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Abstract: This study focuses on the use of superhydrophilic titanium dioxide (TiO₂) coatings applied to the surfaces of water-bearing systems to prevent surface colonization and biofilm formation. Biofilms in water-bearing systems are a problem in many industrial areas and are associated with risks to hygiene and health, material damage, and high costs for cleaning and maintenance. We investigated the suitability of TiO₂ coatings activated by UVA irradiation to achieve a superhydrophilic surface. The well-adherent coatings were deposited on flat and curved substrates (stainless steel, Al₂O₃) by pulsed magnetron sputtering. Surface characteristics, wettability, and the influence on microbial surface colonization were evaluated by WCA measurements, SEM, and XRD. For microbiological evaluation, *Escherichia coli* and *Staphylococcus warneri* were used. An adapted and specialized regime for sample conditioning and testing was developed that allows comparability with upcoming studies in this field. The superhydrophilicity was stable for up to 4 days, and an additional UVA reactivation step revealed comparable results. The microbiological studies proved a successful prevention of bacterial colonization on the activated coatings, which is attributed to their superhydrophilicity. The results demonstrate the potential of UV-activated TiO₂ as a long-term coating of water-bearing systems, like pipes, on which it assists in avoiding biofilm formation.

Keywords: titanium dioxide; photo-induced superhydrophilicity; long-term stability; reactivation; biofilm prevention; surface sanitation; bacteria-repelling effect; water-bearing pipes; *Escherichia coli; Staphylococcus warneri*

1. Introduction

Titanium dioxide (TiO_2) is a suitable candidate for superhydrophilic surface equipment as it is a well-known photocatalyst. It can be deposited by sol-gel processes but also by various physical vapor deposition methods, such as pulsed magnetron sputtering [1-3]. The photoactive phases of TiO₂ can be activated by the absorption of ultraviolet (UV) radiation, which results in the formation of electron-hole pairs. This can initiate two mechanisms on the surface: (1) photocatalytic oxidative decomposition, often described as photocatalysis, and (2) photo-induced hydrophilicity or superhydrophilicity [4,5]. Photocatalysis refers to various oxidation and reduction reactions induced by the electron-hole pairs reacting with molecules adsorbed on the surface. As a result, radicals and ions are formed, which leads to the decomposition of organic matter present at the surface. The final products of photocatalytic decomposition reactions are carbon dioxide, water, and inorganic ions [4,6,7]. In addition to photocatalytic decomposition, UV radiation induces hydrophilicity. Superhydrophilicity describes a condition where a water contact angle (WCA) of less than 10° can be observed on a surface [5,8]. The underlying mechanisms are still not fully understood, and various mechanisms have been proposed that attempt to explain photo-induced hydrophilicity [5,8–10]. One theory describes a superhydrophilic surface in its initial state directly after TiO₂ deposition that becomes more hydrophobic by the adsorption of organic molecules from its surroundings. By photocatalytically



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Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). driven degradation of these adsorbed organic molecules, the surface can return to its initial superhydrophilic state [9]. Another proposed theory connects the occurrence of superhydrophilicity to the thermal effect of irradiation. In this theory, the infrared component of the UV radiation source heats the surface, causing water molecules bound by hydrogen bonds to desorb and additionally causing adsorbed hydrocarbons to be partially degraded by photocatalysis. This increases the surface energy and, thus, the hydrophilicity of the surface. If the surface cools down after irradiation, water and hydrocarbons reabsorb, which results in the formation of the original structure of the hydrated layer and a reduction in surface energy and hydrophilicity [9]. The oldest and most widely accepted theory relies on the photoexcitation of electrons and the associated formation of surface defects or vacancies in terms of electron–hole pairs caused by UV irradiation [5,8–10]. Water molecules are bound to the surface due to the reaction of oxygen and water with the electron–hole pairs (surface defects), which results in an increase in the hydrophilicity of the surface [5,8].

Both photo-induced mechanisms, photocatalytic degradation and hydrophilization, have the potential to contribute to the sanitation of surfaces. Photocatalytic oxidative decomposition is a frequently used mechanism for the partial removal or even inactivation of microorganisms and other organic contamination. Currently, research focuses on nanostructured TiO_2 materials, composite coatings with additional photocatalysts, or doping of the coatings to improve the photocatalytic activity [2,3,11].

However, we consider the effect of superhydrophilicity to be the most suitable for use in water-bearing systems. No permanent activation by irradiation but only intervals of reactivation are necessary. For this reason, we investigated the superhydrophilicity of TiO_2 and its long-term stability as a means to enable the permanent prevention of microorganism adhesion and, hence, subsequent biofilm formation. We also checked whether the superhydrophilic behavior could be reactivated after a certain time. This fact is rarely considered in the literature but represents a key factor when addressing a long-term stable effect.

Adhesion and adsorption of microorganisms to a surface are influenced by physicochemical and morphological parameters as well as by molecular and cellular interactions. Significant influence on bacterial adhesion can be achieved by controlling any of the related surface parameters, such as charge, roughness, topography, or wetting properties [12]. Therefore, superhydrophilic surfaces can be used to develop easy-to-clean surfaces. Influencing microbial adhesion can help to prevent biofilm formation, as biofilm formation always has its origin in the adhesion of single microorganisms [12–14].

Biofilms on surfaces in general, and especially fluid-carrying systems, are a widespread problem and occur in many industries, such as the food and dairy, marine, medical supplies, water, optical, paper, and oil industries [13]. For example, biofilms are involved in the contamination of food processing equipment, which can impair food quality and safety [15,16]. In the oil industry, corrosion caused by biofilms is responsible for 40% of all corrosion events in oil pipelines [17]. From a medical point of view, biofilms have critical implications, as they are major contributors to infection transmission and are estimated to be responsible for the development of 65% of all nosocomial infections [16]. Especially disease spreading through the water systems of hospitals, which is directly connected with biofilm formation in these systems, is a significant risk to the health of hospitalized patients [18,19].

Biofilm formation enables microorganisms to survive in adverse environmental and nutritional conditions, as well as in the presence of high concentrations of antimicrobial agents such as antibiotics and disinfectants [16,20]. Additionally, microorganisms are more persistent in humid settings than on dry surfaces, where they may already live for months [21]. Thus, biofilm growth and its negative effects lead to high costs for cleaning and maintenance, especially in water-bearing systems like pipes, where mechanical cleaning is often impossible. This demonstrates the need for the development of materials that help to prevent bacterial adhesion and the formation of biofilms [13]. Due to limited access to many pipe systems, the mechanical cleaning of them is considerably impaired. It has been

shown that after chemical disinfection of such difficult-to-clean systems, microbiological contamination was still apparent [22].

