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A new silver based composite material for SPA water disinfection

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ABSTRACT

A new composite material based on alumina (Al_2O_3) modified by two surface nanocoatings – titanium dioxide (TiO_2) and silver (Ag) – was studied for spa water disinfection. Regarding the most common microorganisms in bathing waters, two non-pathogenic bacteria *Escherichia coli* (Gram-negative) and *Staphylococcus epidermidis* (Gram positive) were selected as surrogates for bacterial contamination. The bactericidal properties of the Al_2O_3–TiO_2–Ag material were demonstrated under various operating conditions encountered in spa water (temperature: 22–37 °C, presence of salt: $CaCO_3$ or $CaCl_2$, high oxygen content, etc.). Total removal of 10^8 CFU mL^{-1} of bacteria was obtained in less than 10 min with 16 g L^{-1} of material. Best results were observed for both conditions: a temperature of 37 °C and under aerobic condition; this latest favouring Reactive Oxygen Species (ROS) generation. The $CaCO_3$ salt had no impact on the bactericidal activity of the composite material and $CaCl_2$ considerably stabilized the silver desorption from the material surface thanks to the formation of AgCl precipitate. Preliminary tests of the Al_2O_3–TiO_2–Ag bactericidal behaviour in a continuous water flow confirmed that 2 g L^{-1} of material eliminated more than 90% of a 2.0×10^8 CFU mL^{-1} bacterial mixture after one water treatment recycle and reached the disinfection standard recommended by EPA (coliform removal = 6 log) within 22 h.

1. Introduction

Since 1968, when Roy Jacuzzi integrated a mobile hydrotherapy pump in a home bath, hot tubs or spas have transformed bathing into a genuine moment of rest and relaxation. However, the operating conditions of a hot tub (constant temperature of water at 37 °C, aeration, intense agitation during bathing, the presence of organic matter from the body, etc.) are the most favourable for the development of microorganisms (bacteria, viruses, micro-algae, etc.). To protect human health and reduce the risk of infection, all spas, without exception, for public or private use, must be equipped with a water treatment system. Although there are no legislative restrictions for private users, the water quality for public spas has to obey the Statutory Instruments of the Bathing Water Quality Regulations 2008 (Gormley, 2008).

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The majority of hot tubs (over 98%) are traditionally disinfected by adding chemicals with high oxidation potential, such as chlorine (Cl), bromine (Br), etc., into the water. It should be borne in mind that for the same efficiency, it is necessary to add 5 times more Cl or Br to spa water at 37 °C that to pool water at 21 °C. The temperature and the intense aeration of water by bubbling considerably increase the evaporation of the active chemicals. Moreover, the doses are often poorly quantified by users, so these "classic" chemicals generally have a very irregular efficiencies in hot spa waters. When misused, the added chemicals can be hazardous to health and cause very unpleasant effects (irritation of the eyes, respiratory tract, skin, etc. (Kim et al., 2002)). Attempts have been made to use modern electronic devices, such as ozonators or UV-light reactors, in order to reduce the amount of chemicals in water but ozonators are often associated with unwanted effects (exposure to odourless toxic O_3) and UV-light is systematically blocked by the air bubbles. In reality, apart from large doses of chemicals (Cl, Br or O_3) there is no real soft treatment for spa waters. An ideal system has to overcome three main conditions that traditional water treatment methods to date have failed to conquer: highly intense bacterial development, high water temperature and high oxygen content.

Silver (Ag) is an element with very specific bactericidal properties that have attracted scientists for decades. Despite their efforts, the mechanisms by which Ag destroys microorganisms are not completely understood, although several have been discussed. They include interactions between silver ions and thiol groups (-SH) of cytoplasmic proteins (Feng et al., 2000; Brook, 1989), Reactive Oxygen Species (ROS) generation (for example $OH^{\bullet}$, H_2O_2, or $O_2^{\bullet-}$) (Inoue et al., 2002; Matsumura et al., 2003) and the creation of cavities on the external membranes of microorganisms (Li et al., 2008; Sondi and Salopek-Sondi, 2004). On the other hand, silver is successfully applied today as a disinfectant in the medical (Peng et al., 2012), textile (Windler et al., 2013), water treatment (Davies and Etris, 1997) and many other fields. It has been reported that silver ions (Ag^+) are more active that the classical metal silver (Ag^0) (Feng et al., 2000) and, in recent years, the high anti-bactericidal performance of silver nanoparticles has been highlighted (Kheybari et al., 2010). However, as for all disinfectants, we believed that silver concentrations were clearly limited by safety regulations. After a thorough search of European and US legislation (Health Protection Agency, 2006; American National Standards Institute, 2009; World Health Organisation, 2006), we conclude that there is no restriction on the silver concentration in bathing waters. Because of this lack of regulations for bathing waters, the limit recommended by the United States Environmental Protection Agency (US EPA) for Drinking Waters (EPA, 2012), i.e. a silver concentration lower than 0.1 mg L^{-1}, was chosen in this study to ensure that the treated water would be safe for health.

In order to take advantage of the bactericidal capacities silver while keeping it out of the solution, research has focused on silver based composite materials in recent years. Several works (De la Rosa-Gomez et al., 2008; Matsumura et al., 2003) using silver supported on zeolites showed a total inactivation of 2×10^7 CFU mL^{-1} *Escherichia coli* with only 0.1 mg L^{-1} of Ag released in solution. Other studies indicated that the combination of silver with alumina supports allowed an inactivation of about 2×10^8 CFU mL^{-1} *E. coli* along with a desorption of silver ions leading to a concentration of about 0.9 mg L^{-1} (Chang et al., 2008). Even better, silver nanolayers strongly covalently bonded to activated carbon didn't release any traces of silver in solution (Gallion et al., 1998). However, even if this sophisticated material was intended for bactericidal treatment, no bactericidal study has been reported until now to the best of our knowledge. Moreover, silver composite materials can, in some cases, limit silver desorption while maintaining a high bactericidal activity due to the synergistic action of the supported silver and silver ions. For example, a zeolite-Ag material decreased the desorption concentration to 0.1 mg L^{-1} of silver ions while keeping a high bactericidal activity comparable to that achieved with 2 mg L^{-1} of a silver nitrate solution (Matsumura et al., 2003). In spite of their bactericidal performance and chemical stability, these silver based composite materials remain difficult to manufacture and handle (due to their powder form). Expensive and sophisticated methods (such as Cold Plasma, Plasma Assisted Ion-exchange, etc.) are often required and, once made, these particles are very difficult to control in applications such as water treatment. These drawbacks have condemned silver based products to remain at laboratory scale for now. Furthermore, among the silver supported materials cited, there are few studies on alumina supports (Chang et al., 2008; Chen et al., 2007) – the most common porous material – compared with those on zeolites, silica or activated carbon. This lack of interest in alumina-Ag materials is mainly due to the difficulty of binding silver to the alumina supports. These composite materials are often very unstable and, even if they show good bactericidal efficiencies, the concentration of Ag desorbed in solution may, in some cases, exceed the limit concentration required by the regulations, as in the case studied by Chen et al. (2007) with a silver desorption of 11 mg L^{-1} from an Al_2O_3–Ag material.

In this context, the CARDPool Company (France) developed an innovative composite material based on Al_2O_3–TiO_2–Ag as a disinfectant for spa waters. It consists of an alumina support (macro-granular form) treated by two successive nano-coatings, of titanium dioxide (TiO_2) and of silver (Ag) (Chis, 2013). The TiO_2 layer was designed to strongly bind the silver to the alumina in order to stabilize the material and control its desorption. Up to now, a few studies have been performed on Al_2O_3–TiO_2–Ag materials for other applications, related more to their high specific area or photocatalytic properties, such as sorption processes for the desulfurization of jet and diesel fuels (Hussain and Tatarchuk, 2013), reduction of nitrogen monoxide with propene found in automobile emissions (Caplan et al., 2009) and industrial waste (Li et al., 2008), separation of bacteria in groundwater and their inactivation by UV-photocatalysis for drinking water production (Ma et al., 2009), etc.

