Photocatalytic Water Disinfection under Solar Irradiation by D-Glucose-Modified Titania

Agata Markowska-Szczupak [1],*, Paulina Rokicka [1], Kunlei Wang [2], Maya Endo [2], Antoni Waldemar Morawski [1] and Ewa Kowalska [2]

[1] Institute of Inorganic Technology and Environment Engineering, West Pomeranian University of Technology in Szczecin, Pulaskiego 10, 70-322 Szczecin, Poland; rokicka16@poczta.onet.pl (P.R.); amor@zut.edu.pl (A.W.M.)

[2] Institute for Catalysis, Hokkaido University, N21, W10, Sapporo 001-0021, Japan; kunlei@cat.hokudai.ac.jp (K.W.); m_endo@cat.hokudai.ac.jp (M.E.); kowalska@cat.hokudai.ac.jp (E.K.)

* Correspondence: Agata.Markowska@zut.edu.pl; Tel.: +48-91-449-42-30

check for updates

Abstract: Modified titania photocatalysts were synthesized by the pressure method using titanium(IV) oxide from Grupa Azoty Zakłady Chemiczne "Police" S.A., Police, Poland, and D-glucose solution. Characterization of obtained composites was performed by X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), elemental analysis, and measurements of zeta potential and specific surface area (SSA). The possibility of using glucose-titania composites as photocatalysts for simulated solar-assisted disinfection against gram-negative *Escherichia coli* and gram-positive *Stapchyloccocus epidermidis* bacteria were examined in two reaction systems, i.e., for suspended and immobilized photocatalysts (on the concrete). It was found that an increase in the D-glucose concentration, i.e., higher carbon content, led to a decrease in antibacterial properties. The sample obtained from 1% of D-glucose solution at 100 °C (TiO$_2$-1%-G-100) showed superior photocatalytic activity under UV-Vis irradiation toward both bacteria species. Water disinfection was more efficient for suspended photocatalyst than that for supported one, where complete disinfection was reached during 55–70 min and 120 min of irradiation, respectively. For the first time, it has been shown that titania modified with monosaccharides can be efficiently used for water disinfection, and the immobilization of photocatalyst on the concrete might be a prospective method for public water supplies.

Keywords: C/TiO$_2$; photocatalysis; solar radiation; disinfection; immobilized catalyst

1. Introduction

In recent years, the intensification of research directed to delivery new, ecological, and cost-effective disinfection methods that are focused on increased water safety has taken place. It seems that the photocatalytic oxidation with titanium(IV) oxide (TiO$_2$, titania) fulfills above conditions. Unfortunately, the majority of commercially available titania photocatalysts are the first-generation catalyst, which means that their activity occurs only under UV irradiation, i.e., with wavelengths (λ) shorter than 400 nm. Therefore, the amount of solar radiation reaching Earth's surface might be insufficient for photocatalyst activation. In order to enhance the photocatalytic activity of TiO$_2$ under solar radiation, what is essential from the economical point of view, different ways of titania modification have been widely studied, e.g., doping, surface modification, and heterojunction. Various physical and chemical strategies, and many kinds of modifiers, such as metals, metal oxides, and non-metals have been used for titania modification, particularly noble metals (e.g., Au, Ag) and nonmetals (e.g., S, C, N) [1–4]. Scientific studies on nonmetal modification started about two decades ago [5]. Among them,

the aliphatic alcohols, urea, thiourea, calcium carbide, activated carbon, multi-wall carbon nanotubes, and graphene have been used. It has been shown that carbon modification of titania resulted in significant enhancement of photocatalytic activity under solar radiation and even under sole visible irradiation [5–9]. The main advantage of carbon modification of TiO_2 is that the simple and fast methods of preparation can be used. It has been proved that C/TiO_2 demonstrates better photocatalytic activity than un-modified TiO_2 for organic (including phenol and organic dyes) or inorganic (e.g., nitrogen oxides) compounds' degradation [6,8,10–12]. However, except our own research [13–15], only a few studies on disinfection using carbon-modified TiO_2 have been performed [16–18]. For example, titania modified with carbon from coconut shell, alcohols, and carbide presents better biocidal properties than un-modified titania, even under irradiation with visible light. Moreover, it should be mentioned that preliminary results on antibacterial properties for other modified titania samples (with graphene oxide) were quite promising [15]. Therefore, the antibacterial properties of sunlight-activated D-glucose-modified titania was evaluated in the present study.

2. Results

2.1. Characteristics of D-Glucose-Modified TiO$_2$

Starting material contained majority of amorphous titania (ca. 75.0%, data from producer), and thus had very high specific surface area (SSA) of 312 m^2 g^{-1}, whereas crystalline part was composed mainly of anatase (95.0% and 5% of rutile). Thermal treatment did not cause significant changes in the crystalline composition of titania, where only slight phase transition was noticed, i.e., from amorphous titania to anatase, resulting in a slight increase in anatase content to ca. 98% and a decrease in SSA from 312 m^2 g^{-1} to 266, 158, and 88 m^2 g^{-1} after annealing at 100 °C, 150 °C, and 200 °C, respectively. Similarly, the presence of D-glucose practically did not influence the crystalline composition of photocatalysts, and anatase phase was predominant form of titania also in those samples (97.6–98.1%), as shown in Table 1. The largest anatase crystallites and the smallest SSA were obtained for the sample that was prepared with the smallest content of D-glucose at the highest temperature (TiO$_2$-G-1%-200). An increase in annealing temperature caused an increase in anatase crystallite size and a decrease in SSA (Figure 1a). It is well known that thermal treatment results in increases in both crystalline and particle sizes (a decrease in SSA), due to transformation of amorphous titania (e.g., amorphous layer on nano-crystals) and particle sintering/aggregation, respectively. In contrast, an influence of D-glucose on surface properties indicates that D-glucose restrains crystal growing (crystallite size of anatase in un-modified samples annealed at 100 °C, 150 °C, and 200 °C were larger, i.e., 12.0, 16.3, and 21.9 nm, respectively). It has been reported that titania modifiers could influence crystal growth either positively or negatively. For example, Grzybowska et al. found an increase in SSA after surface modification of titania with calcium, tungsten, iron, and aluminum, suggesting that adsorbed modifiers on titania surface disturbed in particles' sintering [19]. Usually, a decrease in crystallite size corresponds to an increase in SSA. Contrary, D-glucose-modification resulted in a decrease in both crystallite size and SSA for samples annealed at 100 °C (with an increase in D-glucose content), whereas higher temperatures of annealing resulted in a significant increase in SSA. Therefore, it is proposed that photocatalysts that were annealed at lower temperature could be composed of fine titania nanoparticles (NPs) covered with a thin layer of adsorbed D-glucose, whereas for those annealed at higher temperatures the presence of carbon (decomposed D-glucose) inhibited significantly particles' aggregation, as also proved by a decrease and an increase in micropore volume, respectively (Figure 1c). Interestingly, in the case of modified samples annealed at higher temperatures, the largest values of SSA were obtained for those with middle content of D-glucose (5%), suggesting that smaller content (1%) was not sufficient to prevent particles' aggregation, whereas too large content (10%) could form a thick layer on titania surface, as confirmed by pore size distribution (a decrease in total pore volume and mesopore volumes, Figure 1b,d). The carbon content increased with an increase in D-glucose content, as shown in Table 1.

Table 1. Physicochemical properties of photocatalysts used in this study (commercial photocatalysts: starting TiO$_2$ (from Grupa Azoty Zakłady Chemiczne "Police" S.A., Police, Poland) and KRONOClean 7000), and starting TiO$_2$ modified with D-glucose.