To counteract the formation of biofilms in water-bearing systems, it is reasonable to prevent microbial adhesion in the first place, as the adhesion of microorganisms is assumed to be the start of biofilm formation [12–14]. This can be achieved by adapting the surface wettability using TiO₂ coatings and their activation by UV irradiation to establish superhydrophilic surfaces for microbial adhesion prevention, as described above. This is the aim of our study. For this purpose, we investigated microbial colonization on superhydrophilic TiO₂-coated half pipes and the time-dependent stability of superhydrophilicity. We chose half pipes with a diameter of 36 mm as a model system for biofilm-burdened water-bearing pipe systems. Initial microbial adhesion has its origins usually in the lower half of pipes due to gravity effects and a continuous abundance of liquid residue. The half pipes were coated with TiO_2 by pulsed magnetron sputtering. The coated samples were activated using UVA irradiation with a wavelength of 365 nm until superhydrophilicity was confirmed. For the evaluation of the influence of superhydrophilicity on microbial colonization, a specialized test regime was developed to represent the usual loading and unloading of liquid in a pipe-based water-bearing system. As test organisms, both Gram-negative and Gram-positive bacteria strains were used: Escherichia coli (E. coli) and Staphylococcus warneri (S. warneri). Both strains are reported to be involved in healthcare-associated infections [18,23,24]. S. warneri was chosen as a representative for Staphylococcus aureus (S. aureus) and its methicillin-resistant variant (MRSA), one of the most dangerous hospital germs. MRSA and human pathogenic strains of E. coli are among the most common biofilmforming bacteria that cause hospital-related infections [24]. S. warneri likewise tends to form biofilms and, hence, is a suitable representative to investigate the initial events of biofilm formation, in particular microbial adhesion [23]. E. coli is a commonly identified organism in water-bearing system-related outbreaks in hospitals and was therefore selected as a second microorganism to be investigated [18].

2. Materials and Methods

2.1. UV Irradiation Setup for Surface Activation

Two UV irradiation setups (planar and curved) using UVA LEDs were constructed to activate the coatings on flat (Figure 1a) and curved samples (Figure 1b). The setup for flat samples in Figure 1a uses a 365 nm LED (NDU1104ESE-365-TR, Stanley Electric, Meguro, Japan), and the setup for curved samples in Figure 1b uses two 365 nm LEDs (ELUA3535OGB series, Everlight Electronics, New Taipei, Taiwan). The irradiation distance, the distance between the UVA LED and the sample surface, was 36 mm. This was based on the standardized diameter of a common drainage pipe (DN 40) according to standard specification [25].





2.2. Determination of UV Dose Distribution

For surface activation, the coated samples were irradiated with UVA radiation using the setup described in Section 2.1 and Figure 1. UVA irradiation results in dose application onto the sample surface. To evaluate the relative dose distribution during sample irradiation, B3 film dosimeters (WINdose B3, GEX Corporation, Palm City, FL, USA) were applied to several positions on the samples (see Figure 2). The film dosimeters (10 mm \times 10 mm) were irradiated for 25 min and afterwards stored in the dark under ambient conditions for 24 h. During this storage time, irradiation-induced color development took place. The film dosimeters were scanned (Epson Perfection 4490 Photo, Seiko Epson Corporation, Suwa, Japan), and their resonance was determined via gray shades (Risø Scan, Risø High Dose Reference Laboratory, Roskilde, Denmark). The resonance was used as measure for the applied UVA dose. To evaluate the relative dose distribution, the central measurement point was set to 100%, and all other points were normalized to this one.



Figure 2. Relative dose distribution of the 365 nm LEDs on curved samples (**left**) and schematical drawing of the setup (**right**). The measured values were normalized to the dose measured at the central point (100%).

The irradiance of the LEDs on the center of the sample surface was measured by UV radiometer equipped with a UVA sensor (RMD Pro, UV Messtechnik Opsytec Dr. Göbel, Ettlingen, Germany). This point represents the measurement point "c" (see Figure 2) and was set to 100%, as described above.

2.3. Sample Coating, Conditioning, and Activation

Sample Coating: The coating deposition process was bipolar pulsed magnetron sputtering in the medium frequency range (some 10 kHz) [26]. The plant UNIVERSA of Fraunhofer FEP was used for coating the samples used in this study. A pair of rectangular magnetron sources arranged side by side were equipped with titanium (Ti) targets of dimensions 508 mm \times 127 mm and reactively sputtered in an argon–oxygen atmosphere at pressures in the range of 1.03–1.13 Pa.

The TiO₂ coating was deposited on flat substrates by rotating the substrates around an axis parallel to the target surface in front of the magnetrons, as described by Fietzke and Zywitzki (deposition parameters in Table 1) [27]. The flat samples were used to determine the effect of UVA irradiation during coating activation in terms of surface hydrophilization, investigated by WCA measurements. Pieces of aluminum oxide ceramic (Al₂O₃; Rubalit[®] 710, 50 mm × 50 mm) and stainless steel (X5CrNi18-10, material No. 1.4301, surface finish 2R: cold rolled and bright annealed, 100 mm × 60 mm) with a thickness of 0.95 mm were selected as substrates. These two substrate materials with differences in surface roughness and chemical composition were chosen to evaluate coating deposition on strongly varying substrates and its influence on layer properties. Stainless steel represents a relevant material type for application in water-bearing pipes. The TiO₂ coating was deposited on these substrates directly or with an additional adhesion-promoting Ti interlayer. For coating of the curved samples (X5CrNi18-10, 0.5 mm thickness), the concave side was coated using analog process parameters (Table 1).

Table 1. Data of the titanium dioxide (TiO_2)-coated samples and the coating parameters for deposition by pulse magnetron sputtering. Coating times for the TiO_2 coatings were 55–60 min (flat and curved) and 5 min for the Ti interlayer.

Sample Type	Substrate	T Heater [°C]	Pulse Pattern [µs]	Γ _{Ar} [sccm]	Γ _{O2} [sccm]	p [Pa]	Coating Rate [nm/min]	Coating
SS-TiO ₂	stainless steel	250	16:10	100	38–39	1.12	47–51	pure TiO ₂
SS-Ti-TiO ₂	stainless steel	250	16:10	100	40	1.03	50–51	Ti interlayer
					39	1.13	200–210	TiO ₂ coating
Al ₂ O ₃ -TiO ₂	Al ₂ O ₃ ceramic	250	16:10	100	38–39	1.12–1.13	47–51	pure TiO ₂
Al ₂ O ₃ -Ti-TiO ₂	Al ₂ O ₃ ceramic	250	16:10	100	40	1.03	50–51	Ti interlayer
						1.13	200–210	TiO ₂ coating
curSS-TiO ₂	stainless steel	250	15.5:10: 16.5:10	100	38	1.12	53	pure TiO ₂ on curved samples

Sample Conditioning: To obtain a comparable initial state of the coating surface, the TiO_2 -coated samples were conditioned by 90 min UVA irradiation using the irradiation setup described in Section 2.1. Afterwards, the samples were stored in water under dark conditions for 24 h. Then, the water was drained off, and the samples were stored for a further 5 days under ambient conditions in the dark. This procedure for optimal sample conditioning was evaluated in preliminary tests to achieve similar initial wetting characteristics (see Section 3.2). Until usage, the samples were stored under ambient conditions in the dark. No further change in WCA was observed under these storage conditions.

For microbiological investigations, the samples were autoclaved (121 $^{\circ}$ C, 20 min, 210 kPa) before use and stored under sterile conditions.

Sample Activation: The TiO_2 -coated samples were activated by UVA irradiation directly before the start of the respective microbiological experiments using the setup described in Section 2.1 and underwent continuous irradiation for 30 min.

2.4. Sample Characterization

2.4.1. Determination of Layer Thickness

The layer thickness was determined by weighing the samples on an analytical balance (AT261 DeltaRange, Mettler-Toledo, Greifensee, Switzerland) before and after coating. The weight gain after the coating process was normalized to the overall coated surface per sample, and, considering the estimated density of the coating (anatase: 3.89 g/cm³ [28]), the layer thickness was calculated.