To the best of our knowledge, the present paper reports the first use of an Al_2O_3–TiO_2–Ag material for the disinfection of spa waters. The main aim of this study is to evaluate the bactericidal capacities of this new material under the influence of key operating parameters specific to spa waters: high bacteria concentration, high temperature, presence of salts and high oxygen content.

2. Materials and methods

2.1. Material synthesis

The Al_2O_3–TiO_2–Ag material samples were synthesized using activated alumina supports (form: extruded trilobite 10 ± 1 mm long and 1.5 ± 0.2 mm diameter, specific area: 250 $m^2.g^{-1}$), modified by two nanocoatings, one of titanium dioxide (TiO_2) and one of silver (Ag), followed by calcination (Chis, 2013). The first TiO_2 nanolayer was deposed by a specific Chemical Vapour Deposition Molecular Layered (CVD-ML) method using titanium chloride ($TiCl_4$) as precursor. The second Ag nanoaggregates layer was then deposed on the surface of the Al_2O_3–TiO_2 support by the Dry Impregnation method using silver nitrate salt ($AgNO_3$) as precursor. The Al_2O_3–TiO_2–Ag material samples contained approximately 3 wt % TiO_2 and 7 wt % silver. These concentrations were obtained after leaching of the samples with a solution of sulphuric acid (50%), ten times dilution and filtration (Millipore 0.2 μm cellulose acetate). Then the samples were measured by Inductively Coupled Plasma – Mass Spectroscopy (ICP-MS) using a Thermo Scientific X Series II analysed by Inductively Coupled Plasma – Mass Spectrometry (ICP-MS). In order to characterize the contribution of each nanolayer to the disinfection performance of the material, Al_2O_3–TiO_2 and Al_2O_3–Ag samples were prepared under the same conditions as the standard Al_2O_3–TiO_2–Ag product.

Concerning the Al_2O_3–Ag, it has to be noted that the Ag attached to the alumina surface and after calcination was very breakable and simple handling led to the detachment of tiny foils of metallic silver. This Al_2O_3–Ag material contained approximately 0.3–0.6 wt % Ag.

2.2. Material surface characterization

The surface morphology and chemical composition of the samples were studied using Scanning Electron Microscopy (Hitachi S4800 SEM), coupled with an Energy-Dispersive X-Ray probe (EDX at 15 kV). Each EDX spectrum shown is representative of three analyses. Before analysis, the samples were incubated for 2 h at 80 °C to remove residual moisture, then metallized with platinum.

The silver layer characterization was completed with XPS analysis obtained using a X-Ray Photoelectron Spectrometer model Esca Kratos Axis Ultra. Samples were irradiated with an Al Kα source ($h\gamma = 1486.6$ eV) and spectra were collected with a pass energy of 40 eV. This technique analyses a maximum depth of the order of 5–10 nm. Spectra calibration was achieved from the contamination Carbon spectra at 284.3 eV. This characterization was reproduced twice.

The porosity of the tested samples was measured by Autopore IV 9500 (Micromeritics) automatic mercury intrusion porosimeter, which can measure mesopores and macropores of over 5 nm.

2.3. Cultivation and counting of bacteria

2.3.1. Strains and culture media

Non-pathogenic *E. coli* (K12 DSM 423, from DSMZ, Germany) and *Staphylococcus epidermidis* (CIP53.124, from Pasteur Institute Laboratory, Lyon, France) were chosen as surrogate microorganisms for bacterial contamination because of their difference in parietal composition linked to their Gram difference (Helbling and VanBriesen, 2007; Shang et al., 2011). Different media were used for the cultivation and counting of bacteria. Lysogeny broth (LB) Miller culture medium (ref. n°1214662, from Fischer Scientific, France) was principally used for broth bacterial growth. Two other specific solid culture media were used for counting the bacteria: Tergitol/TTC medium (BK123HA, from Biokar, France) for *E. coli* and Chapman medium (BK030HA, from Biokar, France) for *S. epidermidis*.

2.3.2. Preparation of bacterial suspensions

For each experiment, a new bacterial suspension was prepared from frozen aliquots of *E. coli* and *S. epidermidis* stored at −20 °C. The aliquots were first rehydrated separately in LB medium for 3 h at 37 °C under constant stirring (110 rpm). Then, the rehydrated aliquots were inoculated individually into fresh LB medium (10% v/v for *S. epidermidis* and 5% v/v for *E. coli*) and incubated at 37 °C under constant stirring (110 rpm) until the bacteria reached the stationary growth phase. After this, the cultivated bacteria were collected by centrifugation (10 min at 12 °C and 1500 g). The recovered pellets were washed by centrifugation (10 min at 12 °C and 1500 g) with a phosphate buffer (13 mM, pH = 7.0 ± 0.1) to remove nutrients from the LB medium and thus avoid bacterial development in the reactor. After centrifugation, the washed bacterial pellets recovered were suspended separately in spring water ($[Ca^{2+}] = 39$ mg L^{-1}, $[Mg^{2+}] = 25$ mg L^{-1}, $[Na^{+}] = 19$ mg L^{-1}, $[K^{+}] = 1.5$ mg L^{-1}, $[F^{-}] < 0.3$ mg L^{-1}, $[HCO_3^{-}] = 290$ mg L^{-1}, $[SO_4^{2-}] = 5$ mg L^{-1}, $[Cl^{-}] = 4$ mg L^{-1}, $[NO_3^{-}] < 2$ mg L^{-1}) and the absorbance of the suspensions was measured at 600 nm to determine the bacterial concentration according to calibration curves obtained previously. The bacterial cells were finally diluted in spring water to obtain a bacterial suspension where the cell concentration was $1.0 \pm 0.2 \times 10^9$ CFU mL^{-1} for each strain.

2.3.3. Counting of cultivable cells and expression of bacterial removal

The bacteria were counted by the conventional Plaque Assay method. Each sample was immediately diluted by decades in 0.9% saline solution to neutralize the effect of any Ag that may have been desorbed. Each dilution was spread onto specific nutrient agar to allow the bacterial colonies to be counted. Negative controls were run in parallel for each experiment. All plates were incubated at 37 °C (24 h for the Tergitol/TTC medium for *E. coli* and 48 h for the Chapman medium for *S. epidermidis*). Once the cultivable bacteria had grown on plates, the colonies were counted, knowing that each colony stemmed from one initial bacterium. All experiments were performed twice and the concentrations of bacteria in the sample were calculated as the average of the number of colonies divided by the volumes inoculated on the specific agar, with the corresponding dilution factor taken into account. The quantification limit was 25 cfu mL^{-1}.

The counting results allowed the concentrations of cultivable bacteria in the test suspensions to be monitored and their evolution was correlated to the bactericidal performances of the material. The concentration decrease was

expressed in log-removal values, as given in the US EPA and European regulations. The log-removal was defined as the logarithm (base 10) ratio of the bacterial concentration C ($CFU.mL^{-1}$) measured at a given time relative to the initial bacterial concentration C_0 ($CFU.mL^{-1}$). A log-removal value of -log (C_0) was attributed to the particular case of total removal of the cultivable bacteria. For instance, when the initial concentration C_0 was fixed at 10^8 CFU mL^{-1}, a log-removal value of −8 thus corresponded to total removal.