Sample Name	Anatase Content [%] [a]	Anatase Crystallite Size [nm]	SBET [m^2/ g]	Carbon Content (wt %)	Zeta Potential ζ [mV]
Starting TiO$_2$	95.0	11.0	312	0.0	−24.13
KRONOClean7000	100.0	11.0	242	0.96	−17.71
TiO$_2$-G-1%-100	97.8	11.7	268	0.59	−13.49
TiO$_2$-G-5%-100	97.6	11.4	252	2.23	−25.28
TiO$_2$-G-10%-100	97.6	11.2	214	4.49	−26.49
TiO$_2$-G-1%-150	98.0	16.0	155	0.49	−17.04
TiO$_2$-G-5%-150	98.1	13.4	206	2.10	−20.24
TiO$_2$-G-10%-150	97.8	11.4	195	3.91	−23.70
TiO$_2$-G-1%-200	98.0	21.8	87	0.29	−16.80
TiO$_2$-G-5%-200	97.9	17.2	120	1.90	−18.72
TiO$_2$-G-10%-200	97.9	17.3	110	3.37	−23.34

[a] anatase content considering only crystalline forms (anatase + rutile).
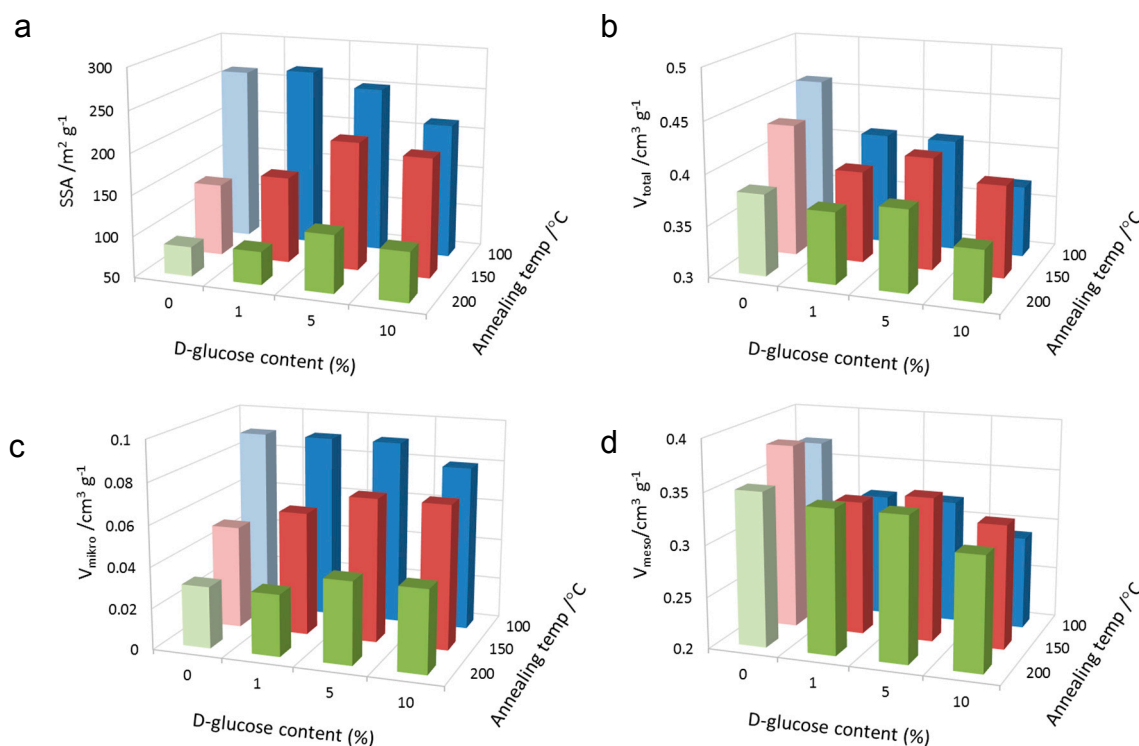

Figure 1. BET (Brunauer, Emmett and Teller) results for un-modified (D-glucose content: 0) and modified with D-glucose (1%, 5% and 10%): (**a**) specific surface area (SSA), (**b**) total pore volume (V$_{total}$), (**c**) micropore volume (V$_{micro}$), and (**d**) mesopore volume (V$_{meso}$).

However, an increase in annealing temperature resulted in a decrease in carbon content, especially for samples that were prepared at 200 °C (Table 1). This decrease in carbon content should be caused by thermal decomposition of D-glucose, which usually starts at ca. 165 °C [20]. Therefore, it is proposed that high pressure in an autoclave could accelerate D-glucose decomposition (at lower temperatures), similarly as reported for D-glucose instability during HPLC analysis [21]. Obviously, the presence of D-glucose on titania surface resulted in changes of zeta potential from −19.49, −21.09 and −21.44 mV for samples annealed at 100 °C, 150 °C, and 200 °C, respectively, to values shown in Table 1.

To investigate surface composition of titania after modification, X-ray photoelectron spectroscopy (XPS) analysis has been performed for three samples: starting TiO_2, TiO_2-100 (starting TiO_2 thermally treated at 100 °C), and TiO_2-G-1%-100 (starting TiO_2 modified with 1% of D-glucose and annealed at 100 °C; the most active sample), and the obtained results are shown in Figure 2 and Table 2. It was found that the surface of titania was enriched with oxygen as the ratio of oxygen to titanium exceeded the stoichiometric ratio (2) being 2.2, which is typical for various titania samples, due to adsorption of water and carbon dioxide on the titania surface. For example, oxygen enrichment of titania was reported for samples that were prepared by the laser ablation (O/Ti = 2.5) [22], the microemulsion (O/Ti = 4.6) [23], the hydrothermal (O/Ti = 5.2) [24] and the gas-phase (O/Ti = 7.7) [24] methods. Carbon is present in all titania samples (and also other oxides), mainly due to adsorption of carbon dioxide from air (forming bicarbonate and mono- and bidentate carbonate [25]), but also from precursors that are used for titania synthesis, such as titanium alcoholates. XPS analysis proved the surface modification of titania with D-glucose since the ratio of carbon to titanium increased by almost double after sample modification with 1 wt % of D-glucose. The banding energies of titanium, oxygen, and carbon were estimated by deconvolution of Ti $2p_{3/2}$, O 1s, and C 1s peaks, respectively, accordingly to published reports on XPS analysis of titania samples [26–31]. Titanium consisted mainly Ti^{4+}, and the reduced form of titanium (Ti^{3+}) did not exceed 2.2%. D-glucose presence and thermal treatment practically did not change form of titanium. Deconvolution of oxygen peak into three peaks at ca. 529.6 eV, 531.3 eV, and 533.3 eV, respectively, confirmed the co-existence of three forms of oxygen, i.e., (i) lattice oxygen in TiO_2, (ii) oxygen bound to: carbon (C=O), titanium in Ti_2O_3, and hydroxyl groups bound to two titanium atoms, (iii) hydroxyl groups that were bound to titanium or carbon (Ti-OH/C-OH). A significant increase in the content of oxygen in the form of hydroxyl groups (from 1.4 to 6.8) and an increase in the carbon content in the form of carbon bound to carbon (C-C) for TiO_2-G-1%-100 sample confirmed the presence of adsorbed D-glucose on the titania surface.

To examine photocatalytic activity of prepared samples, the generation of hydroxyl radicals under irradiation was performed, and the obtained data are shown in Figure 3. It was found that titania modification with glucose resulted in a significant enhancement of photocatalytic activity (more than twice), where the most active sample contained the smallest content of glucose (1 wt %) and was annealed at lowest temperature (100 °C), i.e., TiO_2-G-1%-100. An increase in annealing temperature resulted in a decrease in activity, which is probably due to glucose decomposition, as discussed above.

Table 2. Surface composition and fraction of oxidation states of Ti, O, and C from deconvolution of X-ray photoelectron spectroscopy (XPS) peaks.

Sample name	Content (at. %)			Ratio	
	Ti $2p_{3/2}$	O 1s	C 1s	O/Ti	C/Ti
Starting TiO_2	17.71	43.95	38.34	2.5	2.2
TiO_2-100	15.05	43.3	41.65	2.9	2.8
TiO_2-G-1%-100	12.1	36.06	51.84	3.0	4.3

Fraction of oxidation states								
Sample name	Ti $2p_{3/2}$ (%)		O 1s (%)			C 1s (%)		
	Ti^{4+}	Ti^{3+}	TiO_2	=O[a]	-OH[b]	C-C	C-OH	C=O
Starting TiO_2	97.9	2.1	47.2	50.0	2.8	58.7	30.1	11.2
TiO_2-100	98.1	1.9	45.0	53.6	1.4	54.7	37.2	8.1
TiO_2-G-1%-100	97.8	2.2	46.0	47.2	6.8	61.5	28.5	10.0