2.4.2. Atomic Force Microscopy (AFM), Scanning Electron Microscopy (SEM), and X-ray Diffraction (XRD) Measurement

AFM: The surface roughness was determined by AFM measurements in non-contact mode at the center of the flat samples according to [29] using NaniteAFM (Nanosurf, software: C3000 controller (version 3.8.4.2), Liestal, Switzerland). The evaluation of the measurements was performed in the program MountainsSPIP Expert (version 8.0.9286, DigitalSurf, Besançon, France).

SEM: Characterization of the surface morphology and layer structure of the TiO_2 coatings was performed using a field emission scanning electron microscope (SU8000, Hitachi, Tokyo, Japan). Cross sections were obtained by breaking the substrates, which, in individual cases, was followed by ion polishing. This method was also used to determine the approximate lateral size of the anatase crystals.

XRD: Measurements were performed using a D8 Discover (Bruker Corporation, Billerica, MA, USA) with a grazing incidence of 1° and copper K α -radiation on one representative sample of each coating deposited on flat substrates. Measurements were conducted to determine the effects of the substrate and/or the interlayer on the composition and phase of the final TiO_2 surface. The JCPDS-International Center for Diffraction standard plots #21-1272 (anatase) and #21-1276 (rutile) were used to evaluate the crystal phases [30].

2.4.3. Contact Angle Measurement, Superhydrophilicity, and Stability

WCA measurements were used to examine the wetting behavior of the TiO_2 coatings. The measurements were performed using an OCA 20/6 (software: SCA20.2 (version 4.1.16 build 1023), DataPhysics, Filderstadt, Germany) and the sessile drop method with a drop volume of 0.3 μ L of deionized water. The water contact angle was measured ten times in the middle of the flat samples; the average value and standard deviation were calculated. The OCA 20/6 device has a minimum measurable contact angle of 4°, meaning that actual contact angles below 4° are presented as 4° as well.

WCA measurements were used to determine the optimal sample conditioning procedure, to evaluate hydrophilicity upon UVA activation, and to investigate the long-term stability. Samples were activated by 90 min UVA irradiation. Stability investigations regarding the hydrophilicity were conducted for up to 8 days at the following intervals: before UVA irradiation, directly after irradiation, 0.5 h and 1.0 h after irradiation, and every 24.0 h after irradiation.

According to the literature, samples were designated as superhydrophilic if the WCA was below or equal to 10° [5,8].

Each type of sample was measured in triplicate in three independent experiments.

2.5. Microbiological Evaluation

2.5.1. Stock Culture and Sample Inoculation

Microbiological tests were performed using *E. coli* (Gram-positive bacteria) and *S. warneri* (Gram-negative bacteria).

Cultivation of *E. coli*: For the stock culture, 100 mL of standard I nutrient broth (15 g/L peptone, 3 g/L yeast extract, 6 g/L sodium chloride, 1 g/L glucose, Carl Roth, Karlsruhe, Germany) were inoculated with a stock aliquot of *E. coli* K12 (DSM 498) (stored at -80 °C) in a 300 mL Erlenmeyer flask and incubated for 24 h at 37 °C under ambient conditions in a shaking incubator at 125 rpm.

Cultivation of *S. warneri*: For the stock culture, 100 mL of CASO broth (16 g/L peptone from casein, 3 g/L peptone from soy, 2.5 g/L di-potassium hydrogen phosphate, 5 g/L sodium chloride, 2.5 g/L glucose monohydrate, Carl Roth) were inoculated with a stock aliquot of *S. warneri* (DSM 20036) (stored at -80 °C) in a 300 mL Erlenmeyer flask and incubated for 24 h at 37 °C under ambient conditions in a shaking incubator at 125 rpm.

To determine bacterial density, after 24 h, the cultures were diluted 1:100 with the respective culture medium, and 10 μ L aliquots each were pipetted into a Neubauer counting chamber (Neubauer-Improved, Paul Marienfeld, Lauda-Königshofen, Germany). The number of apparent bacteria in the counting grid was counted under a light microscope (B3 professional series, MoticEurope, Barcelona, Spain), and bacterial density in the stock was calculated considering the dilution.

For impedance calibration (Section 2.5.2) as well as for every experiment during microbiological investigation of the coated curved samples (Section 3.3), a new stock of *E. coli* and *S. warneri* was grown for each.

For inoculation of the coated curved samples (curSS-TiO₂), the stock cultures were diluted with $1 \times$ PBS (Dulbecco's, Capricorn Scientific, Ebsdorfergrund, Germany) to a bacterial density of 10^3 mL⁻¹.

Curved samples were inoculated (after their activation) with bacterial suspension, which was dropped centrally onto the concave side of the sample. For a detailed explanation of the sample preparation and subsequent microbiological procedures, please see Section 3.3.

2.5.2. Impedance Measurement

Impedance describes the resistance to flow of an alternating electric current through a conducting material. It is a complex quantity consisting, amongst others, of a conductive component [31]. In microbiology, impedance measurement can be used to determine initial bacterial concentration in solutions by recording the change in impedance of the solution over time and relating the results to a calibration. The change in impedance is caused by the release of ionic metabolites from viable bacteria in the solution. It is usually measured by the so-called direct method, with a pair of electrodes in direct contact with the solution and the bacteria therein [31,32]. Impedance meters measure the relative or absolute changes in impedance, capacitance, or conductivity at a given temperature (incubation temperature) at regular time intervals. The impedance meter used in the experiments, BacTrac 4100 (software: Y1.49r/D7 (5.10.2004), SY-LAB, Neupurkersdorf, Austria), uses the impedance splitting method. The total impedance is the sum of two components, the electrode or interface impedance (E value) and the electrolyte or media impedance (M value), which can be measured in different frequency ranges. The BacTrac systems are capable of measuring both components at 1 kHz [33]. Due to its higher sensitivity to changes as well as its reliability in media within a wide range of salt concentrations, the E value was selected for our experiments [32].

It has already been shown that this method is suitable for the detection of *E. coli* and *S. warneri*, e.g., to record the delay in impedance curves of microorganism suspensions with different initial densities of microorganisms [34]. The usual detection limit of the device is 1 bacteria/mL, which therefore allows the detection of low bacterial concentrations in liquids.

The time the impedance value of a sample needs to reach or exceed the previously defined threshold is called the detection time. It depends directly on the used microbial strain and the initial number of viable bacteria. The detection thresholds were chosen in such a way that the exceedance occurs in the lower third of the impedance curves for all bacterial concentrations according to [35,36]. Within preliminary investigations, thresholds of E% = 10 (*E. coli*) and E% = 7 (*S. warneri*) were determined.

Direct impedance measurement was used to determine the amount of viable bacteria on the sample surface after activation and UVA irradiation. The method was calibrated for both bacterial strains.

Calibration was performed separately for both bacteria strains in the respective culture medium (see Section 2.5.1) in the impedance meter. Stock cultures were diluted in a 1:10 series with the respective medium from 10^9 to 10^0 bacteria/mL. Each impedance cell was filled with 6 mL of the dilution. Each dilution was measured in triplicate. As the negative control, triplicates of 6 mL of the respective sterile culture medium were used. All impedance samples were incubated in the impedance meter for 24 h at 37 °C, recording the impedance value every 10 min. In parallel, an aliquot of all dilutions was plated in triplicate on nutrient agar to determine the actual number of viable bacteria by counting colony-forming units (CFUs) after incubation (for details, see Section 2.5.3).

