

Influence of titanium dioxide modification on the antibacterial properties

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Antibacterial properties of 15 titania photocatalysts, mono- and dual- modified with nitrogen and carbon were examined. Amorphous TiO₂, supplied by Azoty Group Chemical Factory Police S.A., was used as titania source (Ar-TiO₂, C-TiO₂, N-TiO₂ and N,C-TiO₂ calcined at 300°C, 400°C, 500°C, 600°C, 700°C). The disinfection ability was examined against *Escherichia coli* K12 under irradiation with UV and artificial sunlight and in dark conditions. It has been found the development of new photocatalysts with enhanced interaction ability with microorganisms might be a useful strategy to improve disinfection method conducted under artificial sunlight irradiation. The efficiency of disinfection process conducted under artificial sunlight irradiation with carbon (C-TiO₂) and carbon/nitrogen (N,C-TiO₂) photocatalysts was similar as obtained under UV irradiation. Furthermore, during dark incubation, any toxicity of the photocatalyst was noted.

Keywords: antibacterial properties, *Escherichia coli* K12, modified photocatalysts, titanium dioxide, visible-responsive photocatalysts.

INTRODUCTION

Almost one-fifth of the world population lives in areas of physical scarcity of fresh water, and additionally one quarter of humanity is lacking of basic sanitation systems. It is estimated that poor quality of water is one of the leading causes of people death in the developing countries, where the microbial pollution is considered to be of serious health concern. According to World Health Organization (WHO) nearly 3.4 million people die each year of waterborne diseases – an illness commonly caused by fecal contamination of water. The mortality of children under the age of five is the highest (6.000 deaths per day). Moreover, increasing appearance of antibiotic-resistant bacteria has been reported¹.

The situation is only expected to worsen, due to global climate changing, population growth and inefficient use of existing resources restricting the amount of water available to people². With increasing microbial contamination of water new and more potent antibacterial agents and materials are needed.

Water disinfection is a process of removal, deactivation or killing of living pathogens, including viruses, bacteria and protozoa. Disinfection methods are not forceful as sterilization since resistant bacterial spores are not eliminated from water^{1, 3}. Moreover, the conventional methods of water disinfection are not completely effective and they present many drawbacks. Chlorination gives relatively low protection against protozoa and requires usage of chemicals which can form carcinogenic and toxic chlorine by-product, e.g., trihalomethanes and haloacetic acids formed from the reaction of chlorine with natural organic matters in water⁴. Ozonation, one of the advanced oxidation processes (AOPs, in-situ generating hydroxyl radicals), is very efficient, but has a few major drawback such as ozone instability (must be generated immediately before use), high cost of equipment and complicated operating systems⁵. Whereas, water disinfection by UV-irradiation (another AOP) does not prevent bacterial regrowth after the removal of UV-light source².

Therefore, very effective, environmentally friendly and cost effective processes are in greater demand. It is proposed that the photocatalytic oxidation with titanium dioxide (also AOP) fulfills above mentioned tasks.

Titanium dioxide has attracted tremendous attention due to its high photoreactivity, physicochemical stability, market accessibility (there are many manufactures in the world) and affordable prices. The well-known mechanism of TiO₂ photocatalytic degradation of organic compounds involves generation of reactive oxygen species (ROS). The potential applications of the technology include organic matter, anthropogenic and natural organic contaminants (e.g. pesticides, surfactants, organic fertilizers), and degradation in various homogeneous and heterogeneous systems^{6–8}. Over the last 10 years a lot of reviews with the topic of photocatalytic disinfection of water have been published^{2, 3, 9–11}. Although the antibacterial properties of TiO₂ are well documented, there are difficulty in its application since photocatalytic disinfection requires UVA irradiation ($\lambda = 320–400$ nm) and relatively long inactivation times (e.g., 90 and 130 minutes for achieving 1 log inactivation of *Escherichia coli* and bacteriophage MS2, respectively¹²). It has been estimated that ultraviolet radiation ($\lambda < 400$ nm) contributes less than 4% of whole incoming solar radiation, therefore development of visible light active photocatalyst is major challenge¹³. The effective utilization of the visible light (50% of solar spectrum) is main aim of study conducted by many research teams. A numerous modification techniques, which seem to be promising for TiO₂ band-gap narrowing and shift the light absorption capacity towards visible wavelengths have been proposed^{14–16}. The titania modified with noble metals (e.g. Au, Ag), metal oxides (e.g. ZnO, WO₃, SiO₂, CrO) and non-metals e.g. S, C, N are most often investigated photocatalysts^{16–19}.