=O[a]: Ti-(OH)-Ti/Ti_2O_3/C=O; -OH[b]: Ti-OH/C-OH.
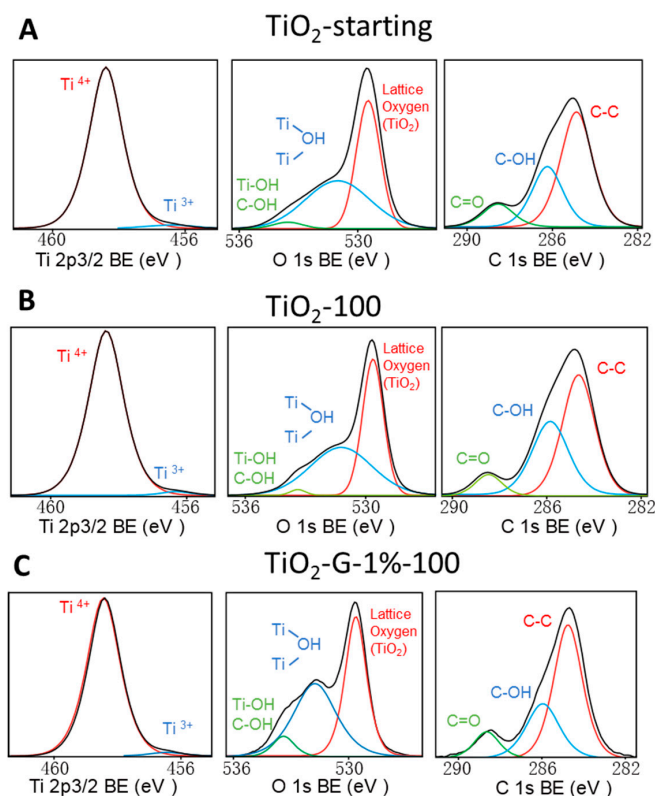
Figure 2. XPS results for Ti $2p_{3/2}$, O 1s, and C 1s for starting TiO_2 (**A**; top), TiO_2-100 (**B**; middle), and TiO_2-G-1%-100 (**C**; bottom).
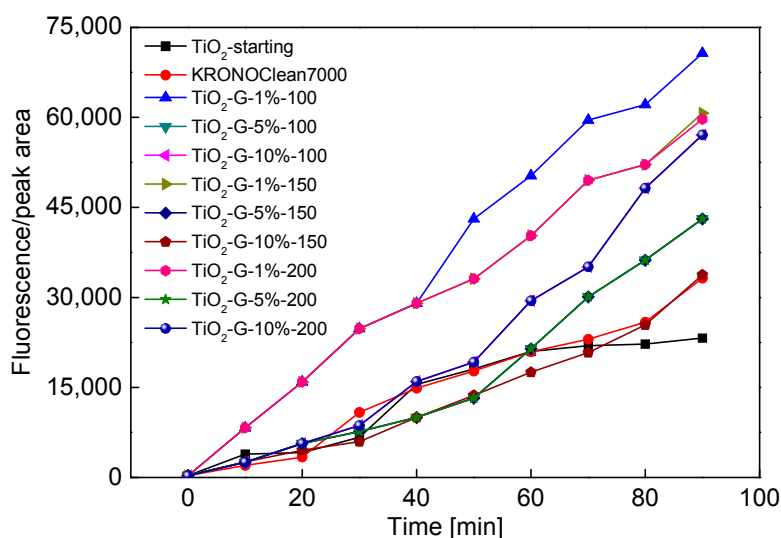

Figure 3. The amount of generated 2-hydroxyterephthalic acid (fluorescence intensity) during 90 min of UV-Vis irradiation.

2.2. Dose-Dependent Photocatalytic Inactivation of Escherichia coli and Staphylococcus epidermidis

The correlation between disinfection duration and dose of the glucose-modified photocatalyst with the highest photocatalytic activity (i.e., rate of hydroxyl radicals' formation), i.e., TiO_2-G-1%-100 sample, was examined. The fastest disinfection process under UV-Vis irradiation (after 70 min) was obtained for photocatalyst dose of 0.1 g $\times$ dm^{-3} (Figure 4a). It was found that both an increase and

a decrease in TiO$_2$-G-1%-100 dose resulted in extension of disinfection duration. All of the differences were statistically significant (Table 3). Taken into account the obtained results, it was assumed that 0.1 g per dm^3 was an optimal dose for photocatalytic disinfection. Next, the influence of bacteria concentration (*E. coli* and *S. epidermidis*) on disinfection duration was studied, and the obtained data are shown in Figure 4b. The necessary duration of disinfection did not differ significantly for both bacteria solutions in the case of their low concentration from 1.5×10^4 CFU $\times$ cm^{-3} to 1.5×10^5 CFU $\times$ cm^{-3} (Table 3), reaching complete disinfection during 60 min of UV-Vis irradiation (Figure 4b). However, an increase in bacteria concentration resulted in a significant extension of necessary disinfection time, even up to 120 min for concentration of 1.5×10^8 CFU.
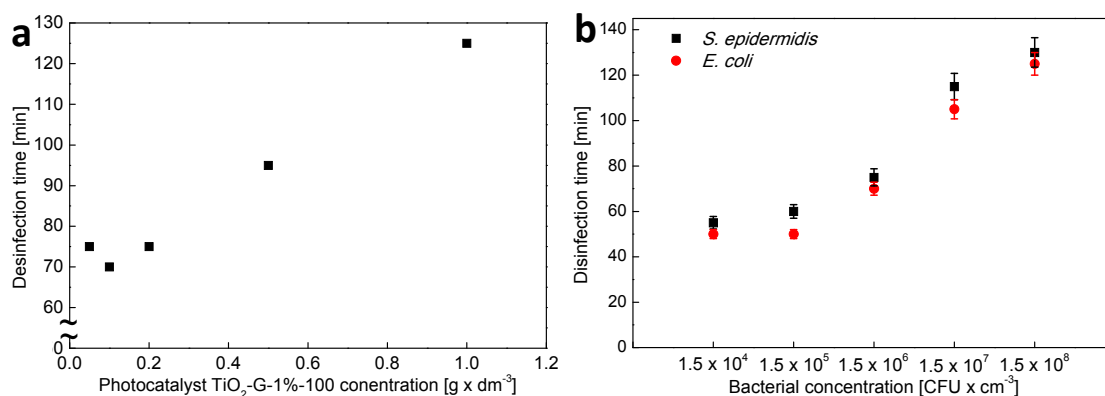

Figure 4. The correlations between disinfection time and: (**a**) photocatalyst TiO$_2$-G-1%-100 content, and (**b**) bacteria concentration.

Table 3. Statistical analysis for factors influencing *E. coli* disinfection time.

Photocatalyst TiO$_2$-G-1%-100 Concentration [g $\times$ dm^3]	0.05	0.1	0.2	0.5	1.0
0.05	-	SI	SI	*	***
0.1	SI	-	SI	*	***
0.2	*	*	*	-	***
0.5					
1.0	***	***	***	***	-
Initial concentration of *E. coli* [CFU $\times$ cm^3]	1.5×10^4	1.5×10^5	1.5×10^6	1.5×10^7	1.5×10^8
1.5×10^4	-	SI	SI	**	***
1.5×10^5	SI	-	SI	**	***
1.5×10^6	SI	SI	-	**	***
1.5×10^7	**	**	**	-	*
1.5×10^8	***	***	***	*	-
Initial concentration of *S. epidermidis* [CFU $\times$ cm^3]	1.5×10^4	1.5×10^5	1.5×10^6	1.5×10^7	1.5×10^8
1.5×10^4	-	SI	SI	**	***
1.5×10^5	SI	-	SI	**	***
1.5×10^6	SI	SI	-	**	***
1.5×10^7	**	**	**	-	*
1.5×10^8	***	***	***	*	-

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; SI—statistically insignificant.

2.3. Influenece of D-*Glucose Content, Used for TiO$_2$ Modification at 100 °C, on Disinfection Properties*

The influence of D-glucose content, used for titania modification at 100 °C, was investigated. It was found that disinfection duration strongly depended on the D-glucose content, as shown in Figure 5. TiO$_2$-G-1%-100 photocatalyst led to the complete inactivation of gram-negative bacteria *E. coli* within 70 min (Figure 5a) and gram-positive bacteria *S. epidermidis* within 85 min (Figure 6a) under UV-Vis irradiation. Scanning electron microscopy (SEM) revealed the significant change in bacterial morphology (e.g., shape, disruption of outer membranes, etc.) after photocatalytic process, as shown