For each dilution, the obtained detection times were plotted against the viable number of bacteria achieved by the plate count method to obtain the calibration curve for both bacterial strains. The calibration curves can be found in Figure 3.

By using the acquired detection time for a solution of unknown bacterial load, this calibration allows the calculation of the initial number of viable bacteria in the solutions and on the investigated samples.

For investigations on coated samples, impedance measurement was used to determine the remaining concentration of viable bacteria on the sample surface after performing the developed experimental regime described in Section 3.3. Five aliquots per sample, with a volume of 6 mL each, were transferred into impedance cells. A triplicate of 6 mL sterile culture medium was used as negative control, and a triplicate of 6 mL stock culture diluted to 10^3 CFU/mL as positive control. The measurement cells remained in the impedance meter for up to 5 days, or until bacterial activity was detected by exceeding the previously



defined threshold. Investigations were performed in triplicate, with three independent experiments per sample.

Figure 3. Calibrations of the direct impedance methodology for (**a**) *Escherichia coli* (*E. coli*) and (**b**) *Staphylococcus warneri* (*S. warneri*). The blue diamonds represent individual measuring points on the basis of which the calibration (black line) was created.

2.5.3. Plate Count Method

The plate count method was used as a reference to determine the amount of viable bacteria for the calibration of the impedance measurement. A total of 100 μ L of all calibration suspensions added to the impedance measurement cells were plated in triplicate on nutrient agar after dilution to 10³ bacteria/mL with the respective nutrient medium. The post-dilutions were done in 1:10 or 1:100 steps with a final volume of 10 mL. The 10^2 , 10^1 , and 10^{0} bacteria/mL suspensions were also plated in triplicate with a volume of 100 μ L each. Positive controls were 100 μ L of the stock solution diluted to 10³ bacteria/mL in triplicate, and negative controls were 100 μ L of sterile nutrient medium on nutrient agar and pure nutrient agar. E. coli was grown on standard I nutrient agar (15 g/L peptone, 3 g/L yeast extract, 6 g/L sodium chloride, 1 g/L glucose, 12 g/L agar, Carl Roth), and S. *warneri* was grown on CASO agar (15 g/L peptone from casein, 5 g/L peptone from soy, 5 g/L sodium chloride, 15 g/L agar, Carl Roth) with 3 g/L yeast extract (Carl Roth). For *E*. *coli*, the agar plates were incubated for 20 h at 27 °C under ambient conditions and 70% humidity, followed by 5 h at 37 °C under ambient conditions and 70% humidity. For S. *warneri* the agar plates were incubated for 24 h at 37 °C under ambient conditions and 70% humidity. The number of CFUs was determined afterwards by optical counting of apparent colonies. The number of viable cells in the sample medium was calculated according to the following formula:

CFU/mL = (mean value colonies of all plates of one dilution level × dilution factor)/(plated volume of bacterial suspension [mL]). (1)

2.6. Statistics

Microsoft 365 Excel (version 2401) was used for the calculation of the mean values and standard deviations within evaluations of the radiation characteristics of the LEDs, the water contact angle measurements, the calculation of the impedance calibrations, and the microbiological analysis of the coatings.

BacEval (version Y1.25r/D7 (19.08.2004), SY-LAB, Neupurkersdorf, Austria) is an analysis program for impedance meters that was used to evaluate the detection times and corresponding concentrations of viable bacteria.

For testing the statistical significance, the data were checked for Gaussian distribution (Kolmogorov/Smirnov test, Lilliefors test: *p*-value) after exclusion of outliers (ROUT, Q = 5%). If the data were normally distributed, statistical significance was tested with an unpaired, two-tailed *t*-test (Welch's correlation, confidence level: 95%). If there was no

normal distribution or it could not be tested, the unpaired, two-tailed Mann–Whitney test (confidence level: 95%) was used. Statistically significant differences are highlighted with *.

3. Results and Discussion

3.1. Dose Distribution and Influence of UV Wavelength on Sample Activation

The determined dose distribution on the curved sample is shown in Figure 2. The measured values are expressed in percent, and the value of the central point of the sample was set to 100%. As can be seen from Figure 2, the highest dose is applied to the uppermost point of the side walls of the curved surface and is 9–10% higher compared to the central point. The reasons for this are the smaller distance to the LEDs compared to the other measurement points and that, due to the central positioning between both LEDs, radiation from both LEDs contributes to the dose input (see Figure 1). The lowest dose is applied in a vertical direction beside the central point at a distance of 15 mm. On these points, the two LEDs do not contribute equally to the applied dose. Accordingly, a dose loss of up to 11% is measured at a vertical distance of 15 mm to the central point. For the envisioned application, the homogeneity of dose distribution on the surface seems acceptable.

It is known that both the light irradiance and the wavelength of the light have a decisive influence on the process of photocatalytic degradation [7]. It is also a known theory that short UV wavelengths increase the photocatalytic efficiency of TiO₂, as it is supposed that the formation of electron-hole pairs is efficiently promoted by suppressing their recombination to a greater extent than at longer UV wavelengths [37,38]. Thus, there is a higher probability of recombination of electron-hole pairs with our experimental setup due to the chosen wavelength of 365 nm, which is longer compared to UVC radiation with wavelengths below 280 nm. It was reported by Herrmann as a hypothesis that the recombination of electron-hole pairs formed by UV radiation is negligible at low light irradiances (far below 25 mW/cm^2) [39]. In our experimental setup, the UVA LEDs apply an irradiance of 8.2 \pm 0.6 mW/cm² (flat samples) and 9.8 \pm 0.7 mW/cm² (curved samples, central point). These values are far below the above-mentioned irradiance level of 25 mW/cm^2 described by Herrmann [39]. Therefore, it can be assumed that the electron– hole pair recombination in our experiments will be correspondingly low. Furthermore, Venkatchalam et al. found that wavelengths of 365 nm enable better activation of TiO_2 than wavelengths of 254 nm, as the longer wavelengths enable deeper penetration of absorbed contaminations on the surface [38]. In our envisioned application, water-bearing pipe systems, it can be expected that other contaminants besides bacteria are present. These could absorb the UV radiation before it reaches the surface of the TiO_2 coatings. With the relatively long wavelength chosen for our setup, this impairment of UV activation is mitigated to some extent.

Assuming the most accepted theory that superhydrophilicity on TiO_2 is caused by the formation of electron–hole pairs upon UV irradiation and avoiding recombination, the superhydrophilicity of the investigated coatings should not be significantly impaired by the chosen wavelength and the irradiance of the UV sources, since the recombination of electron–hole pairs should be low, as explained above.

3.2. Surface Characterization and Superhydrophilicity

The AFM, SEM, and XRD measurements used for surface characterization revealed surface morphological and structural differences between the samples coated on different substrates. This affects the activation of the different coatings and the associated superhydrophilicity.

The AFM measurements reveal that the coatings on ceramic substrates show higher roughness than on stainless steel (Table 2). In addition, the deposition of the Ti interlayer leads to an increased roughness of the final TiO₂ coating. Thus, comparing all coatings, SS-TiO₂ has the lowest roughness with $R_a = 21.3$ nm, and Al_2O_3 -Ti-TiO₂ has the highest with $R_a = 67.4$ nm. Accordingly, the SEM images of the different samples show comparable differences in the surface structure (Figure 4).