2.4. Assessment of bactericidal characteristics of the Al_2O_3–TiO_2–Ag sample

2.4.1. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

2.4.1.1. Measurement of MIC

The MIC is usually defined as the lowest concentration of an antimicrobial agent that inhibits the growth of microorganisms in the matrix. In this study, the MIC was determined by the Broth Dilution technique (Andrews, 2001) and specified as the concentration of Al_2O_3–TiO_2–Ag inducing an inhibition of bacterial growth in a nutritive LB medium within 24 h at 37 °C. The presence of both disinfectant and nutrients led to competition between disinfection and growth. In this work, 1 log-removal of bacteria was chosen to characterize inhibition.

The MIC was measured for isolated strains and for the mixture of bacteria, with mean initial *E. coli* and *S. epidermidis* concentrations of $7.5 \pm 0.2 \times 10^7$ CFU mL^{-1}. Different material concentrations were tested: 2, 5, 7 and 10 g L^{-1}. *E. coli* and *S. epidermidis* suspensions were prepared in LB medium as described in Section 2.3.2 and these suspensions were inoculated into each flask, corresponding to a given concentration of material. All flasks were incubated at 37 °C for 24 h under constant stirring (80 rpm). Then samples were taken from the flasks and the bacteria were counted as described in Section 2.3.3, except that dilutions were performed in LB medium instead of spring water.

2.4.1.2. Measurement of MBC

The MBC is usually described as the lowest concentration of an antimicrobial agent that reduces the initial bacterial inocula by ≥ 99.9%. In this study, the MBC was defined as the lowest concentration of Al_2O_3–TiO_2–Ag material inducing total removal of the *E. coli* and *S. epidermidis* mixture in spring water (initial bacterial concentration of $1.0 \pm 0.3 \times 10^8$ CFU mL^{-1}) within 1 h at 22 °C. Different concentrations of material were tested: 1.5, 3, 5, 8, 10 and 13 g L^{-1}. The bacterial suspension was prepared as described in Section 2.3.2 and then seeded in each flask to correspond to a given concentration of material. All flasks were incubated at 37 °C for 1 h under constant stirring (80 rpm). Then, samples were taken from the flasks for bacteria counting as described in Section 2.3.3.

2.4.2. Determination of contribution of each Al_2O_3–TiO_2–Ag nanolayer to the bactericidal effect

In order to evaluate the bactericidal influence of each active layer, different samples corresponding to the material at various phases of its elaboration (Al_2O_3, Al_2O_3–TiO_2 up to its final form Al_2O_3–TiO_2–Ag compared with Al_2O_3–Ag without TiO_2 layer) were tested. Sixteen g L^{-1} of each ceramic sample were emerged in a 1 L batch reactor (breathable closed) containing 350 mL of seeded spring water. Both *E. coli* and *S. epidermidis* suspensions were inoculated at a final concentration of $1 \pm 0.2 \times 10^8$ CFU mL^{-1} and the reactor was incubated at 22 ± 0.1 °C under constant stirring (80 rpm) on a rotary shaker. Water samples were taken at defined times during 1 h in order to follow the kinetics of bacteria removal. To determine the initial bacterial concentration, sampling was always performed before the material was introduced. These experiments were carried out in sterile conditions and without addition of nutriments. The reactor was protected from light in order to reproduce the conditions of spa water tanks, given that a normal spa is kept covered more than 95% of the time. The solution pH was measured during the experiments and appeared to be constant at 7.8 ± 0.1. The control tests (bacteria without material, in the same operating conditions) were achieved for each kinetics run. Simultaneously, at the end of each bactericidal kinetics (after an hour), a quantitative analysis of the silver desorbed from the material was performed. For these analyses, each liquid sample was diluted ten times in spring water and acidified with 2.5% HNO_3 to completely solubilize the silver. The samples were sometimes filtered (on Millipore 0.2 μm cellulose acetate filters) to distinguish soluble from total silver. Then, the samples were analysed by Atomic Absorption Spectrometry (Perkin Elmer). These results were expressed in mg L^{-1} and/or % wt corresponding to the desorbed silver mass divided by the total deposited silver mass, multiplied by 100.

2.4.3. Highlighting the bactericidal action of the Al_2O_3–TiO_2–Ag surface

To underline the bactericidal surface action of the silver nanocomposite material, two agar contact tests were run: a Direct Biofilm Contact test and a Stamping test. The Direct Biofilm Contact test was performed by separately spotting *E. coli* and *S. epidermidis* suspensions at 10^8 CFU mL^{-1} onto the nutrient LB agar plates. Four material granules (previously dried at 40 °C for 2 h) were put on the plates, which were then incubated at 37 °C for 24 h. Negative controls (plates without materials) were also performed.

For the Stamping test, a small volume (10 μL) of a suspension of *E. coli* or *S. epidermidis*, at a high concentration (10^8 CFU mL^{-1}), was directly inoculated on the material surface, previously deposited on an adhesive tape. The inoculated material was put in contact with an LB nutrient agar and was removed after 1 h of incubation at 37 °C. The plates were then incubated at 37 °C for 24 h. For both tested bacteria, control samples were also achieved with non-modified Al_2O_3 granules.

Finally, *E. coli* and *S. epidermidis* colonies were observed on each plate to assess the bactericidal effect of the material surface.

2.5. Study of bactericidal performance of the Al_2O_3–TiO_2–Ag material under the key operating parameters of a SPA

2.5.1. Influence of temperature, salts and oxygen

The effects of different operating parameters on the bactericidal performance of the material were assessed in a batch

reactor as described in Section 2.4.2. Spa water is always maintained close to body temperature, so the influence on the disinfection efficiency of the material was also studied at 37 °C.

The influence of common water salts on the bactericidal activity of the material was also evaluated. Because some regions, such as the South of France, have hard water with high limestone concentrations and varying chlorine concentrations, two representative salts ($CaCO_3$ and $CaCl_2$ at 100 ppm) were added independently in the spring water and then incubated for 3 days with the material under permanent agitation. Because a material concentration of 16 g L^{-1} did not show any differences in kinetics between the two temperatures tested (22 and 37 °C), a lower concentration (8 g.L^{-1}) was used to highlight the effect of temperature on the bactericidal activity.

Finally, to evaluate the action of oxygen on the material bactericidal activity, some kinetics were compared in aerobic conditions (air bubbling) and in anaerobic conditions (nitrogen bubbling). Measurements of dissolved oxygen were carried out before and after each kinetics to check the anaerobic conditions (WTW OXI340I oximeter). Controls were carried out to check that the gas bubbling did not affect cell viability, and confirmed that *E. coli* and *S. epidermidis* are usually aero-anaerobic facultative bacteria (Brook, 1989). No difference was observed in aerobic or anaerobic conditions for a material concentration of 16 g L^{-1}. As for the temperature, these experiments were