in Figures 5c and 6c. In contrast, all of the photocatalysts (commercial and modified) did not cause the change in bacterial number in dark conditions (Figures 5b and 6b).
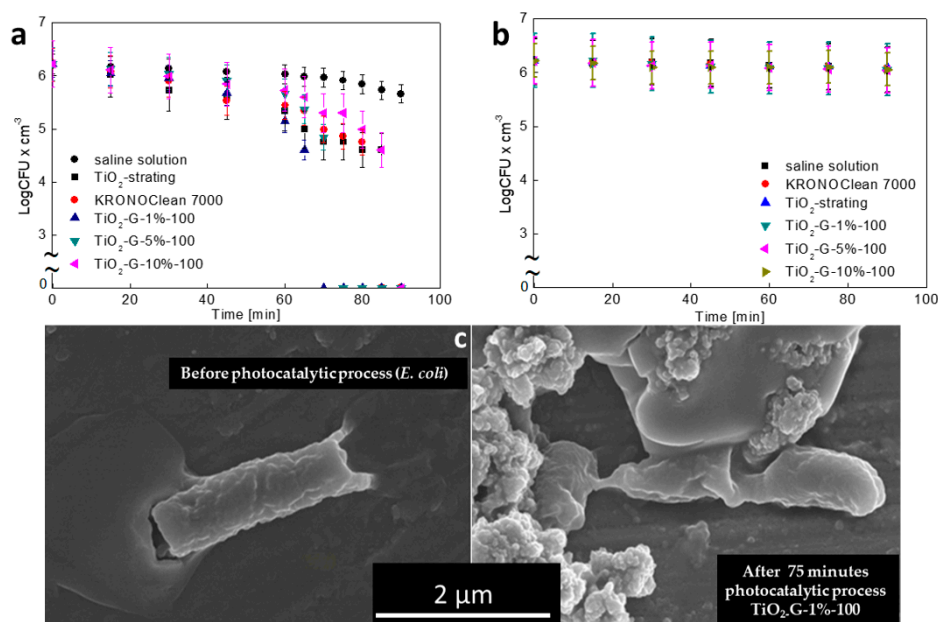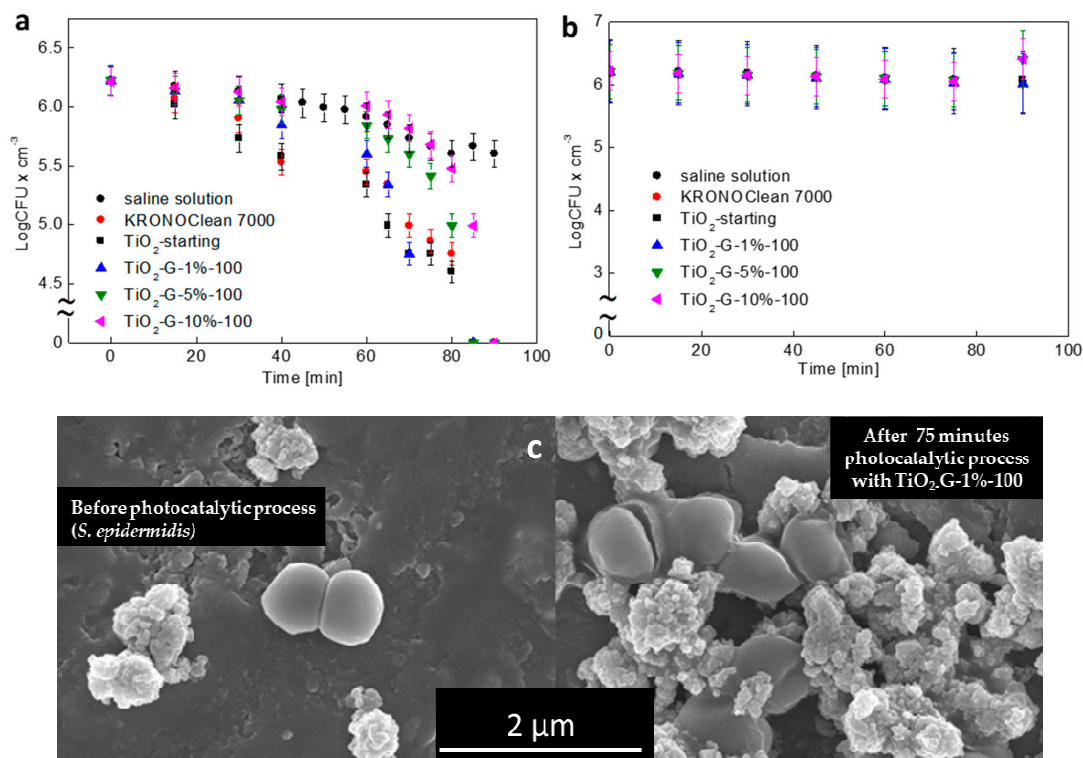

Figure 5. Antibacterial activity against *E. coli* of commercial and D-glucose-modified titania: (**a**) under UV-Vis irradiation, and (**b**) in dark conditions; and, (**c**) SEM images of *E. coli* before (left) and after 75 min of UV-Vis irradiation (right) in the presence of 0.1 g × dm^3 TiO$_2$-G-1%-100.

Figure 6. Antibacterial activity against *S. epidermidis* of commercial and D-glucose-modified titania: (**a**) under UV-Vis irradiation, and (**b**) in dark conditions; and, (**c**) SEM images of *S. epidermidis* before (left) and after 75 min of UV-Vis irradiation (right) in the presence of 0.1 g × dm^3 TiO$_2$-G-1%-100.

2.4. Influenece of D-Glucose Content, Used for TiO₂ Modification at 100 °C, on Enzymatic Activity

The enzymatic activity, i.e., catalase activity (CAT) and superoxide dismutase activity (SOD), secreted by *E. coli* and *S. epidermidis*, under UV-Vis irradiation and in the dark, was investigated for commercial and D-glucose-modified (at 100 °C) photocatalysts. The tests were performed before and after 30, 60, 90, and 120 min of process. The enzymatic activity was not observed from 90 min of process, and thus the obtained results were only presented till 60 min, as shown in Figures 7 and 8. It was demonstrated that without activation (dark conditions) all of the photocatalysts did not influence the CAT and SOD activity (Figures 7 and 8). However, 30-min UV-irradiation caused an increase in CAT and SOD enzyme activities, whereas next 30-min UV-Vis irradiation resulted in a decrease in those activities below the initial levels.
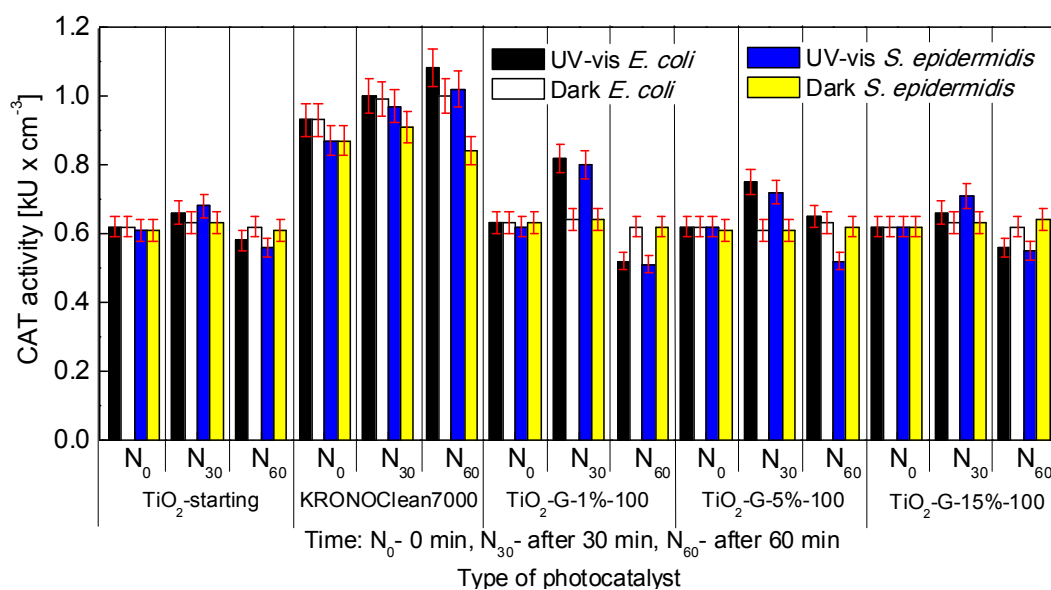

Figure 7. Influence of commercial and D-glucose-modified titania under UV-Vis irradiation and in dark conditions on *E. coli* and *S. epidermidis* catalase activity (CAT).
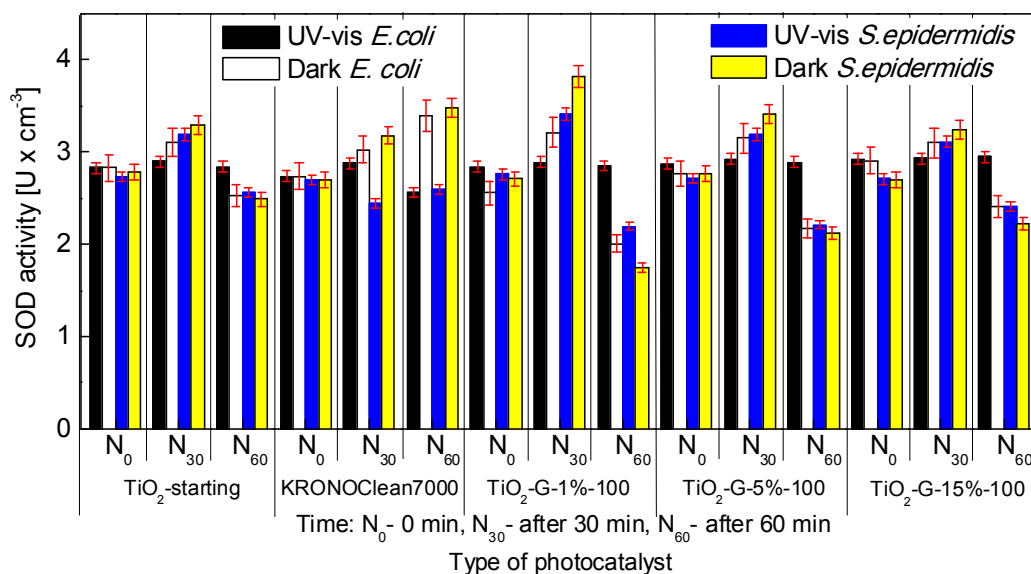

Figure 8. Influence of commercial and D-glucose-modified titania under UV-Vis irradiation and in dark conditions on *E. coli* and *S. epidermidis* superoxide dismutase activity (SOD).