Sample Type	Coating Thickness [nm]		Roughness R _a [nm]	Lateral Size TiO ₂ Crystallites [nm]
SS substrate	-		5.0	-
Al ₂ O ₃ substrate	-		120.0	-
SS-TiO ₂	2600		21.3	≈ 150
SS-Ti-TiO ₂	1200 2800	(Ti interlayer) (TiO ₂ coating)	44.5	≈ 500
Al ₂ O ₃ -TiO ₂	2600		56.4	≈200
Al ₂ O ₃ -Ti-TiO ₂	1200 2800	(Ti interlayer) (TiO ₂ coating)	67.4	≈350
curSS-TiO ₂	3200		-	≈ 200

Table 2. Thickness of deposited coatings, arithmetic average deviation of the roughness profile, and lateral size of TiO₂ crystallites.



Figure 4. Scanning electron microscope (SEM) images of TiO_2 coating on (**a**,**b**) aluminum oxide ceramic substrate (Al₂O₃); (**c**,**d**) Al₂O₃ substrate with Ti interlayer; (**e**,**f**,**l**) stainless steel substrate; (**g**,**h**) stainless steel substrate with Ti interlayer; (**i**–**k**) curved stainless steel substrates. (**k**,**l**) Higher magnifications of the coatings on the geometrically different stainless steel substrates (flat and curved) for better comparison of the influence of substrate geometry (flat (**l**) or curved (**k**)) on coating formation. The red arrows in (**d**) mark gaps in the coating.

The layer thicknesses given in Table 2 were determined by weighing. Compared with the SEM cross sections in Figure 4, there are deviations of a maximum of 20%, which are attributed to the porosity of the coatings.

The SEM images reveal significant differences in the layer structure that depend on the substrate material and the deposition of an interlayer (Figure 4). The substrate material has the greatest impact on the layer formation, as it affects the structure of the Ti interlayer and, as a result, the structure of the final coating. The stainless steel substrate has a lower roughness, in the range of $R_a = 5$ nm, while the Al₂O₃ ceramic has a higher roughness, in the range of $R_a = 120$ nm, due to its grain structure (manufacturer's data). This leads to the formation of gaps in the TiO₂ layer, which are wider and deeper than those between the individual crystallites (Figure 4d). These gaps extend into the Ti interlayer on Al₂O₃ substrates, while the interlayer on stainless steel is uniformly dense and pore-free (Figure 4h).

The interlayer influences the grain growth of the final coating and thus affects the lateral grain size of the crystallites. On the Al_2O_3 substrate, this influence is less pronounced than on stainless steel, on which at least a doubling of the lateral expansion of the crystallites and thus an increase in surface roughness is observed (Figure 4 and Table 2).

TiO₂ occurs in different phases with different facets of the crystallites. XRD measurements can be used to investigate the layer composition with respect to these two aspects [3,40]. The results of the XRD measurements show that TiO₂ consisting of pure anatase is deposited on both substrate materials without interlayers (Figure 5a,c). The final coating on stainless steel substrates (Figure 5a) shows a very strong texture; for the coatings on ceramic substrates, this effect is less pronounced (Figure 5c). The texturing is also evident on the coating surface visualized by SEM (Figure 4). TiO₂ coatings deposited on Ti interlayers (Figure 5b,d) are predominantly anatase phase but contain an amount of rutile phase that appears to be independent from the substrate material. Accordingly, the Ti interlayer has a significant effect on the texture and phase of the TiO₂ coating. The texture of anatase deposited on Ti interlayers is clearly visible only on stainless steel substrates (Figure 5b), whereas no preferred orientation of anatase is observed on the ceramic substrates (Figure 5d).



Figure 5. X-ray diffraction (XRD) determined composition of TiO_2 coatings on (**a**) stainless steel substrate, (**b**) stainless steel substrate with Ti interlayer, (**c**) Al_2O_3 substrate, (**d**) Al_2O_3 substrate with Ti interlayer.

It is also evident from the diagrams in Figure 5 that the faceting of the anatase crystallites is influenced by both the substrate material and the interlayer. The (101) facets occur on stainless steel only in combination with the Ti interlayer, while they are observed on

In summary, the substrate material leads to differences in the surface morphology of the final coatings, but the greater influence results from the deposition of a Ti interlayer, which additionally influences the phase of the final TiO_2 coating.

ceramic substrates independently from the interlayer.

The layer formation on flat or curved geometries is strongly affected by the substrate geometry. The geometry influences the angular distribution of incident particles during layer deposition as well as the associated energy distribution. A complex geometry like the one used for the curved samples can induce shadowing effects and lead to alterations in the deposited coatings. This results in an inhomogeneity of the layer thickness, roughness, and crystallite structure (Figure 4i,j and Table 2). Taking changes in interference color as a qualitative sign for inhomogeneity in coating thickness, a slight but symmetrical decrease in coating thickness from the center to the top and bottom of the curved samples was observed.

Compared to the TiO_2 coating on flat samples, no gaps formed between the crystallites in the curved geometry (Figure 4k,l).

WCA measurements were performed to investigate the wetting behavior and superhydrophilicity of the TiO₂ coatings after UVA activation. From the literature, it is known that major problems for the investigation of photo-induced hydrophilicity on TiO₂ are the non-reproducibility of the initial state of a coating and the different methods for the pretreatment of the coatings ("sample conditioning") as well as conditions during sample storage. All of this leads to WCA variations in the range of 20–60°. This severely compromises the determination of relevant factors influencing the hydrophilicity of the surface and a comparison with results obtained by different research groups [9]. By conditioning all our coatings in a defined procedure (see Section 2.3), we created comparability of all samples per sample type and additionally enabled the reproduction of this way of sample conditioning in other studies. This good comparability within our own study is supported by very low standard deviations in WCA measurements before UVA activation, i.e., at point "–1.5 h" (max. 4° for each sample type (Figure 6)).



Figure 6. Water contact angle (WCA) before and after activation by UVA irradiation of the samples. Activation was performed a second time after 8 days (192 h). Samples: TiO_2 on stainless steel substrate (SS-TiO_2, triangle, blue) and with Ti interlayer (SS-Ti-TiO_2, circle, red), TiO_2 on Al_2O_3 ceramic substrate (Al_2O_3 -TiO_2, diamond, green) and with Ti interlayer (Al_2O_3 -Ti-TiO_2, square, yellow). Significant differences are marked with an * and assigned to the color of the respective sample.

In the first step, we investigated the initial WCA of our conditioned samples. With 12° (Al₂O₃-Ti-TiO₂) and 9° (Al₂O₃-TiO₂), the initial WCAs of the conditioned TiO₂ coatings on ceramic substrate are lower than the WCA of the coatings on stainless steel substrate with 19° (SS-Ti-TiO₂) and 13° (SS-TiO₂) ("-1.5 h" in Figure 6). Additionally, the WCA is higher on the samples with Ti interlayer. It is well known that surface morphology has a great

influence on the contact angle [10,41]. It has been shown that rough TiO₂ surfaces have a lower contact angle than smooth TiO₂ surfaces (roughness approx. \leq 10 nm) [10,14]. In our case, this relationship can be generally confirmed: TiO₂ coatings (SS-TiO₂, SS-Ti-TiO₂) with lower roughness show higher WCA than coatings (Al₂O₃-TiO₂, Al₂O₃-Ti-TiO₂) with higher roughness (Table 2 and Figure 6). However, when comparing the coatings regarding the presence of the Ti layer, the opposite trend is observed. The coatings with higher roughness (due to the interlayer) show a higher WCA. The surface roughness thus cannot be the sole cause of these observed WCAs. In addition, the chemical composition has a major influence on surface hydrophilicity. With increasing amounts of chemisorbed OH groups, the hydrophilicity increases as well [42].