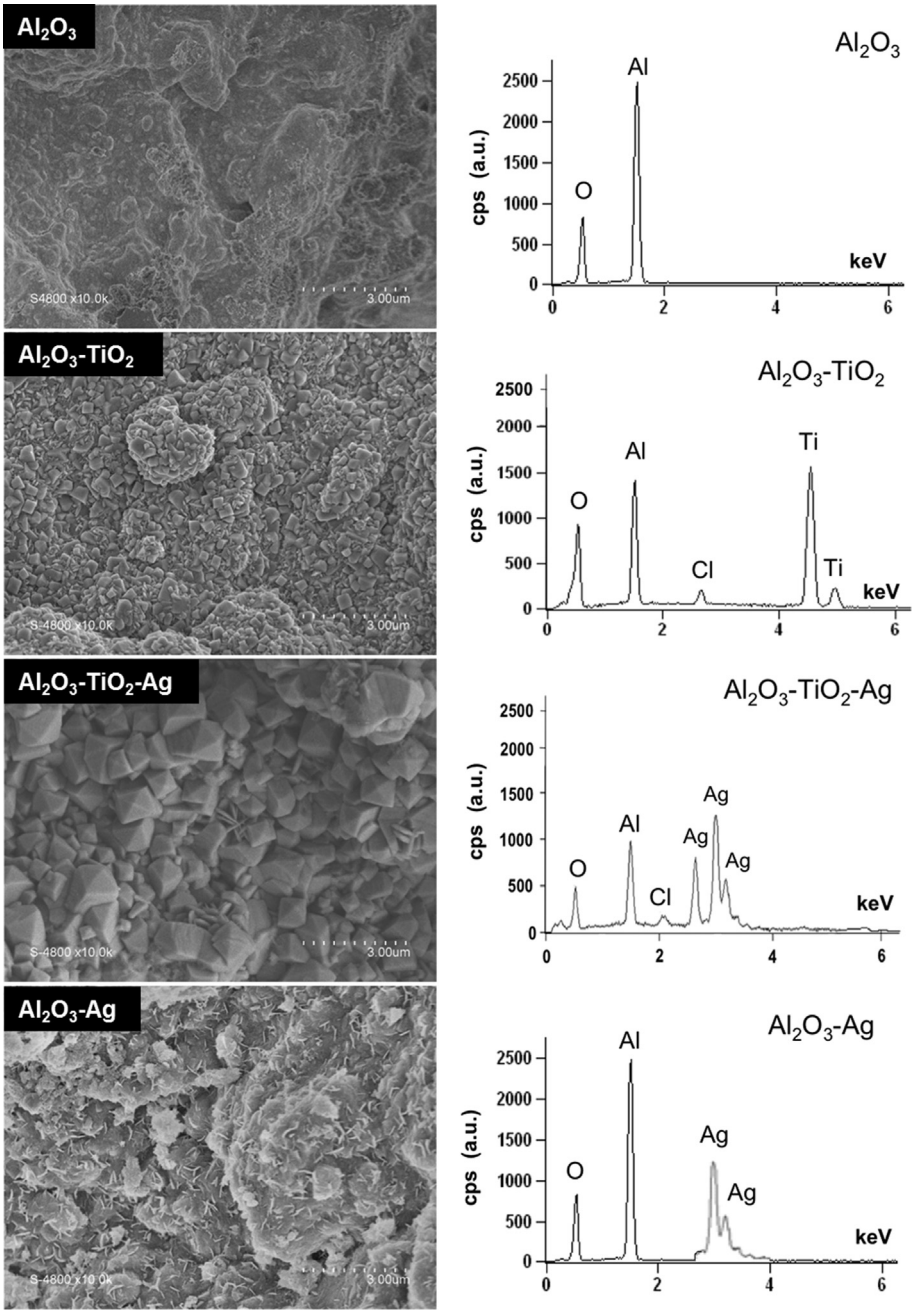

Fig. 1 – SEM micrographs (left) and EDX spectra (right) of alumina based samples.

achieved in the same conditions (aerobic and anaerobic) with a lower material concentration of 8 g L^{-1} to observe the influence of oxygenation on the bactericidal activity.

2.5.2. *Determining the bactericidal behaviour of Al_2O_3–TiO_2–Ag in a continuous water flow reactor: preliminary tests*

Dynamic experiments were performed in a closed circuit using a glass column (2.5 cm in diameter and 10 cm in length) filled with 4 g of Al_2O_3–TiO_2–Ag and a tank (2 L) containing the bacteria mixture. Bacterial suspensions were inoculated into the reservoir containing spring water to a final concentration of $1 \pm 0.2 \times 10^8$ CFU mL^{-1} and incubated at 22 °C under constant stirring (80 rpm). Water was pumped through the column at 0.03 L min^{-1} using a peristaltic pump (Masterflex I/P) until the mixture of bacteria had been totally eliminated. Water samples were taken from the reactor and spotted onto agar plates, following the same method as described in Section 2.3.3, in order to establish the kinetics of bacterial decline. The pH was measured and appeared to be constant at 7.8 ± 0.1. The experiment was carried out in the dark. A control on alumina without silver in the same hydrodynamics conditions was achieved and showed that the bacteria circulation onto the alumina bed did not have any effect on the bacteria.

3. Results and discussion

3.1. *Physicochemical characteristics of the Al_2O_3–TiO_2–Ag samples*

3.1.1. *Chemical composition and morphology*

Fig. 1 presents both the surface morphology (SEM micrographs) and the surface elementary composition (EDX spectra) of the material used in this study at the different phases of its elaboration. As expected, the EDX spectra of the different samples show the presence of titanium on Al_2O_3–TiO_2 and Al_2O_3–TiO_2–Ag samples, and silver on Al_2O_3–TiO_2–Ag and Al_2O_3–Ag. At the same time, the presence of some Chloride (Cl) traces can be observed for samples coated with titania (Al_2O_3–TiO_2 and Al_2O_3–TiO_2–Ag), as a result of the $TiCl_4$ precursor used for this coating.

The SEM micrographs highlight the morphological changes of Al_2O_3, Al_2O_3–TiO_2, Al_2O_3–TiO_2–Ag and Al_2O_3–TiO_2–Ag. The alumina support presents an amorphous surface characteristic of porous ceramics, while the Al_2O_3–TiO_2 surface is completely covered with very regular, well-shaped TiO_2 nanocrystals of roughly 200 nm. The Al_2O_3–Ag has some fine stick shaped silver crystals that are poorly bonded at the alumina surface. Confirming the EDX results, the Al_3O_3 surface is practically silver free. In contrast, the surface of Al_2O_3–TiO_2–Ag is full of pyramid shaped Ag crystals (size: 500–800 nm) that completely cover the TiO_2 nanoaggregates. It can be concluded that, besides the Al_2O_3–Ag, each coating leads to a homogeneous layer that profoundly changes the surface morphology of samples.

In order to complete the characterization of the silver layer, XPS analyses were performed on two samples and showed an Ag/Cl atomic ratio of 0.94 ± 0.09 (nearby to 1). The Ag 3d spectra given in Fig. 2 exhibited one contribution of Ag $3d_{5/2}$ at 367.5 eV of Ag^+ probably, which evidenced an active AgCl surface.

3.1.2. *Porosity and average pore diameter*

As can be seen in Table 1, the material porosity was constant (61 ± 1%) as well as the average pore diameter (0.01 μm). These results revealed thus that successive coatings of titanium dioxide and silver had no influence on the material porosity.

3.2. *Bactericidal characteristics of the Al_2O_3–TiO_2–Ag nanocomposite material*

3.2.1. *Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)*

Fig. 3 shows the growth inhibition effect of several material concentrations on *Escherichia coli* and/or *Staphylococcus*

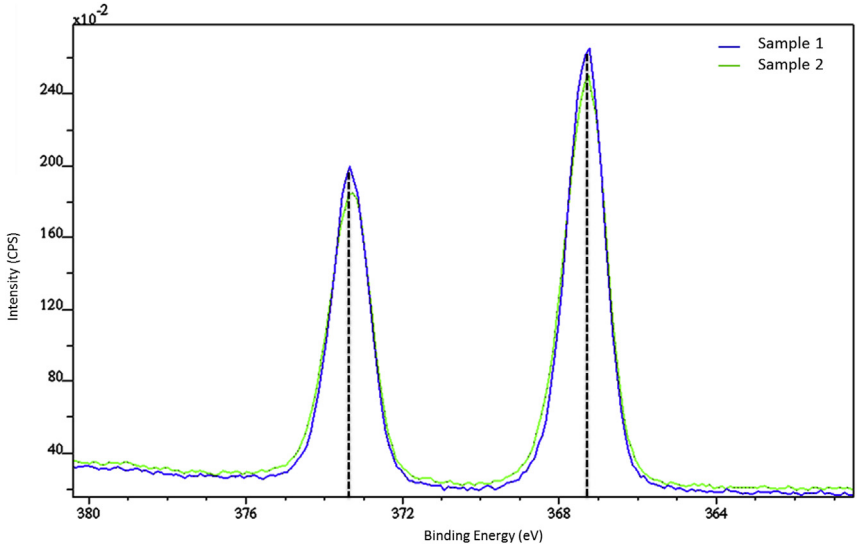

Fig. 2 – XPS spectrum Ag 3d of the material.