In the present study, the antibacterial properties of titania photocatalysts modified with nitrogen and carbon under UV and artificial sunlight irradiation have been examined. There are only a few studies on water disinfection on visible active titania photocatalysts modified

with nitrogen and/or carbon. The aim of this study was to assess the role of modifiers and post-treatment conditions (temperature of calcination) in microbial inactivation mechanisms. *Escherichia coli* K12 was chosen as an indicator microorganism in photocatalytic water disinfection systems^{3, 10}.

EXPERIMENTAL

Material

An amorphous TiO₂ supplied by Azoty Group Chemical Factory Police S.A., Poland was used as a starting material. Fifteen modified titania by nitrogen, carbon and nitrogen/carbon were prepared in Institute of Chemical and Environment Engineering, West Pomeranian University of Technology in Szczecin (Poland). In order to modify, 20 g of TiO₂ suspended in water was placed in a tubular furnace. Modification was conducted in one-step process with benzene (Sigma-Aldrich Co., USA) and gaseous ammonia (Messer, Poland) as carbon and nitrogen precursors respectively. Samples were heated to desired temperature in inert gas-argon (Messer, Poland) and calcined at 300, 400, 500, 600 and 700°C (Ar-TiO₂) in the presence of ammonia (N-TiO₂), benzene (C-TiO₂) and ammonia with benzene (N,C-TiO₂) for 4 hours. Then samples were cooled down to room temperature in an argon atmosphere. After that samples were rinsed with water and dried for 12 h at 100°C to remove residual ammonia. Photocatalysts calcined in an argon atmosphere were regarded as a control (Ar-TiO₂) for nitrogen (N-TiO₂), carbon (C-TiO₂), and nitrogen/carbon co-modified (N,C-TiO₂) photocatalysts. The phase characteristics of starting TiO₂ and N-TiO₂, C-TiO₂ and co-modified N,C-TiO₂ calcined at different temperatures was investigated by XRD analysis. The BET specific surface areas were calculated on the basis of the N₂ adsorption measurements conducted at 77 K using a Quadrasorb SI analyzer (Quantachrome Instruments, USA).

Bacterial inactivation

Glass beakers of 25 mL were used as reactors. The experiments were carried out under UVA (six bulbs 20 W Philips) or artificial sunlight (one bulb 300 W OSRAM Ultra Vitalux) irradiation. The emission spectra of artificial sunlight and UV sources were presented in previous papers¹⁹. The radiant flux was monitored with a Radiation Intensity Meter LB901/WCM3 & PD204AB cos. sensor meter. The distance between the solution and the light source was fixed at ca. 15 cm. The suspension was continuously stirred (using a magnetic stirrer at speed of 250 rpm) throughout the experiment to ensure homogeneity.

Bacteria *Escherichia coli* strain K12 (ACCT 25922) was used for study. Before the experiments, bacteria were inoculated into Enriched Broth (Biocorp, Poland) and were cultured at 37°C for 24 h. The cell concentration of inoculum was adjusted to final cell density of 1.0 in MacFarland standard (bioMérieux, Poland) equivalent to 3.0 × 10⁸ CFU/ml. Five μL of the bacterial suspension was added into the reactor which contained 10 mL NaCl solution (0.9%) and 0.1 g × L⁻¹ photocatalyst. The reaction mixture was illuminated with UVA or artificial

sunlight for 45 min. Samples were collected in every 15 min. The control experiments in darkness and for NaCl solution were also performed. Serial dilutions were prepared in saline solution (0.9%). The samples were placed on Plate Count Agar (PCA agar, BTL, Poland). The plates were incubated for 24 h at 37°C and then colony forming unit (CFU/mL) was counted.

Statistical analysis of obtained results was conducted using Excel spreadsheet and Statistica 8.0. The comparisons among means and the statistical significance of differences between means were evaluated by Tukey's test at P ≤ 0.05.