2.5. Influenece of D-Glucose Content, Used for TiO_2 Modification at 100 °C, on Bacteria Mineralization

To study the probability of complete decomposition of bacterial cells (mineralization), the liberation of carbon dioxide during UV-Vis irradiation and in the dark, were investigated. It was found that, indeed, bacterial cells could be efficiently decomposed only under photocatalytic process (Figures 9a and 10a), whereas no changes in carbon dioxide concentration were noticed in the dark (Figures 9b and 10b). Moreover, the fastest evolution of carbon dioxide was observed for TiO_2-G-1%-100 sample with the lowest content of carbon among the modified samples, confirming its highest photocatalytic activity.
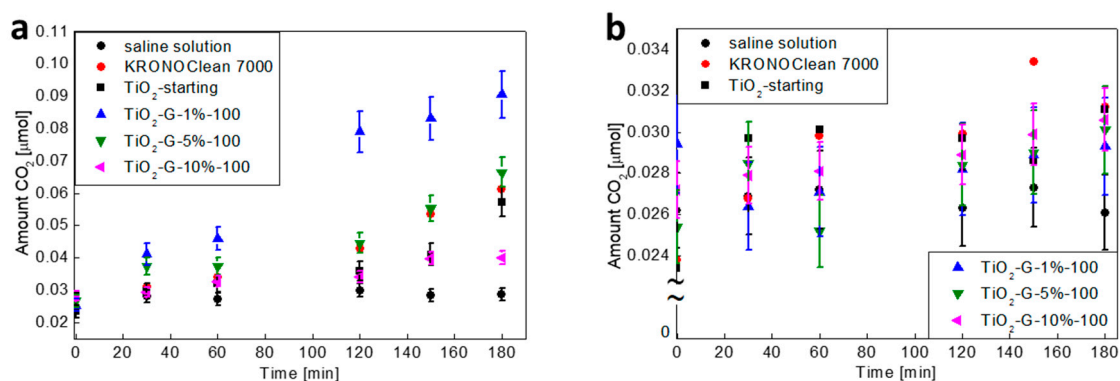

Figure 9. Evolution of CO_2 during mineralization of *E. coli* cells under: (**a**) UV-Vis irradiation, and (**b**) in the dark on commercial and D-glucose-modified titania.
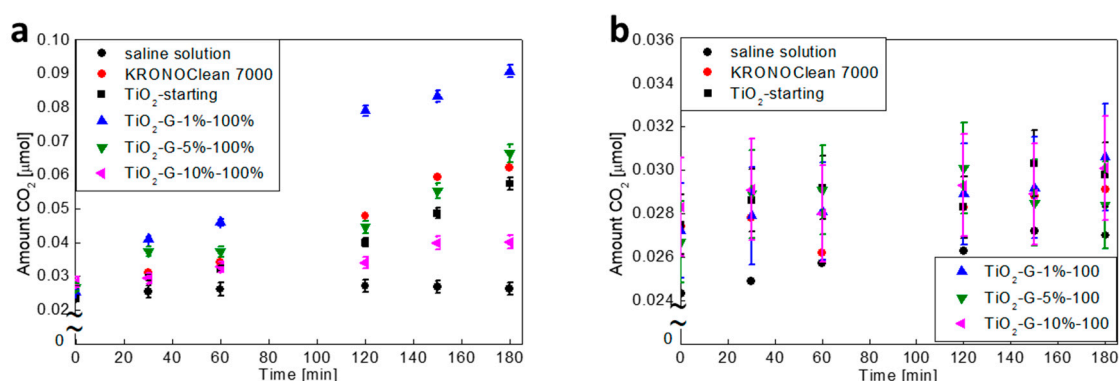

Figure 10. Evolution of CO_2 during mineralization of *S. epidermidis* cells under: (**a**) UV-Vis irradiation, and (**b**) in the dark on commercial and D-glucose-modified titania.

2.6. Commercialization of Antimicrobial Photocatalysts for Building Materials

To check the commercial feasibility of antimicrobial photocatalysts, cement mortars were supplemented with 10 wt % of TiO_2-G-1%-100 photocatalyst and tested for *E. coli* inactivation. It was clearly demonstrated that D-glucose-modified titania contributed to the additive benefits of the final product. Under UV-Vis irradiation the obtained concrete exhibited disinfection potential, as shown in Figure 11. The control experiments (concrete without photocatalyst) of *E. coli* inactivation did not show any significant differences in the number of bacteria after 120-min incubation in dark condition and under light irradiation. In contrast, in water being in contact with the plate, made of concrete supplemented with TiO_2-G-1%-100, the complete disinfection was achieved after only 120 min under UV-Vis irradiation (Figure 11).
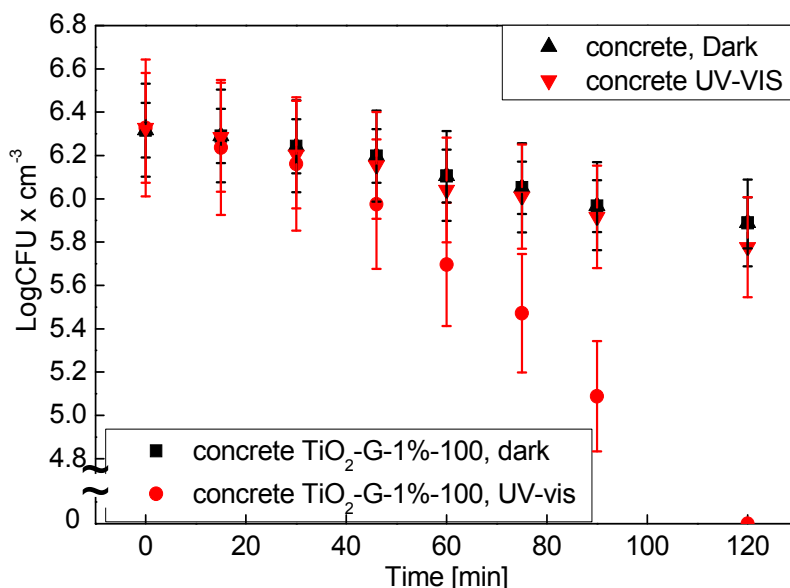
Figure 11. Antibacterial activity (against *E. coli*) of concrete supplemented with 10% TiO_2-G-1%-100 and reference samples (concrete without TiO_2) under UV-Vis irradiation and in dark condition.

3. Discussion

Antibacterial properties of titania have received a great attention by many researchers [2,14,18,32–34], and thus studies on TiO_2 disinfection under solar radiation are mainstream research and development [33]. Therefore, in this study it has been hypothesized that surface modification of titania by D-glucose could result in development of antimicrobial agent towards both gram-negative (*Escherichia coli*) and gram-positive (*Staphylococcus epidermidis*) bacteria under solar radiation. Physicochemical characteristics of D-glucose-modified titania at 100 °C showed that this group of samples has various advantageous, such as large SSA (214–268 $m^2 \times g^{-1}$), large content of anatase (97.6–97.8%), and small size of anatase crystallites (11.2–11.7 nm). The photocatalytic activity of the D-glucose-modified TiO_2 was examined for the generation of •OH radicals under UV-Vis light irradiation. The most reactive photocatalyst, inducing the fastest *E. coli* and *S. epidermidis* inactivation, was obtained by titania modification with 1% of D-glucose solution at 100 °C (TiO_2-1%-100). The enhancement of photocatalytic activity (in the visible range of solar spectrum ($\lambda > 400$ nm)) has been reported for TiO_2 modified with glucose by Kim at al. [35]. According to these studies, glucose-adsorbed TiO_2 can form the charge transfer complex by ligand-to-metal charge transfer (LMCT) sensitization mechanism. Moreover, enhanced photocatalytic activity under UV irradiation could be achieved by the hindering of electron–hole recombination, since photogenerated holes could be trapped by hydroxyl groups [36]. Indeed, the enrichment of titania surface with hydroxyl groups was observed after its modification with D-glucose (TiO_2-1%-100), as shown in Table 2.