Table 1 – Porosity and average diameter of the alumina based samples.

Sample	Porosity (%)	$d_{average}$ (μm)
Al_2O_3	62 ± 1	0.01
Al_2O_3–TiO_2	60 ± 1	0.01
Al_2O_3–TiO_2–Ag	60 ± 1	0.01
Al_2O_3–Ag	60 ± 1	0.01

epidermidis. In the absence of an Al_2O_3–TiO_2–Ag sample, the bacterial concentration increases naturally to almost 10^{10} CFU mL^{-1} (Log (C/C_0) = 1.9, Fig. 3) for both species due to the nutrients provided by the culture medium. This bacterial growth was stopped as soon as the material was introduced at 2 g L^{-1} and, from 5 g L^{-1}, the bacterial concentration started to decline. Results showed that the MIC value for both isolated and mixed bacteria ranged between 5 and 7 g L^{-1}.

Moreover, the curves show that there is no significant difference between the results obtained for isolated bacteria (Fig. 3, a) or the mixture of bacteria (Fig. 3, b). In other words, the Al_2O_3–TiO_2–Ag product has the same bacteriostatic effect for both.

The MBC was evaluated only for the bacteria mixture (*Escherichia coli* and *Staphylococcus epidermidis*). Among the material concentrations tested (1.5, 3, 5, 8, 10 and 13 g L^{-1}), a total bacteria removal was induced within 1 h from 8 g L^{-1}. The Al_2O_3–TiO_2–Ag MBC value is thus comprised between 5 and 8 g L^{-1}.

The small difference between the MIC and the MBC highlights the powerful bactericidal capacity of Al_2O_3–TiO_2–Ag: only 5 g L^{-1} of material is sufficient to inhibit the growth of 10^8 UFC.mL^{-1} and 8 g L^{-1} is enough for total removal of this high concentration of bacteria.

3.2.2. *Contribution of each Al_2O_3–TiO_2–Ag nanolayer to the bactericidal effect*

Fig. 4 presents the bacteria removal kinetics for a mixture of *E. coli* (Fig. 4a) and *S. epidermidis* (Fig. 4b) put in contact with the different samples of material corresponding to Al_2O_3–TiO_2–Ag at various phases of its elaboration, and also with the material without a TiO_2 layer (Al_2O_3–Ag).

The results show that Al_2O_3 and Al_2O_3–TiO_2 have no bactericidal activity. Given that the porosity constancy (Table 1), there is not even a sorption phenomenon on pure Al_2O_3 or Al_2O_3–TiO_2 because the concentration of bacteria remains constant at its initial level in the presence of both ceramic supports. Moreover, in spite of the well-known photocatalytic properties and bactericidal activity of titanium dioxide at the nanometre scale, the Al_2O_3–TiO_2 sample shows no effect in dark conditions.

In contrast, the Al_2O_3–TiO_2–Ag material led to total removal of both bacteria in about 10 min and no re-growth of the bacteria was observed after cultivating the supernatant collected at the end of the experiment. Furthermore, the Al_2O_3–Ag sample showed the highest bactericidal efficiencies, achieving total removal of the bacteria in less than 3 min (log (C/C_0) = −8). The results clearly show that the bactericidal effects are induced only by silver but they have to be related to the chemical stability of the nanolayers (Table 2).

Moreover, at the end of the experiment involving the Al_2O_3–Ag material, the total and soluble silver concentrations in solution were 26.0 and 5.1 ppm respectively (corresponding to 36.1 and 7.1% wt of the deposited Ag, Table 2). These concentrations were much higher than those measured at the end of the experiment conducted with the Al_2O_3–TiO_2–Ag. These results demonstrate that the TiO_2 nanolayer (being strongly bonded to the alumina surface) helps to stabilize the release of silver from the ceramic support and the nanocomposite material contains more than 98.8% of the silver deposited at its surface (Table 2). It is thus likely that a combined action of supported and desorbed silver occurred. The contribution of the Al_2O_3–TiO_2–Ag surface was notably evidenced qualitatively by agar contact tests that are presented in the next section.

3.2.3. *Al_2O_3–TiO_2–Ag bactericidal surface action*

The results of the Direct Biofilm Contact test are presented in Fig. 5. The solid-gel contact between the Al_2O_3–TiO_2–Ag samples and the nutrient agar seeded with bacteria (*Escherichia coli* and *Staphylococcus epidermidis* on Fig. 5b and c respectively) considerably limited silver desorption comparatively to the experiments in a batch reactor.

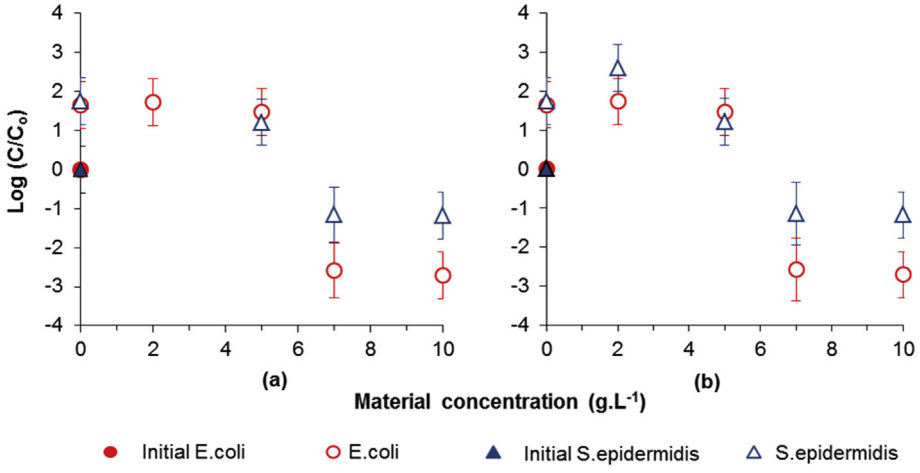

Fig. 3 – Evolution of bacterial concentration in LB broth according to Al_2O_3–TiO_2–Ag concentration for isolated bacteria (a) and bacteria mixture (b) (C_0 *E. coli* = 1.0 ± 0.3 × 10^7 CFU mL^{-1}; C_0 *S. epidermidis* = 1.0 ± 0.3 × 10^8 CFU mL^{-1}).