RESULTS

The precise characteristic of modified titania photocatalysts with carbon, nitrogen and carbon and nitrogen will be presented in our further papers. Here, only data essential for interpretation of microbiological results are presented in Figure 1. It is clear that properties of photocatalysts, such as anatase content (a), anatase crystallite size (b), specific surface area (c) and the content of modifiers (d), differ significantly among the samples. The photocatalysts calcined at 300°C consist of predominantly anatase of small crystallites (9–10 nm), and thus with large specific surface area (182–194 m²/g). On the contrary, calcination at 700°C results in significant growth of crystals reaching 82 nm (more than 8 times larger crystallite size than at lower calcination temperature), and a decrease in specific surface area (16.4 smaller BET, i.e., 12 m²/g and 15 m²/g for N-TiO₂ and N,C-TiO₂, respectively). As expected, the contribution of rutile phase (6–44.7%) increases with an increase in calcination temperature (Fig. 1a) since rutile is the most stable polymorph of titania. In consequence, different antibacterial activities are expected for titania photocatalysts prepared at varied calcination temperature.

The most important structural properties of examined photocatalyst appear to correlate with calcination temperature, which strongly determines the structural composition. Due to this reason the antibacterial activity of photocatalyst is presented for individual calcination temperature. The antibacterial properties of unmodified (Ar-TiO₂) and modified (N-TiO₂, C-TiO₂ and N,C-TiO₂) photocatalysts as function of time are demonstrated in Figures 2–4. Under UVA irradiation, almost all examined Ar-TiO₂, N-TiO₂, C-TiO₂ and N,C-TiO₂ photocatalysts presented similar antibacterial activity. After 30 min of irradiation, 100% of *Escherichia coli* was killed, with the exception of the control sample calcined in argon (Ar-TiO₂) at the highest temperature of 700°C, which needed longer irradiation time of 45 min (Fig. 2e).

In Figure 2 the results obtained in experiments conducted under UV irradiation are presented. All examined photocatalyst (unmodified and C- and N-modified and C,N co-modified) have antibacterial high activity. The bacterial killing process started in 15 min and increased with further increase in exposure time. After 30 minutes the total reduction count in all PCA plates was observed.

Figure 3 shows *E. coli* inactivation in the presence of examined photocatalyst under artificial sunlight (VIS) irradiation. The best activity towards *E. coli* possessed carbon modified photocatalyst (C-TiO₂) calcined at 700°C

