2003, **156**, 227.
9 S. Lee, M. Nakamura and S. Ohgati, *J. Environ. Sci. Health, Part A*, 1998, **33**, 1643.
10 R. Cai, Y. Kubota, T. Shuin, H. Sakai, K. Hashimoto and A. Fujishima, *Cancer Res.*, 1992, **52**, 427.
11 R. Armon, N. Laot and N. Narkis, *J. Adv. Oxid. Technol.*, 1998, **3**, 145.
12 W. A. Jacoby, P. C. Maness, E. Wolfrum, D. M. Blake and J. A. Fennell, *Environ. Sci. Technol.*, 1998, **32**, 2650.
13 V. Keller, N. Keller, M. C. Lett, M. J. Ledoux and F. Garin, *French Patent Application*, 0413152, 2004.
14 W. D. Griffiths and D. J. Birch, *J. Aerosol Sci.*, 1994, **25**, 573.