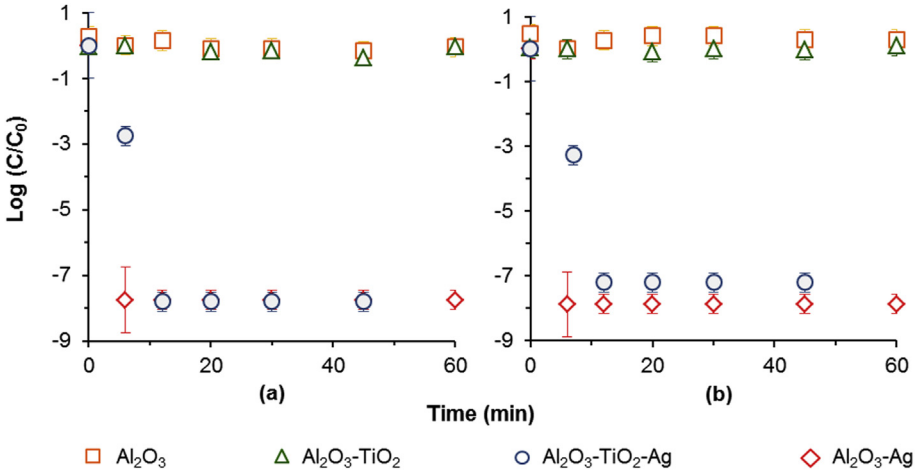

Fig. 4 – Kinetics of *E. coli* (a) and S. epidermidis (b) removal in the bacteria mixture by the material (16 g L^{-1}) at various phases of its elaboration, compared with Al_2O_3–Ag (C_0 *E. coli* and C_0 S. epidermidis = 1 ± 0.2 × 10^8 CFU mL^{-1}).

As can be seen in the figure, an inhibition zone for both *Escherichia coli* and *Staphylococcus epidermidis* appeared very close to the Al_2O_3–TiO_2–Ag granules (b and c, Fig. 5) while the biofilm formed at the LB surface without the bactericidal ceramics was continuous (a, Fig. 5). In view of the width of the inhibition zone, the bactericidal activity must be related more to the Al_2O_3–TiO_2–Ag surface than to silver desorption on the nutrient gel. This result is qualitative evidence of the bactericidal capacities of the nanocomposite material surface, which allowed no bacterial growth.

Moreover, for the Stamping test, when the bacteria were directly seeded over the Al_2O_3–TiO_2–Ag surface and the granules were then stamped onto the nutrient agar for 1 h only, the action of the desorbed silver became limited. The results of the Stamping test show a total absence of bacterial colonies on the plates stamped with seeded Al_2O_3–TiO_2–Ag granules (b and d, Fig. 6) compared with the results obtained with the non-modified alumina, where the stamp mark is completely covered with bacteria (a and c, Fig. 6). The absence of bacteria highlighted once again qualitatively the bactericidal abilities of the Al_2O_3–TiO_2–Ag surface and demonstrated the absence of bacteria re-growth.

3.3. Al_2O_3–TiO_2–Ag bactericidal behaviour under the key SPA operating parameters

3.3.1. Influence of oxygen

The influence of oxygen on the bactericidal activity is presented in Fig. 7. In aerobic conditions, the Al_2O_3–TiO_2–Ag material achieved total removal of the bacteria mixture

Table 2 – Elements desorbed from the surface of the ceramic samples.

Sample	Ti concentration (% wt)	Ti concentration (±0.3 ppm)	Ag concentration (±0.1% wt)		Ag concentration (±0.3 ppm)	
			$[Ag]_{total}$	$[Ag]_{soluble}$	$[Ag]_{total}$	$[Ag]_{soluble}$
Al_2O_3–TiO_2	0.00	<0.1	–	–	–	–
Al_2O_3–TiO_2–Ag	0.00	<0.1	1.2	0.2	13.0	2.0
Al_2O_3–Ag	–	–	36.1	7.1	26.0	5.1

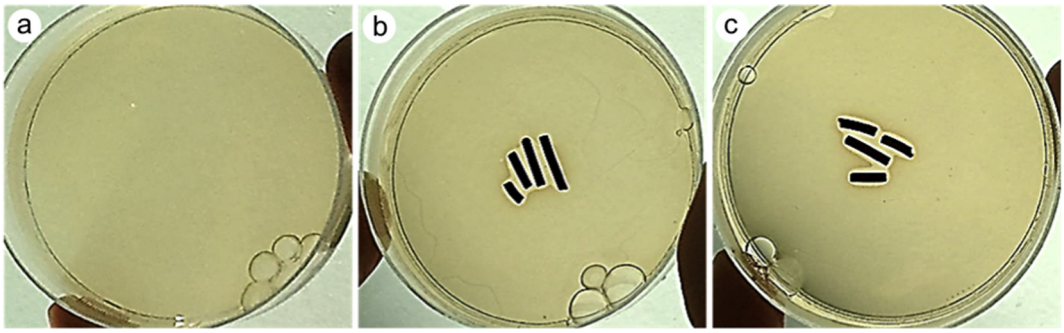

Fig. 5 – Bacterial biofilm on the surface of LB nutrient agar: a) Negative control; b) *E. coli* with Al_2O_3–TiO_2–Ag granules; c) *S. epidermidis* with Al_2O_3–TiO_2–Ag granules (C_0 for both bacteria = 1 × 10^8 CFU mL^{-1}).

(*Escherichia coli and Staphylococcus epidermidis*) in less than 45 min. In anaerobic conditions, total removal of the bacteria mixture was reached after 2 h.

These results show that the absence of oxygen inhibits the bactericidal activity of the Al_2O_3–TiO_2–Ag samples. This is in accordance with some studies highlighting a drastic decrease of bactericidal activity in non-aerated conditions for silver based materials (Chen et al., 2007; Inoue et al., 2002). These studies demonstrated the role of oxygen in bacterial disinfection by supported silver through the formation of Reactive Oxygen Species (ROS) such as superoxide anions (O_2^-), hydrogen peroxide (H_2O_2) or hydroxyl radicals (OH°) under aerobic conditions. According to many authors, ROS production could indeed lead to cell lysis, due to its highly oxidative effect, and corresponds to one of the most important mechanisms responsible for the bactericidal activity of silver.

3.3.2. *Influence of common salts*

The bacterial removal kinetics of the Al_2O_3–TiO_2–Ag material in presence of the two common salts from drinking water ($CaCO_3$ and $CaCl_2$ at 100 ppm) are presented in Fig. 8.

The bactericidal efficiencies of the nanocomposite material were not affected by the presence of $CaCO_3$. In both cases, without salt or with $CaCO_3$, total removal (for *Escherichia coli* and *Staphylococcus epidermidis*) was reached after 5 min. There is no evident chemical reaction between the Al_2O_3–TiO_2–Ag surface and $CaCO_3$ because the concentration of the desorbed silver is similar with or without $CaCO_3$: 0.2% wt in both spring water and the salt solution (Table 3).

On the other hand, in presence of $CaCl_2$, the bacteria removal ranged between −2 and −3 log, i.e. 99.99%, during the 60 min test period. This inhibition of the bactericidal activity in presence of $CaCl_2$ could be explained by the formation of a silver chloride (AgCl) precipitate at the Al_2O_3–TiO_2–Ag surface and potentially in solution. The solubility equilibrium of Ag^+ and Cl^- ions is characterized by a reaction quotient ($Q_{sp} = 1.4 \times 10^{-6}$ M) that is higher than the solubility constant of AgCl ($Ks = 1.8 \times 10^{-10}$ M), which is in accordance with the formation of AgCl precipitate in our aqueous solution. Moreover, these precipitates stabilized the Al_2O_3–TiO_2–Ag surface, leading to a soluble silver concentration (1.0 ppm) half that obtained in pure spring water (1.9 ppm) (Table 3). These results suggest that optimized concentrations of $CaCl_2$ may be used to chemically stabilize the material. Even if some of its bactericidal capacities are inhibited by the AgCl precipitants, new fields of application, more restrictive in terms of standard limitations but having smaller bacterial concentrations upstream, can be considered, such as drinking water sanitation.

It should be noted that the almost seven-fold difference between the total and soluble silver is mainly due to the broken surface that detached from the material granules during the agitation and possibly to the formation of AgCl in solution. These values are not reached in, for instance, a fixed bed reactor (3.3.4).