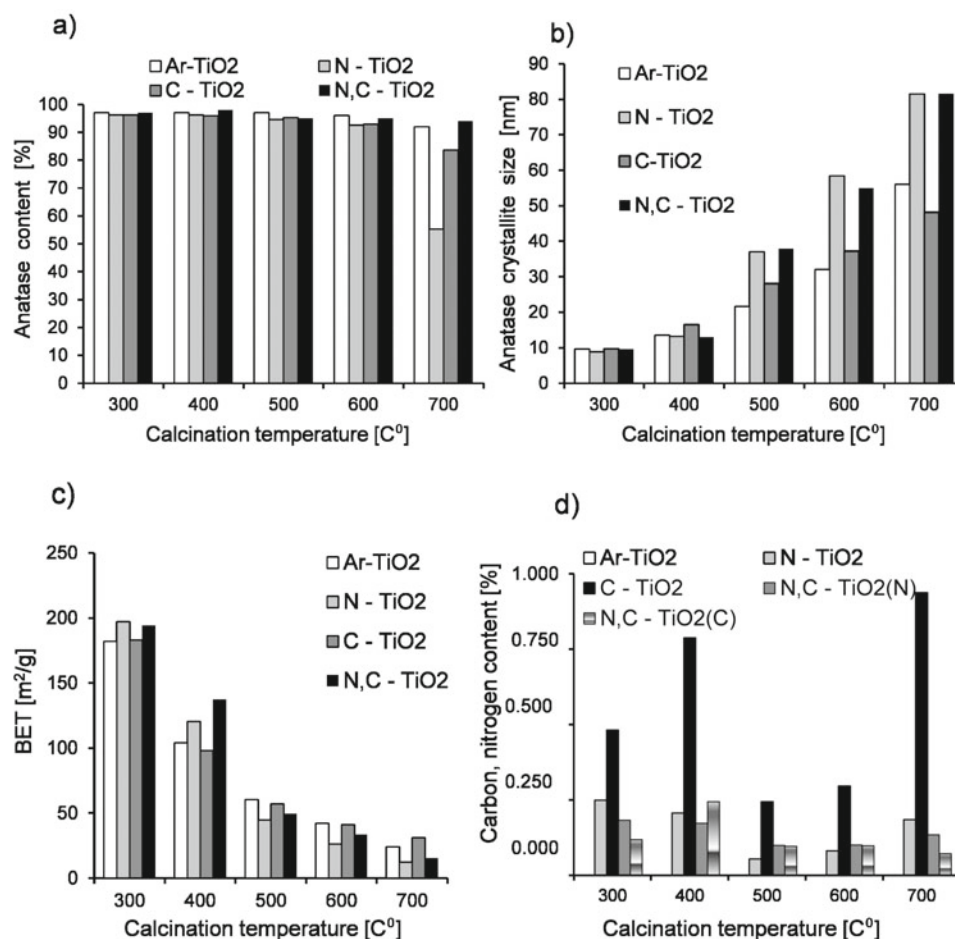


Figure 1. The characterization of used photocatalyst: a) the crystal form b) anatase crystallite size of Ar-TiO₂, N-TiO₂, C-TiO₂ and NC-TiO₂ c) BET Ar-TiO₂, N-TiO₂, C-TiO₂ and NC-TiO₂ d) nitrogen, carbon content

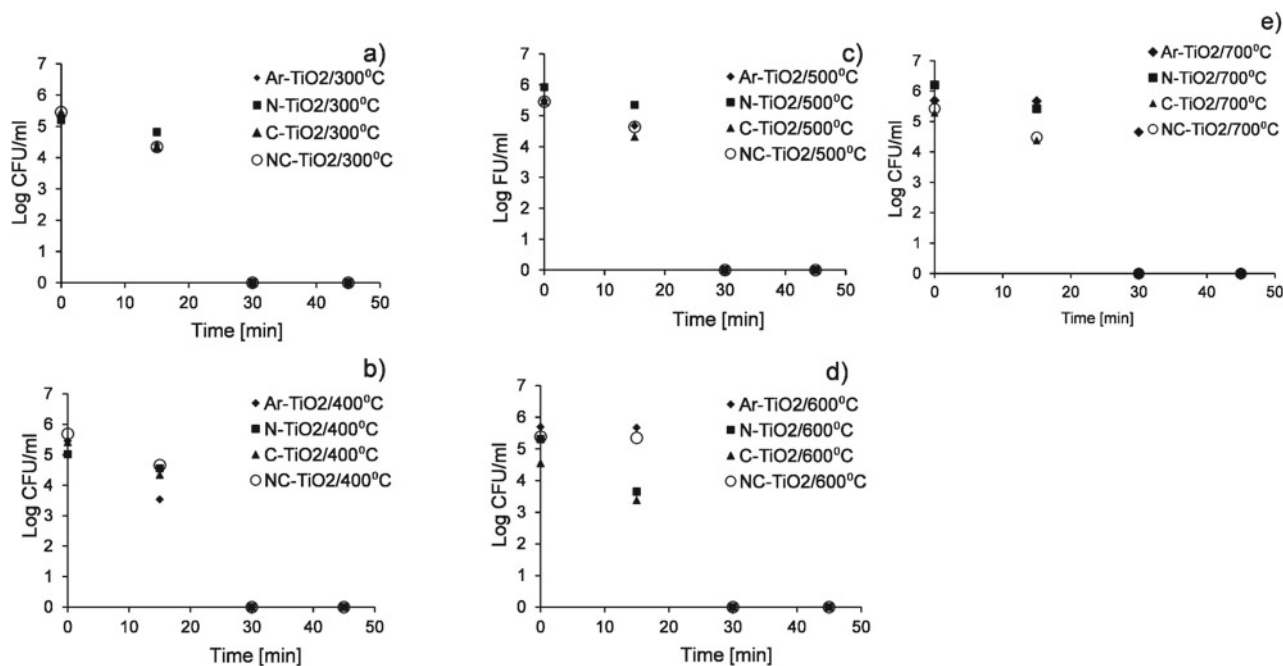


Figure 2. Antibacterial activity of modified titania: calcined in argon (Ar-TiO₂) ammonia (N-TiO₂), benzene (C-TiO₂) and ammonia with benzene (N,C-TiO₂) atmosphere at 300 °C (a), 400 °C (b), 500 °C (c), 600 °C (d) and 700 °C (e) under UV irradiation

(Fig. 3e). No live bacteria cells were found just over 15 min under VIS irradiation. A slight lower antibacterial performance demonstrated other photocatalysts: C-TiO₂ and N,C-TiO₂ obtained at 300 °C and 400 °C temperatures,

C-TiO₂ calcined at 600 °C and N-TiO₂ calcined at 700 °C (Fig. 3a-e).