In the literature, there are many reports concerning the optimization procedures for photocatalyst and bacterial doses used for water disinfection. The results that were obtained by various teams differ significantly. For example, Herrmann [37] found that 2.5 g $TiO_2 \times dm^{-3}$ was the best value corresponding to the maximal photon absorption (all of the particles could be illuminated). However, Cho et al. [38] determined that the bactericidal activity of P25 at concentration of 1.0 g $\times dm^{-3}$ was two times more effective than that at 0.1 g $\times dm^{-3}$, whereby increasing the TiO_2 concentration from 1.0 to 2.0 g $\times dm^{-3}$ did not enhance the inactivation efficiency. Therefore, it is proposed that for disinfection process less content of titania is required than that for decomposition of organic compounds. The presented results in this report have confirmed this hypothesis, but much less content of titania (0.1 g $\times dm^{-3}$) than that by Cho et al. [38] appeared to be an optimal value.

The most effective concentration of bacteria (*E. coli* and *S. epidermidis*) was in the range from 1.5×10^4 CFU $\times$ cm^{-3} to 1.5×10^5 CFU $\times$ cm^{-3}, confirming the previous findings by Benabbou et al. [39]. Necessary time for complete inactivation of microorganisms was longer in the case of more concentrated bacterial suspension due to the shielding effect by bacteria cells. It was found that conditions of disinfection, such as photocatalyst content and bacterial concentration, have had a critical influence on the obtained results, and the optimal conditions must be experimentally determined.

Unfortunately, there are no reports on the antibacterial properties of glucose-modified titania prepared at temperatures lower than 300 °C, and thus the direct comparison of our results with others was impossible. The best antibacterial activity was obtained for the sample that was modified with 1% of D-glucose at 100 °C (TiO$_2$-1%-100). Under UV-Vis irradiation, the complete disinfection of *E. coli* and *S. epidermidis* was achieved after 70 and 75 min, respectively, whereas carbon-modified commercial photocatalyst KRONOClean 7000 was less efficient, needing 85 min and 90 min, respectively, of UV-Vis irradiation. Therefore, it is proposed that the form of carbon is crucial for antimicrobial properties, i.e., hydroxyl groups in glucose structure responsible for holes' trapping.

Antioxidant enzymes, secreted by bacteria, are considered to be important indicators for the oxidative stress. Catalase (CAT) and superoxide dismutase (SOD) are two commonly investigated intracellular enzymes [35,40]. CAT is an antioxidant enzyme catalyzing the decomposition of H$_2$O$_2$ to water and oxygen, whereas SOD is a metalloenzyme catalyzing the dismutation of •O$_2$ into H$_2$O$_2$ and O$_2$. Accordingly, the loss of their activity accelerated the accumulation of reactive oxygen species (ROSs) and led to the deficiency of cell viability. On the basis of the obtained results, the two-step mechanism of response to the presence of photocatalyst, activated under UV-Vis irradiation, was described. It was found that the induced levels of these enzymes increased during the first 30 min, and then decreased (after 60 min). The changes in enzyme activity during the photocatalytic disinfection process indicated that defense capacity was overwhelmed by the rapidly created ROSs at the initial stage, and then suppressed gradually. The correlation between high amount of generated hydroxyl radicals (Figure 3) during 90 min of UV-Vis irradiation and the loss of enzymatic activity suggested that oxidative stress could act as an important pathway by which photocatalyst induced bacterial death. Interestingly, no significant differences in the oxidative stress response between two examined bacteria species was found. The protein nature of enzymes determines their quick and explicit reaction on various environmental factors whether naturally or artificially introduced, such as photocatalyst. Despite short lifetime of ROSs, generated on the surface of photoexcited photocatalyst, they may probably cause conformational changes (in secondary structure), damage of the active sites or irreversible loss of the activity.

It was found that carbon content was the main factor influencing antibacterial efficiency of D-glucose modified photocatalysts, and larger than 1% content of D-glucose was detrimental for activity. Similarly, it was reported that too high carbon content on the photocatalyst surface could block the active sites decreasing photocatalytic decomposition of organic compounds, such as phenol [41] and methylene blue [42]. The results of mineralization of the bacterial cells clearly indicated that the amount of CO$_2$, liberated from TiO$_2$-1%-100 suspension under UV-Vis irradiation, was 1.5 times higher than that from suspension containing commercial photocatalyst KRONOClean 7000 or starting TiO$_2$. Although an increase of CO$_2$ concentration proved the mineralization of bacteria cells, this did not guarantee water "microbial purity", as reported by Kacem at al. [43]. Photoexcited TiO$_2$ could cause only the partial mineralization of bacterial cells that could lead to bacteria transition to viable, but nonculturable (VBNC) state. This apparent dormant state, in which bacterial cells are metabolically active, maintains their pathogenic features, but bacteria cannot grow on culture media. Therefore, it is necessary to introduce genetic methods, based on the quantification of DNA (e.g., polymerase chain reaction PCR), which may justify the complete destruction of bacterial DNA.

The interaction between bacteria and photocatalyst was observed by SEM observations. It was found that modified samples with 1% of D-glucose showed stronger adhesion to the bacterial cells. The carbon content of 0.59 (TiO$_2$-G-1%-100) and enrichment of the titania surface with hydroxyl groups,

resulted in a significant modification of surface properties, as clearly shown by zeta potential changes, and thus increased bacterial adhesion to the photocatalyst surface. It is very important finding since, according to the literature reports, the titania NPs with particle sizes larger than 8 nm cannot penetrate bacteria through the cell-wall porins of ca. 1.2-nm diameters [44]. Accordingly, the electrostatic interaction between photocatalyst and bacteria has a crucial significance for antibacterial activity and should be extensively investigated in future. It is well-known that gram-negative bacteria contain both anionic and zwitterionic phospholipids, whereas gram-positive bacteria contain predominantly anionic lipids. When considering the observation that was made by Clogston and Patri [45], that NPs with a zeta potential between -10 and $+10$ mV were considered as neutral, whereas NPs with zeta potentials that were lower than -30 mV were strongly anionic, and thus cationic particles would attract negatively charged bacteria (e.g., *E. coli* ζ ~35 mV). The obtained results confirmed these findings since the best antibacterial properties were found for TiO_2-G-1%-100 photocatalyst with the least negative zeta potential of -13.49 mV. Deterioration of antibacterial properties was observed for photocatalyst characterized by more negative ζ (<-20 mV), due to worse contact between bacterial cells and photocatalyst NPs. It is important to notice that bacteria cells are much larger than NPs of photocatalysts (approx. 180 times bigger), and thus stronger adsorption of photocatalyst on bacteria is advisable for better interaction. An attractive feature of this mechanism is that it can be used to design antimicrobial agents with selective toxicity against certain organisms.

The present work fulfilled previous studies on disinfection process. First time, the application of D-glucose-modified photocatalyst in a cement mortar for water disinfection was presented. The complete disinfection was achieved during 120-min contact of contaminated water with a small concrete plate, made of cement mortar and supplemented with 10 wt % of TiO_2-G-1%-100 photocatalyst. According to the obtained results, it would be possible to use solar energy to activate the D-glucose-modified photocatalyst, and thus disinfect water in a concrete container used for storage of drinking water or fish farms (e.g., trout ponds). Last mentioned application should improve health conditions in fish farms since the microbial purity of water is the most important factor affecting performance in aquaculture production systems.

4. Materials and Methods

4.1. Preparation of C/TiO₂

The intermediate product, taken directly from the production line of titanium(IV) oxide, from the Grupa Azoty Zakłady Chemiczne "Police" S.A., Police, Poland), was used as the starting material in the process of photocatalysts preparation. Titanium dioxide was treated with D-glucose (Chempur, Piekary Śląskie, Poland) solutions of various concentrations (1%, 5%, 10%) at various annealing temperature (100 °C, 150 °C, and 200 °C) in a pressure autoclave BHL-800 (Berghof, Eningen, Germany).

4.2. Characterisation of Photocatalyst

The characteristic of commercial and obtained photocatalyst was studied by measurement of specific surface area (SSA) by the N_2 adsorption-desorption method on a Quadrasorb SI analyzer (Quantachrome Instruments, Boynton Beach, FL, USA). The crystalline phase and crystal structures of prepared samples were identified by X-Ray Diffraction analysis (PANalytical Empyrean X-ray diffractometer, Malvern Panalytical Ltd., Melvern, UK) using Cu Kαradiation (λ = 1.54056 Å). Total carbon amount in the samples was calculated using CN628 elemental analyzer (LECO Corporation, St Joseph, MI, USA). Chemical composition of the surface (chemical state and content of titanium, oxygen, and carbon) was estimated by XPS JEOL JPC-9010MC with MgKα X-ray (JEOL, Tokyo, Japan). The zeta potential was determined by using ZetaSizerNanoSeries ZS (Malvern Instruments, Melvern, UK).