After activation by UVA irradiation, all coatings show a WCA below 4° ("0 h" in Figure 6), which is below the defined threshold for superhydrophilicity. This superhydrophilicity manifests as a strongly increased spreading of aqueous fluids on the surface (Figure 7b). During 8 days of storage, the WCAs almost recovered, and after 8 days, the second activation by UVA irradiation was performed (Figure 6, "192 h"), and again, WCAs below the threshold for superhydrophilicity were observed. Thus, reproducible activation of superhydrophilicity by multiple UVA irradiation is possible for all TiO₂ coatings investigated, regardless of the coating composition and the existence of an interlayer.



Figure 7. Spreading behavior of water on the TiO_2 coating on stainless steel before (**left**) and after (**right**) activation: (**a**) WCA measurement with 0.3 µL H₂O; 1 mL H₂O (**b**) on flat samples and (**c**) curved geometries.

Furthermore, the long-term stability of the achieved superhydrophilic effect was investigated by WCA measurements (Figure 6). In the first hour after UVA activation, the WCA increases only by a few degrees for all coatings. In the following four days, the WCA of the TiO₂ coating on stainless steel substrates (SS-TiO₂) continues to increase slightly, up to about 10°. After exceeding this threshold for superhydrophilicity, the coating's WCA further increases slowly, reaching a value of 12° after 7 days ("168 h"). The WCA of SS-Ti-TiO₂ already reaches the threshold for superhydrophilicity after 24 h. Up to 96 h, this coating shows no further increase in WCA; afterwards, an increase up to 17° is observed ("144 h" in Figure 6).

The coatings on Al_2O_3 ceramic substrates also show a further increase in WCA. The samples of Al_2O_3 -Ti-TiO₂ are superhydrophilic up to 48 h after activation by UVA irradiation; after 72 h, the threshold of 10° is exceeded. For Al_2O_3 -TiO₂, the WCA reaches the threshold for superhydrophilicity 48 h after activation by UVA irradiation, but during the following two days ("72 h" and "96 h" in Figure 6), the WCA decreases again and reaches values below this threshold. After 6 days ("144 h"), the WCA increases up to 15° and stagnates in this range. No further change in WCA was observed for any coating 7 days ("168 h") after irradiation. After the second activation by UVA irradiation, similar observations of the WCA for all coatings are made (see Figure 6). Due to the low standard deviations of the WCA measurements after the two activations, we assume that the reproducibility of the wetting behavior is also given with further reactivation have already been described in the literature [43,44]. Therefore, we assume that our coatings offer the prospects of long-term use by periodic reactivation.

It is known that the anatase phase of TiO₂ has better photocatalytic efficiency compared to other pure phases, such as rutile, brookite, amorphous TiO_2 and mixed anatase/rutile phases [3]. With regard to the formation of superhydrophilicity, coatings of rutile, but also anatase and mixtures of the two phases, have been increasingly investigated in the past [40–42,45]. Due to the fast electron–hole pair recombination of pure TiO₂ coatings, these surfaces lose their hydrophilic properties immediately when UV radiation is turned off [10,46]. For example, Watanabe et al. showed that their polycrystalline anatase coatings and rutile single-crystal coatings on glass reach the superhydrophilic state, but the effect lasts only a few minutes [45]. Ly et al. observed on thin TiO_2 coatings that, for the same irradiation time, the superhydrophilicity of the anatase coating could be activated by UV radiation, while the WCA of the rutile coating increased, i.e., no superhydrophilicity was observed [40]. The group of Vrakatseli et al. investigated anatase–rutile mixed coatings. Superhydrophilicity was observed for all coatings, with the most stable superhydrophilicity lasting up to 2 days on coatings with the lowest rutile fraction (2%). Both the pure rutile layer and the other mixed layers exhibited superhydrophilicity only for a few hours [41]. Yu et al. showed on thin anatase, rutile, and anatase (77%) and rutile (23%) mixed coatings that the mixed layer maintained superhydrophilicity for up to 24 h, followed by pure anatase and rutile. They explained the differences in the long-term stability of superhydrophilicity with the fact that fine roughness or porous structures and the number of hydroxyl groups on the surface favor the adsorption of water molecules and reduce the rate of conversion from the photo-induced hydrophilic to the hydrophobic state. Accordingly, the lowest hydrophobicity rate of the mixed layer is due to the synergistic effects of the chemical and geometric properties of the surface. Rutile exhibited the lowest stability because, first, the hydroxyl content on the surface was the lowest, and second, water evaporated more easily from the comparably larger pores of rutile [42].

Compared with the literature, our coatings show good properties in terms of superhydrophilicity, as even the coatings with the lowest stability showed superhydrophilicity for up to 24 h (SS-Ti-TiO₂, Figure 6). Lasting up to 4 days, SS-TiO₂ shows significantly better stability in terms of superhydrophilicity than the coatings discussed in the literature. It is remarkable that apparently pure anatase coatings or a very high anatase content in mixed coatings normally show the highest stability [40–42]. This observation also correlates with our findings, as the pure anatase coatings (coatings without Ti interlayer) show a longer stability of superhydrophilicity than the coatings with rutile fraction. Based on the statements of Yu et al., the lower stability in our investigations of superhydrophilicity can be attributed to the larger pores (higher roughness) of the coatings with rutile fraction, and the 4-day-stable superhydrophilicity can be attributed to the absence of these pores and the absence of rutile.

In addition to the phases of TiO_2 , the facets of the crystallites have an influence on the photoactivity of TiO_2 [10,45,47]. It has been proven both theoretically and experimentally that the (101) facets of anatase surfaces are expected to be rather unreactive. Furthermore,

the (001) facets are expected to have higher reactivity due to their larger size compared to the (101) facets. Because of the higher reactivity of (001) facets, an increased formation of electron–hole pairs is expected [47]. For rutile TiO₂, the crystal planes (110) and (100) with bridging oxygen sites were found to have higher efficiency for hydrophilic conversion than (001) planes without bridging oxygen sites [10]. In addition, the adsorbed OH groups on surfaces without bridging oxygen are considered unstable, so they tend to desorb more easily, and the surface hydrophilicity decreases [45]. Thus, our coatings consist of rutile with higher reactivity, but unreactive anatase was also proportionally deposited in the cases of SS-Ti-TiO₂, Al₂O₃-Ti-TiO₂, and Al₂O₃-TiO₂, as can be seen from Figure 5. The fact that anatase (101) is rather unreactive is supported by our findings. Samples of SS-TiO₂ that revealed no anatase (101) have the highest reactivity and the most stable superhydrophilicity. This also applies to the reactivation of these samples (Figure 6). Therefore, the SS-TiO₂ system was chosen for deposition on curved samples and application in microbiological studies (see Sections 3.3 and 3.4).