To better understand photocatalytic effect, control experiments were carried out in the darkness. Under these conditions almost all examined photocatalysts did

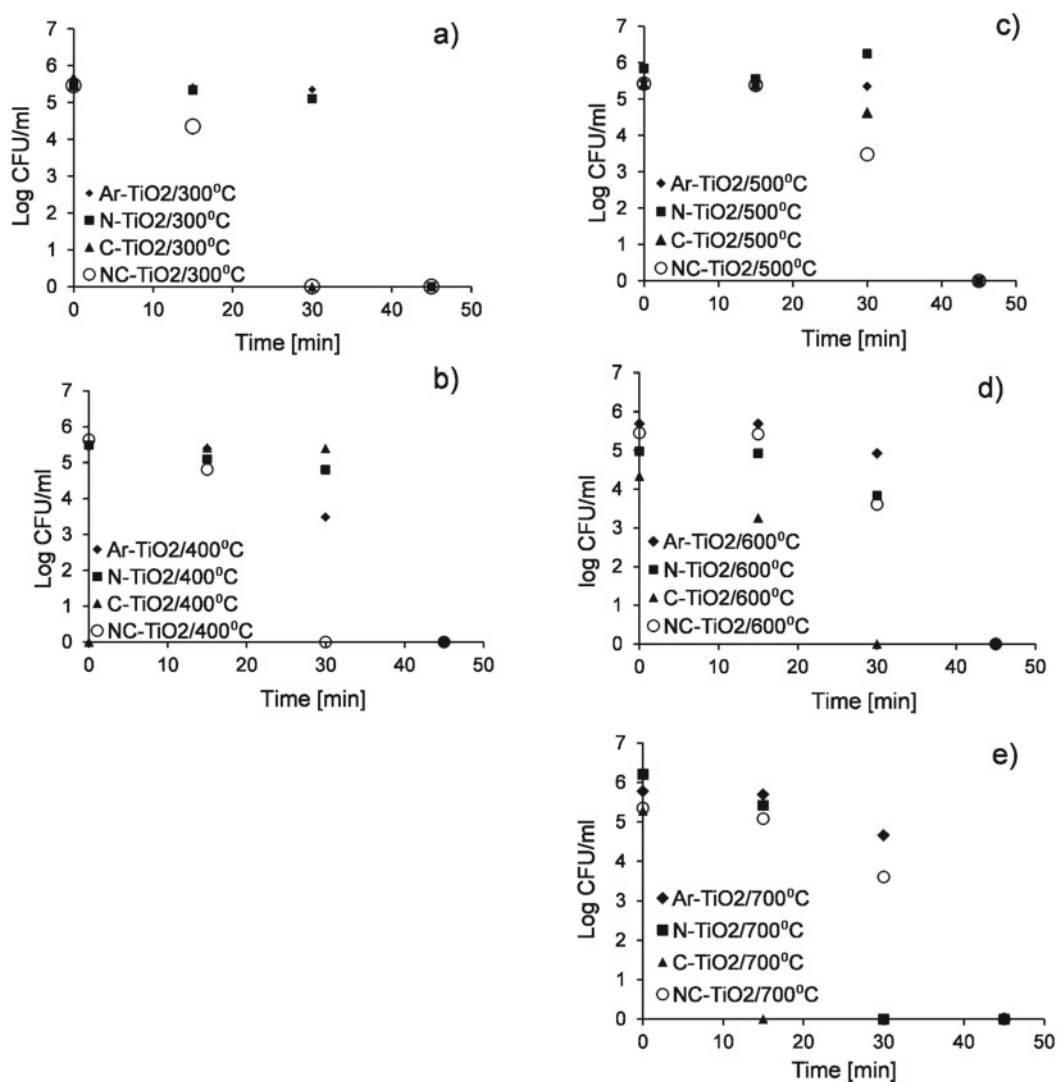


Figure 3. Antibacterial activity of modified titania: calcined in argon (Ar-TiO₂) ammonia (N-TiO₂), benzene (C-TiO₂) and ammonia with benzene (N,C-TiO₂) atmosphere at 300°C (a), 400°C (b), 500°C (c), 600°C (d) and 700°C (e) under artificial sunlight irradiation

not influence on survival of bacteria (Fig. 4a–e). The only exception was unmodified catalyst (Ar-TiO₂) calcined at 400°C. After 45 minutes of incubation the percentage of killed bacteria achieved approx. 17% (Fig. 4b).