4.3. Antimicrobial Properties of Dispersed C/TiO$_2$

D-glucose-modified titania, starting TiO$_2$ (Grupa Azoty Zakłady Chemiczne "Police" S.A., Police, Poland), commercial photocatalyst KRONOClean 7000 (International Inc., Leverkusen, Germany) were dispersed in 10 cm^{-3} *Escherichia coli* (*E. coli*) K12 ATCC29425 or *Staphylococcus epidermidis* (*S. epidermidis*) ACCT 49461 suspension in a sterile sodium chloride aqueous solution (8.5 g dm^{-3}) in a glass test tube, and irradiated with lamp emitting artificial solar light (250 W OSRAM) under continuous stirring (using a magnetic stirrer at speed of 250 rpm) at 37 °C. The distance between the solution and the light source was fixed at ca. 15 cm. The radiant flux was monitored with a Radiation Intensity Meter LB901/WCM3 & PD204AB cos. sensor meter. The photocatalyst and bacterial cell concentration was adjusted, according to the results described in Section 2.2. The control experiments in darkness and for NaCl solution were also performed. Serial dilutions (10^{-1} to 10^{-6}) were prepared in saline solution (0.9%). The samples were placed on Plate Count Agar (PCA, BTL Polska Sp. z.o.o, Warszawa, Poland). The plates were incubated for 24 h at 37 °C and then colony forming unit (CFU $\times$ cm^{-3}) was counted. Statistical analysis of obtained results was conducted using Excel spreadsheet. The comparisons among the means and the statistical significance of differences were evaluated by Tukey's test. Microscopic bacteria specimen were prepared according to general protocol and dehydrated by graded series of ethanol (30, 50, 70, 90, 95, and 99.5% (*v/v*)). Samples were sputtered with gold for 60 s from three-directions and observed by SEM (SU8020 UHR FE-SEM, Hitachi, Tokyo, Japan). The bacteria mineralization rate was calculated as amounts of liberated CO$_2$ (gas chromatography; GC 8610C; SRI Instrument Inc., Torrance, CA, USA) after 180 min irradiation or under dark condition. The catalase (CAT) and superoxide dismutase (SOD) in bacterial suspension were determined before photocatalytic process (N$_0$) and after 30 (N$_{30}$) and 60 (N$_{60}$) min of irradiation and in dark conditions. Catalase activity was determined spectrophotometrically at 25 °C (1-cm cuvette, spectrophotometer F-2500, Hitachi, Tokyo, Japan) by monitoring the change in absorbance A (λ = 374 nm), according protocols that are given in [46] and own modification. One unit (U) of activity was defined as the amount of enzyme that catalyses the oxidation of 1 mmol H$_2$O$_2$ in 1 min under the assay conditions. Superoxidase dismutase activity was determined spectrophotometrically at 25 °C (1-cm cuvette, spectrophotometer F-2500, Hitachi, Tokyo, Japan) by monitoring the change in absorbance A (λ = 420 nm), according to [47] and own modification. One unit of SOD activity inhibits the rate of pyrogallol oxidation at 25 °C by 50% in 1 min.

4.4. Antimicrobial Properties of Employed in Concrete TiO$_2$-G-1%-100

The bactericidal properties of cement mortars supplemented with modified titania (TiO$_2$-G-1%-100) were examined in an own-designed photoreactor according patented procedure [48]. Cement mortar Fix M-15 Kreisel (Kreisel Technika Budowlana Sp. z.o.o., Poznań, Poland) was used to prepare reference concrete plates. The same material was used to prepare concrete plated with 10% weight of TiO$_2$-G-1%-100. The antimicrobial activity was evaluated against *Escherichia coli* K12 (ATCC 25922) under artificial solar light (UV-Vis) and in dark condition. The bacterial concentration was calculated as CFU per milliliter according the procedure described in Section 4.3.

5. Conclusions

Titania modification with cheap carbon source, i.e., D-glucose, resulted in the appearance of high antimicrobial activity against both gram-positive and gram-negative bacteria. It is thought that strong interaction between bacterial cells and photocatalyst surface are responsible for inactivation and complete decomposition of bacteria cells. It is proposed that D-glucose-modified titania photocatalyst is highly prospective material, and may be commercially used for cheap and effective water disinfection and wastewater treatment since its immobilization on a concrete results in the efficient removal of bacteria under solar radiation.

References

1. Rengifo-Herrera, J.A.; Mielczarski, E.; Mielczarski, J.; Castillo, N.C.; Kiwi, J.; Pulgarin, C. Escherichia coli inactivation by N, S co-doped commercial TiO_2 powders under UV and visible light. *Appl. Catal. B Environ.* **2008**, *84*, 448–456. [CrossRef]

2. Kowalska, E.; Mahaney, O.O.P.; Abe, R.; Ohtani, B. Visible-light-induced photocatalysis through surface plasmon excitation of gold on titania surfaces. *Phys. Chem. Chem. Phys.* **2010**, *12*, 2344. [CrossRef] [PubMed]

3. Liu, G.; Han, C.; Pelaez, M.; Zhu, D.; Liao, S.; Likodimos, V.; Ioannidis, N.; Kontos, A.G.; Falaras, P.; Dunlop, P.S.M.; et al. Synthesis, characterization and photocatalytic evaluation of visible light activated c-doped TiO_2 nanoparticles. *Nanotechnology* **2012**, *23*, 294003. [CrossRef] [PubMed]

4. Asahi, R.; Morikawa, T.; Irie, H.; Ohwaki, T. Nitrogen-doped titanium dioxide as visible-light-sensitive photocatalyst: Designs, developments, and prospects. *Chem. Rev.* **2014**, *114*, 9824–9852. [CrossRef] [PubMed]

5. Asahi, R.; Morikawa, T.; Ohwaki, T.; Aoki, K.; Taga, Y. Visible-light photocatalysis in nitrogen-doped titanium oxides. *Science* **2001**, *293*, 269–271. [CrossRef] [PubMed]

6. Palanivelu, K.; Im, J.-S.; Lee, Y.-S. Carbon doping of TiO_2 for visible light photo catalysis—A review. *Carbon Lett.* **2007**, *8*, 214–224. [CrossRef]

7. Wu, G.; Nishikawa, T.; Ohtani, B.; Chen, A. Synthesis and characterization of carbon-doped TiO_2 nanostructures with enhanced visible light response. *Chem. Mater.* **2007**, *19*, 4530–4537. [CrossRef]

8. Wu, Z.; Dong, F.; Zhao, W.; Wang, H.; Liu, Y.; Guan, B. The fabrication and characterization of novel carbon doped TiO_2 nanotubes, nanowires and nanorods with high visible light photocatalytic activity. *Nanotechnology* **2009**, *20*, 235701. [CrossRef] [PubMed]

9. Zhong, J.; Chen, F.; Zhang, J. Carbon-deposited TiO_2: Synthesis, characterization, and visible photocatalytic performance. *J. Phys. Chem. C* **2010**, *114*, 933–939. [CrossRef]

10. Xie, C.; Yang, S.; Shi, J.; Niu, C. Highly crystallized C-doped mesoporous anatase TiO_2 with visible light photocatalytic activity. *Catalysts* **2016**, *6*, 117. [CrossRef]

11. Morawski, A.W.; Janus, M.; Tryba, B.; Inagaki, M.; Kałucki, K. TiO_2-anatase modified by carbon as the photocatalyst under visible light. *Rendus Rendu Chim.* **2006**, *9*, 800–805. [CrossRef]

12. Sakthivel, S.; Kisch, H. Daylight photocatalysis by carbon-modified titanium dioxide. *Angew. Chem. Int. Ed.* **2003**, *42*, 4908–4911. [CrossRef] [PubMed]

13. Janus, M.; Markowska-Szczupak, A.; Kusiak-Nejman, E.; Morawski, A.W. Disinfection of *E. coli* by carbon modified TiO_2 photocatalysts. *Environ. Prot. Eng.* **2012**, *38*, 90–97. [CrossRef]

14. Wanag, A.; Rokicka, P.; Kusiak-Nejman, E.; Markowska-Szczupak, A.; Morawski, A.W. TiO_2/glucose nanomaterials with enhanced antibacterial properties. *Mater. Lett.* **2016**, *185*, 264–267. [CrossRef]

15. Wanag, A.; Rokicka, P.; Kusiak-Nejman, E.; Kapica-Kozar, J.; Wrobel, R.J.; Markowska-Szczupak, A.; Morawski, A.W. Antibacterial properties of TiO_2 modified with reduced graphene oxide. *Ecotoxicol. Environ. Saf.* **2018**, *147*, 788–793. [CrossRef] [PubMed]

16. Li, Y.; Ma, M.; Wang, X.; Wang, X. Inactivated properties of activated carbon-supported TiO_2 nanoparticles for bacteria and kinetic study. *J. Environ. Sci.* **2008**, *20*, 1527–1533. [CrossRef]