3.3. Development of the Microbiological Test Regime for the Application-Oriented Evaluation of Bacterial Colonization in Water-Bearing Pipes

The aim of our study was to investigate whether microbial colonization in waterbearing systems (such as pipes) can be prevented by equipping the surface with superhydrophilic characteristics using TiO_2 coatings. Therefore, the coating with the longest superhydrophilic activity (SS- TiO_2 , see Section 3.2) was chosen and deposited on curved substrates (curSS- TiO_2). The microbiological test regime was adapted to the application scenario of water-bearing pipes: the samples for microbiological evaluation are TiO_2 -coated half pipes, as persistent water contact and microbial adhesion are more likely to take place in the lower half of pipes.

In the literature, bacterial adhesion behavior in relation to the wetting characteristics of surfaces is controversially discussed [48,49]. However, it has been demonstrated by a variety of research groups that extreme hydrophilic surfaces can prevent bacterial adhesion [14,50–54]. Therefore, our microbiological test regime focuses on detecting viable bacteria on the sample surface.

The complete microbiological evaluation was designed as follows: In the first step, the coated and conditioned samples were placed under the LEDs and activated by UVA irradiation (see Section 2.3, Figure 1b). The second step of our microbiological test regime simulated the bacterial contamination of the pipe, with subsequent retention of the contaminated liquid on the surface. Therefore, the samples were removed from the irradiation setup after activation, and the surface was inoculated with 1 mL of bacteria suspension of E. coli (10³ CFU/mL) or S. warneri (10³ CFU/mL). Although in the literature higher concentrations of 10³–10¹⁰ CFU/mL have also been reported [19,55,56], we chose this concentration as we assumed, for the envisioned application, a new and clean pipe where bacterial contamination principally arises from the inflow of liquid, e.g., after hand washing (max. 10^2 CFU/mL) [57–59]. The bacteria suspension was dropped centrally onto the concave side of the sample (Figure 7c). The sample was then incubated for 45 min in the dark at room temperature and under ambient conditions. As there is no liquid flow during the incubation, this setup represents a worst-case scenario where microbial adhesion is very likely due to sedimentation and the absence of shear stresses caused by continuous liquid flow. Additionally, S. warneri tends to form biofilms and hence can be assumed as representative of biofilm formation in a pipe, where microorganism dislocation is impeded due to the good adhesion of the microorganisms to each other and to the substrate [23].

After this incubation, the sample surface was immediately rinsed with 20 mL of sterile deionized water using a sterile syringe. Afterwards, a second activation of the still-moist samples by UVA irradiation was conducted, simulating the step of reactivation of the surface. For non-activated control samples, the irradiation periods were replaced by storage in darkness. Assuming the superhydrophilic surface prevents bacterial adhesion, there should be no viable bacteria remaining on the sample surface. To confirm this

assumption, in the next step of our microbiological test regime, we tested for viable bacteria on the surface.

To evaluate the number of viable bacteria on the sample surface, the samples were overlaid with 40 mL of sterile culture medium and incubated for 22 h at 17 °C and 70% humidity under ambient and sterile conditions in the dark. After incubation, the overlaid medium was investigated concerning bacterial concentration using the direct impedance measurement (see Section 2.5.2). The calibrations created for this purpose are shown in Figure 3.

The results of the study of bacterial colonization on the surface using this test regime can be found in Section 3.4.

In this regime, reactivation of the coating by a second irradiation was conducted since regular reactivation of the coating will also be necessary for the envisioned application of water-bearing pipes to enable long-term use. Based on the results of the WCA measurements (see Section 3.2, Figure 6), it is confirmed that the coatings show superhydrophilicity upon activation by UVA irradiation. The superhydrophilicity was maintained for up to 4 days. Nevertheless, we assume a daily activation would be suitable to guarantee a constantly superhydrophilic surface and avoid bacterial adhesion. Hence, we defined a daily reactivation of the coating by UVA irradiation.

3.4. Investigation of Bacterial Colonization on TiO₂ Coatings

In the microbiological studies, we evaluated whether viable bacteria were present on the surface of the inoculated curved samples. The evaluation was performed using *E. coli* and *S. warneri*, with *S. warneri* as a representative of a biofilm former and *E. coli* as a common pathogen in waterborne system-related outbreaks in hospitals [18,23]. For some samples, no viable bacteria were detected. On the other samples, small amounts were detected (Figure 8 and Table 3). On the positive controls and the samples without UVA activation, both *E. coli* and *S. warneri* were detected using the impedance measurement (example impedance curves, see Figure 8). The detected bacterial concentrations of the non-activated samples were 0.939 CFU/mL (*S. warneri*) and 0.015 CFU/mL (*E. coli*) (Table 3). Thus, the bacterial contamination with the Gram-positive *S. warneri* is 100-fold higher than the Gram-negative *E. coli.*, which can be attributed to the tendency of aggregation and preferred biofilm formation of *Staphylococcus* species [60].



Figure 8. Representatives of impedance curves of incubated liquids recorded by direct impedance measurement on *E. coli*: 10^3 concentrated bacterial culture (orange), sterile culture medium (red), curSS-TiO₂ + bacteria (blue), UVA-activated curSS-TiO₂ + bacteria (green). The black line represents the detection threshold (E% = 10).

	-	-		
Sample Type	Microorganism	UVA Dose [J/cm ²]	Viable Bacteria [CFU/mL]	
	E coli	17.6 ± 1.2	0.000 ± 0.000	
	E. COll	no activation	0.015 ± 0.002	
$curSS-TiO_2$	Ci	17.6 ± 1.2	0.000 ± 0.000	
	S. warneri			

no activation

Table 3. Viable bacteria determined by direct impedance measurement.

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The low number of viable bacteria on the sample without UVA activation (Table 3) indicates that generally only a small fraction of the inoculated bacteria colonizes the surface. From the literature, it is known that the characteristics of the material, such as the surface wettability, the properties of the bacteria, and the environmental conditions, significantly affect bacterial adhesion on the surface [12,14,49,61]. If microbiological adhesion is considered in relation to the wetting properties of a surface, very different statements can be found in the literature [12-14,48,50,61-65]. In general, superhydrophobic (WCA > 150°) and superhydrophilic (WCA < 10°) surfaces have been reported to have an adverse effect on bacterial adhesion, as bacteria are more likely to adhere to moderately wettable surfaces (WCA 40–130°) than to extremely wettable surfaces like superhydrophilic surfaces [12,14,48,63]. Studies with a wetting range of WCA between 54° and 130° showed that the adhesion of bacteria is favored with an increasing WCA because an increase in surface hydrophobicity promotes the adsorption of adhesive proteins like peptidoglycans [61,64]. For E. coli, it is known that hydrophobic surfaces are preferred for adhesion due to the hydrophobic lipopolysaccharide bacterial surface [48,50]. In contrast, Koubali et al. showed that the adhesion of the hydrophilic bacterium S. aureus is better on very hydrophilic surfaces (WCA 15–36°) than on moderately hydrophilic surfaces (WCA 46–68°) [48,62]. Based on the WCA measurements of our samples (Figure 6), an initial WCA below 20° is to be expected for all our samples. Thus, even without UVA activation, the samples show a strongly hydrophilic surface, which, based on the above-mentioned results of other research groups, explains the higher bacterial concentration of S. warneri on the samples compared to E. coli. E. coli, as a hydrophobic bacterium, preferentially binds to hydrophobic surfaces, while S. warneri seems to preferentially adhere to hydrophilic surfaces similar to S. aureus [50,62]. The adhesion of microorganisms to a surface is furthermore influenced by hydration effects, which are a result of the balance between hydrophilicity and hydrophobicity between microorganisms and the surface as well as the hydrophobic-hydrophilic properties of the surface [13,65]. The prevention of microbial adhesion on superhydrophilic TiO₂ surfaces is attributed to the hydration layer formed on the TiO_2 surface, as this inhibits the interaction between the bacterium and the surface [14,51–54]. This explains the result of no bacterial colonization on the UVA-activated samples in our studies.