To include the influence of irradiation type, the experiments without photocatalysts under UV and artificial sunlight irradiation were also conducted. It was found that both kind of irradiations did not cause significant changes in bacteria survival (Fig. 5).

To analyze the influence of calcination temperature on the antimicrobial performance, the bacterial reduction rate after 15 min from start of experiments was calculated and compared by Tukey's test. It was shown that for unmodified samples (Ar-TiO₂), the interaction between photocatalysts structural characteristic (closely related to preparation temperature) and bacteria destroying properties was statistically significant at $P \leq 0.05$ (Fig. 6). Unexpectedly, after 15 min of UV irradiation the similar high antimicrobial performance was obtained by photocatalysts prepared at 400°C and 600°C, which vary in specific surface area (BET) and anatase crystallite size (Fig. 1). In turn, under artificial sunlight irradiation different photocatalysts (treated at 500°C and 700°C) exhibited higher antibacterial properties (Fig. 6).

Since 30 minutes of UV irradiation resulted in complete bacteria inactivation (Fig. 1), to estimate the influence of nitrogen content on the antimicrobial properties, the bacterial reduction rate after 15 min of UV irradiation was compared by Tukey's test. It was found out that nitrogen content is particularly important in nitrogen modified photocatalyst N-TiO₂ (Fig. 7a). The highest antibacterial properties had titania calcined at 600°C and characterized by relatively low N-content (0.081%). All observed differences were statistically significant. The number of killed bacteria decreased with decreasing nitrogen content. In contrary nitrogen content in carbon co-modified photocatalysts did not influenced on antibacterial performance. The only exception was N,C-TiO₂-600°C which possessed the lowest bacterial reduction (Fig. 7b).

The similar analysis was carried out for experiments carried out under artificial sunlight irradiation. The results were presented in Fig. 8(a,b). Only in Figure 8b there is a clearly defined pattern and this can mean that increasing nitrogen content in N-TiO₂, and (N,C-TiO₂) co-modified photocatalysts improves antimicrobial performance. Almost all observed differences were statically significant at $P \leq 0.05$.