17. McEvoy, J.G.; Cui, W.; Zhang, Z. Degradative and disinfective properties of carbon-doped anatase-rutile TiO_2 mixtures under visible light irradiation. *Catal. Today* **2013**, *207*, 191–199. [CrossRef]

18. Sun, D.-S.; Kau, J.-H.; Huang, H.-H.; Tseng, Y.-H.; Wu, W.-S.; Chang, H.-H. Antibacterial properties of visible-light-responsive carbon-containing titanium dioxide photocatalytic nanoparticles against anthrax. *Nanomaterials* **2016**, *6*, 237. [CrossRef] [PubMed]

19.　Grzybowska, B.; Słoczyński, J.; Grabowski, R.; Samson, K.; Gressel, I.; Wcisło, K.; Gengembre, L.; Barbaux, Y. Effect of doping of TiO_2 support with altervalent ions on physicochemical and catalytic properties in oxidative dehydrogenation of propane of vanadia–titania catalysts. *Appl. Catal. A Gen.* **2002**, *230*, 1–10. [CrossRef]

20.　örsi, F. Kinetic studies on the thermal decomposition of glucose and fructose. *J. Therm. Anal.* **1973**, *5*, 329–335. [CrossRef]

21.　Slimestad, R.; Vågen, I.M. Thermal stability of glucose and other sugar aldoses in normal phase high performance liquid chromatography. *J. Chromatogr. Coruña* **2006**, *1118*, 281–284. [CrossRef] [PubMed]

22.　Siuzdak, K.; Sawczak, M.; Klein, M.; Nowaczyk, G.; Jurga, S.; Cenian, A. Preparation of platinum modified titanium dioxide nanoparticles with the use of laser ablation in water. *Phys. Chem. Chem. Phys.* **2014**, *16*, 15199–15206. [CrossRef] [PubMed]

23.　Zielińska-Jurek, A.; Kowalska, E.; Sobczak, J.W.; Lisowski, W.; Ohtani, B.; Zaleska, A. Preparation and characterization of monometallic (au) and bimetallic (ag/au) modified-titania photocatalysts activated by visible light. *Appl. Catal. B Environ.* **2011**, *101*, 504–514. [CrossRef]

24.　Wei, Z.; Janczarek, M.; Endo, M.; Balčytis, A.; Nitta, A.; Mendez Medrano, M.G.; Colbeau-Justin, C.; Juodkazis, S.; Ohtani, B.; Kowalska, E. Noble metal-modified faceted anatase titania photocatalysts: Octahedron versus decahedron. *Appl. Catal. B Environ.* **2018**, *237*, 574–587. [CrossRef]

25.　Baltrusaitis, J.; Schuttlefield, J.; Zeitler, E.; Grassian, V.H. Carbon dioxide adsorption on oxide nanoparticle surfaces. *Chem. Eng. J.* **2011**, *170*, 471–481. [CrossRef]

26.　Fleisch, T.H.; Mains, G.J. Reduction of copper oxides by uv radiation and atomic hydrogen studied by xps. *Appl. Surf. Sci.* **1982**, *10*, 51–62. [CrossRef]

27.　Akhavan, O.; Ghaderi, E. Self-accumulated ag nanoparticles on mesoporous TiO_2 thin film with high bactericidal activities. *Surf. Coat. Technol.* **2010**, *204*, 3676–3683. [CrossRef]

28.　Kruse, N.; Chenakin, S. Xps characterization of Au/TiO_2 catalysts: Binding energy assessment and irradiation effects. *Appl. Catal. A Gen.* **2011**, *391*, 367–376. [CrossRef]

29.　Rossnagel, S.M.; Sites, J.R. X-ray photoelectron spectroscopy of ion beam sputter deposited SiO_2, TiO_2, and Ta_2O_5. *J. Vac. Sci. Technol. A Vac. Surf. Films* **1984**, *2*, 376–379. [CrossRef]

30.　Jensen, H.; Soloviev, A.; Li, Z.; Søgaard, E.G. Xps and ftir investigation of the surface properties of different prepared titania nano-powders. *Appl. Surf. Sci.* **2005**, *246*, 239–249. [CrossRef]

31.　Yu, J.; Zhao, X.; Zhao, Q. Effect of surface structure on photocatalytic activity of TiO_2 thin films prepared by sol-gel method. *Thin Solid Films* **2000**, *379*, 7–14. [CrossRef]

32.　Lonnen, J.; Kilvington, S.; Kehoe, S.C.; Al-Touati, F.; McGuigan, K.G. Solar and photocatalytic disinfection of protozoan, fungal and bacterial microbes in drinking water. *Water Res.* **2005**, *39*, 877–883. [CrossRef] [PubMed]

33.　Wang, L.; Hu, C.; Shao, L. The antimicrobial activity of nanoparticles: Present situation and prospects for the future. *Int. J. Nanomed.* **2017**, *12*, 1227–1249. [CrossRef] [PubMed]

34.　Maletić, M.; Vukčević, M.; Kalijadis, A.; Janković-Častvan, I.; Dapčević, A.; Laušević, Z.; Laušević, M. Hydrothermal synthesis of TiO_2/carbon composites and their application for removal of organic pollutants. *Arab. J. Chem.* **2016**. [CrossRef]

35.　Kim, G.; Lee, S.-H.; Choi, W. Glucose–TiO_2 charge transfer complex-mediated photocatalysis under visible light. *Appl. Catal. B Environ.* **2015**, *162*, 463–469. [CrossRef]

36.　Dong, F.; Wang, H.; Wu, Z. One-step "green" synthetic approach for mesoporous c-doped titanium dioxide with efficient visible light photocatalytic activity. *J. Phys. Chem. C* **2009**, *113*, 16717–16723. [CrossRef]

37.　Herrmann, J. Heterogeneous photocatalysis: Fundamentals and applications to the removal of various types of aqueous pollutants. *Catal. Today* **1999**, *53*, 115–129. [CrossRef]

38.　Cho, M.; Chung, H.; Choi, W.; Yoon, J. Linear correlation between inactivation of *E. coli* and OH radical concentration in TiO_2 photocatalytic disinfection. *Water Res.* **2004**, *38*, 1069–1077. [CrossRef] [PubMed]

39.　Benabbou, A.K.; Derriche, Z.; Felix, C.; Lejeune, P.; Guillard, C. Photocatalytic inactivation of escherischia coli. *Appl. Catal. B Environ.* **2007**, *76*, 257–263. [CrossRef]

40.　Kumar, A.; Pandey, A.K.; Singh, S.S.; Shanker, R.; Dhawan, A. Engineered ZnO and TiO2 nanoparticles induce oxidative stress and dna damage leading to reduced viability of escherichia coli. *Free. Radic. Biol. Med.* **2011**, *51*, 1872–1881. [CrossRef] [PubMed]

41. Wanag, A.; Morawski, A.W.; Kapica-Kozar, J.; Kusiak-Nejman, E. Photocatalytic performance of thermally prepared TiO$_2$/C photocatalysts under artificial solar light. *Micro Nano Lett.* **2016**, *11*, 202–206. [CrossRef]

42. Cui, Y.; Li, H.; Hong, W.; Fan, S.; Zhu, L. The effect of carbon content on the structure and photocatalytic activity of nano-bi2wo6 powder. *Powder Technol.* **2013**, *247*, 151–160. [CrossRef]

43. Kacem, M.; Bru-Adan, V.; Goetz, V.; Steyer, J.P.; Plantard, G.; Sacco, D.; Wery, N. Inactivation of escherichia coli by TiO2-mediated photocatalysis evaluated by a culture method and viability-qpcr. *J. Photochem. Photobiol. A Chem.* **2016**, *317*, 81–87. [CrossRef]

44. Lin, X.; Li, J.; Ma, S.; Liu, G.; Yang, K.; Tong, M.; Lin, D. Toxicity of TiO2 nanoparticles to escherichia coli: Effects of particle size, crystal phase and water chemistry. *PLoS ONE* **2014**, *9*, e110247. [CrossRef] [PubMed]

45. Clogston, J.D.; Patri, A.K. Zeta potential measurement. *Methods Mol. Biol.* **2011**, *697*, 63–70. [CrossRef] [PubMed]

46. Hadwan, M.H.; Abed, H.N. Data supporting the spectrophotometric method for the estimation of catalase activity. *Data Brief* **2016**, *6*, 194–199. [CrossRef] [PubMed]

47. Marklund, S.; Marklund, G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur. J. Biochem.* **1974**, *47*, 469–474. [CrossRef] [PubMed]

48. Markowska-Szczupak, A.; Rokicka, P.; Janus, M.; Kusiak Najman, E.; Morawski, A.W. Method for Testing Antibacterial Properties of Materials Made from Cements Containing Titanium Dioxide. Patent Number 416,872, 19 April 2016.