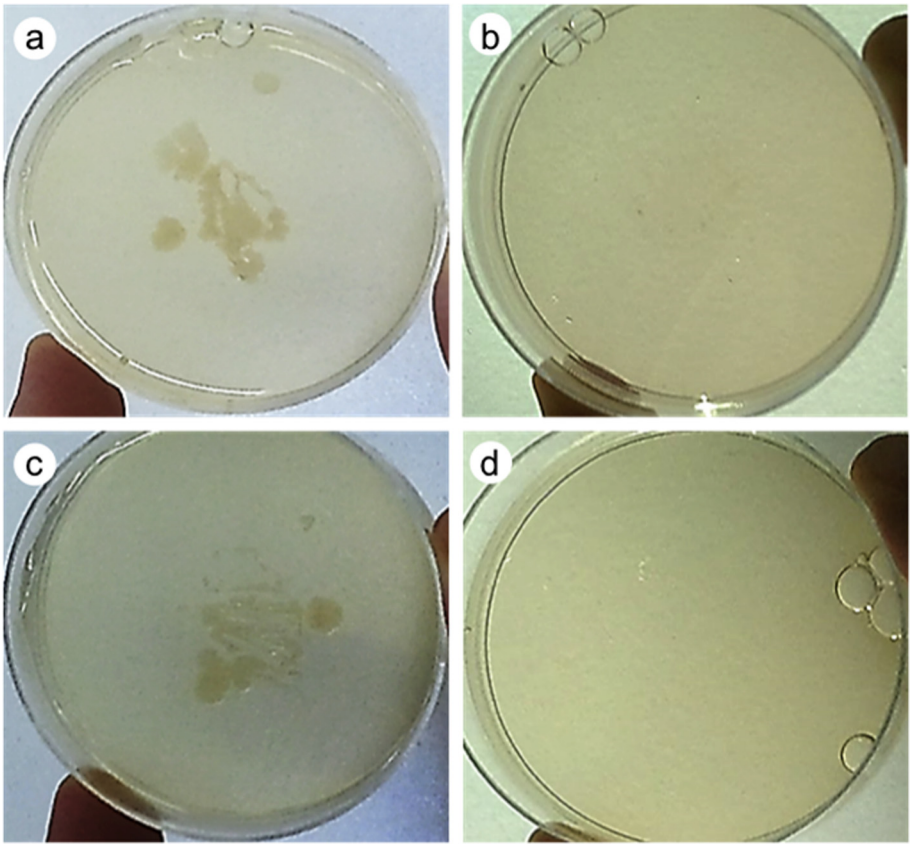

Fig. 6 – Bacterial development on the surface of LB nutrient agar: a) stamps made by the non-modified alumina seeded with *E. coli*; b) stamps made by the Al_2O_3–TiO_2–Ag seeded with *E. coli*; c) stamps made by the non-modified alumina seeded with *S. epidermidis*; d) stamps made by the Al_2O_3–TiO_2–Ag seeded with *S. epidermidis*; (C_0 for both bacteria $= 1 \times 10^8$ CFU mL^{-1}).

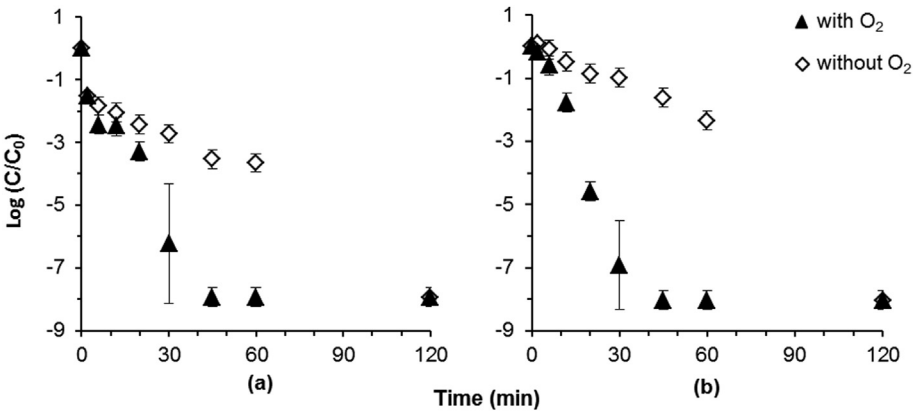

Fig. 7 – Removal kinetics for a bacteria mixture under aerobic and anaerobic conditions: *E. coli* (a) and *S. epidermidis* (b); (C_0 for both bacteria = 1.0 ± 0.2 × 10^8 CFU mL^{-1}; Al_2O_3–TiO_2–Ag concentration = 8 g L^{-1}, T = 22 °C).

3.3.3. *Influence of temperature*

The influence of temperature on the bactericidal material efficiency was investigated by comparing the removal kinetics for a bacteria mixture at 22 °C and at 37 °C, since 22 °C corresponds to typical swimming pool water temperature and 37 °C corresponds to the spa operating temperature. Controls without material at 37 °C showed that the temperature increase did not affect the cell viability.

As can be seen in Fig. 9, at 22 °C, the bacterial mixture was completely inactivated in 30 min while, at 37 °C, total removal was achieved in only 10 min (material concentration 8 g L^{-1}) which proves that increasing the water temperature improved the bactericidal efficiency of the Al_2O_3–TiO_2–Ag material. These results are consistent with the work presented by Chang et al. (2008), who reported that the efficiency of supported silver increased strongly with the temperature, especially around 40 °C (7 × 10^6 CFU mL^{-1} of *E. coli* were inactivated within 60 min at 25 °C but in 5 min at 40 °C). This behaviour of the material is a considerable advantage given that 37 °C is a suitable temperature for bacteria growth and that all the other conventional spa water treatments (mainly addition of chemicals) have more difficulty with this key operating parameters.

3.3.4. *Al_2O_3–TiO_2–Ag bactericidal behaviour in a continuous water flow reactor: preliminary results*

Controls carried out with a non-modified alumina proved that the selected flow (0.03 L min^{-1} inducing a laminar flow, Reynolds Number = 25), did not contribute to the inactivation of the bacteria. Fig. 10 shows that total *Escherichia coli* removal was attained in about 22 h and total *Staphylococcus epidermidis* removal within 8 h. At the same time, it is worth noting that 90% of the bacteria mixture (corresponding to −1 log removal) was deactivated from the first hour. These −8 log total removals are in accordance with the disinfection criteria for bathing water for public use as defined by the US EPA, which require a reduction of 6 log for coliforms (Hilteband and Averell Wancho, 1991). It is interesting to note that, in these conditions, *E. coli* appeared to be more resistant than *S. epidermidis*.

As expected, the concentration of silver desorbed in solution was much lower in the case of a fixed bed with a

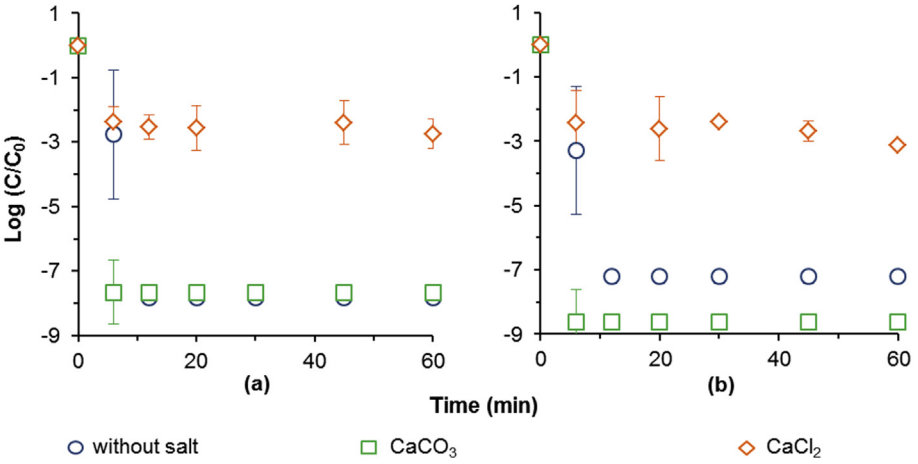

Fig. 8 – Removal kinetics for a bacterial mixture: *E. coli* (a) and *S. epidermidis* (b) in presence of salts commonly present in water: $CaCO_3$ and $CaCl_2$ (100 ppm); (C_0 for both bacteria = 1.0 ± 0.2 × 10^8 CFU mL^{-1}; Al_2O_3–TiO_2–Ag concentration = 16 g L^{-1}, T = 22 °C).