Additionally, surface roughness has an influence on bacterial adhesion to the surface. High values of roughness (values above the lateral size of relevant bacteria) can lead to an increase in surface area, which facilitates adhesion for bacteria [12,14,49,63,65]. In addition, the surface morphology (size and spacing of features) at the scale of the bacterial cells provides protection from the shear forces of fluids and supports initial adhesion on the surface [12,63]. But as the size and shape of bacteria vary and environmental conditions are also subjected to variations, there is reportedly no consistent relationship between bacterial adhesion and surface roughness [12,14,48]. In addition, differences in surface nano-roughness can lead to fundamental changes in adhesion behavior [63]. Since the roughness of the investigated surfaces (<100 nm) is two dimensions smaller than the size of the bacteria used ($\geq 1 \mu m$), we assume that bacterial adhesion is impaired by these rather smooth surfaces.

However, for our study, we cannot exclude that, besides superhydrophilicity, other mechanisms influence microbial colonization of the surface. Besides surface roughness/ smoothness, the effect of photocatalysis due to UVA activation and the inactivating effect of

 0.939 ± 1.359

UVA irradiation itself (during the second activation step) might influence the adhesion and abundance of viable bacteria on the sample surface. For this reason, we want to address these points in the subsequent section.

In our setup, we used UVA irradiation with a wavelength of 365 nm. UVA irradiation can inactivate microorganisms by oxidative stress through the formation of reactive oxygen species, but the mechanisms are much less effective and more time-consuming than the damage mechanisms by UVB or UVC radiation, which damage the DNA directly and lead to the effective inactivation of bacteria [66–68]. Since no literature data for the inactivation of *S. warneri* with UVA radiation were found, we discuss inactivation values for the closely related bacterium S. aureus. Regarding the inactivation doses at 365 nm for S. aureus and E. coli (our second model organism), controversial statements can be found in the literature [66–70]. It has been reported that, for a 1 log reduction of viable bacteria in suspensions containing *E. coli*, doses of 55.2 [68], 13.8 [67], 62.6 [66], and 122.2 J/cm² [70] had to be applied. For S. aureus, a required dose of 44.2 J/cm² was found [68]. However, inactivation below 1 log at 720.0 J/cm² has also been reported for both microorganisms [69]. Prasad et al. also found that the doses required for inactivation depended additionally on the irradiance of the UVA source. The higher the irradiance, the lower the doses required for inactivation. The reported inactivation doses were obtained with higher irradiances $(17-70 \text{ mW/cm}^2)$ [66–70] than the irradiance in our setup (9.8 mW/cm²). Thus, we assume, based on the reported correlation between irradiance and dose, that the doses applied to our samples $(17.6 \text{ J/cm}^2 \text{ to } 19.4 \text{ J/cm}^2)$ are not sufficient for inactivation by 1 log of both bacteria strains investigated. Hence, direct inactivation by UVA radiation within our experiments has a negligible effect.

Even though photocatalysis and superhydrophilicity are two different processes, it is assumed that they occur simultaneously on the surface of TiO_2 coatings upon activation with UVA radiation and the subsequent formation of electron-hole pairs [5,7,8]. The generated electron-hole pairs can either increase hydrophilicity or support photocatalysis. Therefore, it is concluded that one of these two processes is preferred, which in turn depends on the processing and composition of the coating [7]. Thus, it is possible that photocatalysis occurs during the second UVA irradiation in our microbiological regime, which contributes to the inactivation of the bacteria as well. When comparing UVA, UVB, and UVC radiation of the same irradiance, it has been reported in the literature that the efficiency of photocatalytic inactivation of bacteria on TiO_2 is the highest for UVC and UVB [71]. Considering this fact and the observation that our surfaces preferably seem to have a strongly hydrophilic character (Figures 6 and 7), the reduction of bacteria on TiO₂ in our experiments can be attributed mainly to the prevention of adhesion due to superhydrophilicity. However, even if photocatalytic behavior might contribute in small amounts to the inactivation of bacteria, this would pose an advantageous effect for the envisioned application in water-bearing systems, as it additionally assists the cleanliness of the surface by photocatalytic decomposition of adhered particles or other sorts of dirt and organic contaminants. Possible photocatalytic effects only occur during irradiation with UV light [10,40,42]. In the time between two steps of UVA irradiation for activation, the superhydrophilicity provides the long-term sanitation of the surfaces by adhesion prevention, as it lasts more than 24 h to up to 4 days, as shown in Section 3.2 (Figure 6).

We showed with our study that the investigated TiO_2 coatings on stainless steel can be activated by UVA irradiation. This leads to hydrophilization of the surface and thus prevents microbial adhesion and colonization, which are the initial steps of biofilm formation. The stability of superhydrophilicity upon surface activation by UVA irradiation and regular reactivation within 24 h allows this setup to be used for the selective sanitation of water-bearing pipes by the prevention of biofilm formation or spread in pipes. Thus, biofilm-induced problems in pipe systems, such as the spread of infections in hospitals [18,19], the corrosion of pipelines [17], or the contamination of food processing equipment [15,16] can be prevented.

4. Conclusions

Within our study, TiO₂ films were deposited on various substrates, and their surface characteristics were analyzed by WCA, SEM, and XRD. Samples were conditioned for our experiments in a defined manner to guarantee reproducible initial conditions. Using WCA measurements, we demonstrated that the deposited TiO₂ coatings can be activated by UVA irradiation, resulting in superhydrophilicity. Superhydrophilicity (WCA $< 10^{\circ}$) appears regardless of the substrate material or the presence of a Ti interlayer. However, the differences in the coatings, such as roughness, microscopic morphology, and composition, affect the stability of superhydrophilicity. The longest-lasting superhydrophilicity was observed up to 4 days for TiO_2 on stainless steel substrates. Reactivation of the coatings by UVA showed reproducible wetting behavior, which highlights the potential for the longterm stability of superhydrophilicity by periodic reactivation. However, this point should be confirmed by several reactivation cycles in further studies. Because of the good results of TiO₂ coated on stainless steel, this coating–substrate combination was selected for further microbiological investigations on curved samples. In the microbiological studies with a specially developed regime adapted to the envisioned application in water-bearing pipes, we inoculated the activated samples with 10³ CFU/mL of *E. coli* and *S. warneri* and showed that the selected TiO₂ coating completely prevents microbial surface colonization for the waterborne pathogen E. coli and the biofilm former S. warneri. With these results, we were able to show that our developed setup provides a good basis for the envisioned application. To confirm the benefits for other use cases and more complex systems, further applicationrelevant microorganisms, such as Legionella or Pseudomonas aeruginosa, could be tested in future studies. Although our coating has shown very good superhydrophilic performance, to confirm its efficacy in different applications, it should be tested whether applicationdependent parameters of water chemistry (pH, organics, salts) affect the performance of the coating.

The presented results and the long-term stability of superhydrophilicity that can be achieved by reactivation demonstrate the potential of the TiO_2 coating to sanitize waterbearing pipes in terms of preventing microbial surface colonization and biofilm formation.

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