Table 3 – Silver desorbed from Al_2O_3–TiO_2–Ag material in test solutions.

Sample taken after 3 days of material submersion in:	Ag concentration (±0.1% wt in comparison with the deposited Ag)		Ag concentration (±0.3 ppm)	
	$[Ag]_{total}$	$[Ag]_{soluble}$	$[Ag]_{total}$	$[Ag]_{soluble}$
Spring water	1.2	0.2	13.0	1.9
Spring water + $CaCO_3$	1.1	0.2	12.5	2.2
Spring water + $CaCl_2$	0.6	0.1	6.5	1.0

continuous flow (Al_2O_3–TiO_2–Ag concentration = 2 g L^{-1}) than in a batch reactor under agitation (Al_2O_3–TiO_2–Ag concentration = 16 g L^{-1}) and tends to the standards for drinking waters that were chosen in this study (0.1 ppm). The desorbed silver concentration in solution was indeed 1.4 ± 0.1 ppm, i.e. 0.11 ± 0.03% wt, and was 0.7 ± 0.1 ppm, i.e. 0.055 ± 0.03% wt, for ionic desorbed silver. These preliminary results confirm that 2 g L^{-1} of Al_2O_3–TiO_2–Ag material is enough to eliminate more than 90% of 2.0×10^8 CFU mL^{-1} after one water treatment cycle in a closed dynamic circuit (2L/0.03 L min^{-1} = 66.6 min).

4. Conclusion

In this study, the CARDPool Company has developed an innovative Al_2O_3–TiO_2–Ag nanocomposite material that uses the disinfectant properties of silver.

The novelty of this material lies first in its new composition, with an additional TiO_2 nanolayer to bind the silver to the alumina support and thus limit its desorption. Secondly, its macro-granular form, which contrasts with the current powder form of supported silver material, makes it for safe handling and favourable settling in fixed-bed reactors from the pressure loss point of view. Finally, this new nanocomposite material exhibits a bactericidal surface action combined with low silver desorption which permitted that 16 g L^{-1} of Al_2O_3–TiO_2–Ag sample removed 2×10^8 CFU mL^{-1} of a *Escherichia coli* and *Staphylococcus epidermidis* mixture in less than 10 min.

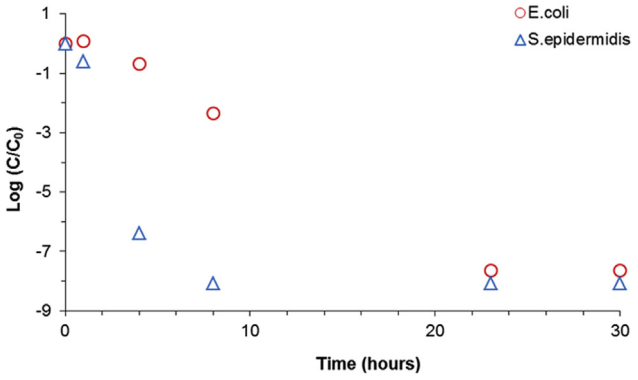

Fig. 10 – Bacteria removal kinetics in a continuous water flow, (C_0 for both bacteria = 1.0 ± 0.2 × 10^8 CFU mL^{-1}; Al_2O_3–TiO_2–Ag concentration = 2 g L^{-1}).

Unlike the classical bactericidal chemical products regularly applied for spa water treatment (such as Cl, Br, O_3, etc.), the Al_2O_3–TiO_2–Ag material shows increased disinfection capacities under the key spa operating parameters. At 37 °C, a suitable temperature for bacterial growth, only 8 g L^{-1} of Al_2O_3–TiO_2–Ag were needed for total elimination of the 2×10^8 CFU mL^{-1} bacteria mixture in 10 min. Furthermore, aerobic conditions allow to generate Reactive Oxygen Species (ROS), which contribute to the bactericidal activity of the material.

The preliminary tests of Al_2O_3–TiO_2–Ag bactericidal behaviour in a continuous water flow confirmed that 2 g L^{-1} of material (corresponding to the actual mass used in a spa decontamination filter) were enough to eliminate more than 90% of 2.0×10^8 CFU mL^{-1} after one water treatment cycle and reached the disinfection standard recommended by the Environmental Protection Agency (coliform removal = 6 log) within 22 h. After 30 h in these operating conditions, 0.7 ppm of soluble silver had desorbed from the Al_2O_3–TiO_2–Ag surface. Even though $CaCl_2$ slightly inhibited the bactericidal effect of the material (which fell from 99.999999% to 99.99%) due to the formation of AgCl precipitate, an optimized concentration of this salt can be probably used to stabilize even better

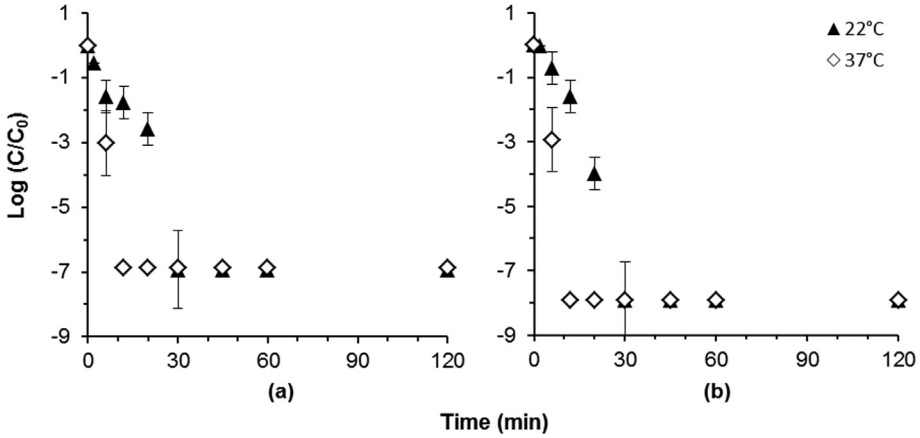

Fig. 9 – Removal kinetics for a bacteria mixture: *E. coli* (a); *S. epidermidis* (b) at 22 °C and 37 °C, (C_0 *E. coli* = 1 ± 0.2 × 10^7 CFU mL^{-1}; C_0 *S. epidermidis* = 1 ± 0.2 × 10^8 CFU mL^{-1}; Al_2O_3–TiO_2–Ag concentration = 8 g L^{-1}).

the surface silver (100 ppm of $CaCl_2$ reduces Ag desorption by half).

In further research work, both bactericidal performances and silver desorption will be studied in continuous mode, paying specific attention to the various operating parameters (such as water flow rate, material concentration, initial bacterial concentration, etc.) in order to optimize the disinfection process. Biomolecular analysis coupled with physicochemical analyses are envisaged to elucidate the mechanisms of silver action more precisely but, for the time being, all results lead to the general conclusion that the CARDPool Al_2O_3–TiO_2–Ag material can be successfully applied for spa water disinfection.

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