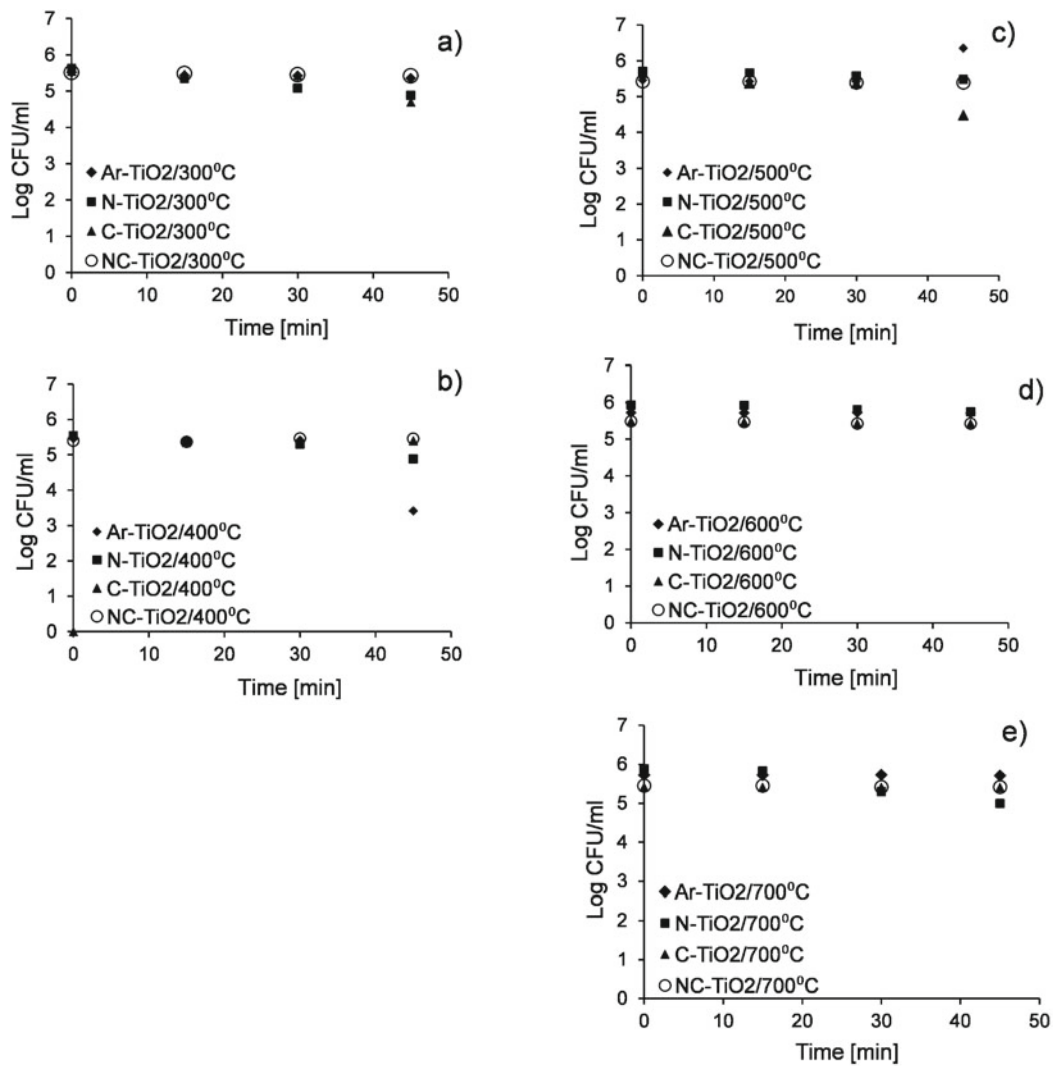


Figure 4. Antibacterial activity of modified titania: calcined in argon (Ar-TiO₂) ammonia (N-TiO₂), benzene (C-TiO₂) and ammonia with benzene (N,C-TiO₂) atmosphere at 300°C (a), 400°C (b), 500°C (c), 600°C (d) and 700°C (e) in darkness

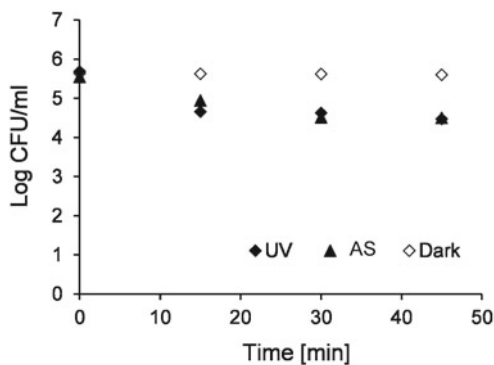


Figure 5. Concentration of bacteria in water under UV or artificial sunlight (AS) irradiation and in dark conditions

To consider the impact of carbon content on the antimicrobial performance, the bacterial reduction rate after 15 min of UV and artificial sunlight irradiation was calculated and compared by Tukey's test. According to the Figure 9, the carbon content in C-TiO₂, and (N,C-TiO₂) photocatalysts did not influence significantly on antibacterial performance under UV irradiation. The only one exception was N,C-TiO₂-600°C photocatalyst that caused lower bacterial reduction after 15 minutes UV irradiation.

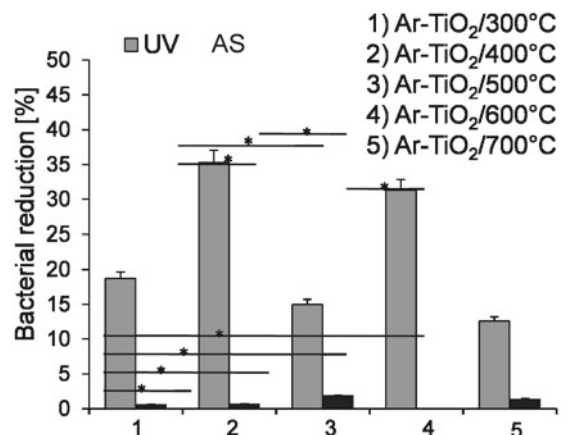


Figure 6. Photocatalytic bacteria reduction of unmodified Ar-TiO₂ catalysts calcined at different temperatures after 15 min under UV or artificial sunlight (AS) irradiation. *Statistical significance of differences between means obtained for catalysts calcined at different temperatures at $P \leq 0.05$ (Tukey's test)

On the other hand, the carbon content strongly influenced on bacteria reduction in carbon modified titania under artificial sunlight irradiation (Fig. 10a). The C-TiO₂-700 photocatalyst characterized by substantially higher carbon content (0.937%) caused complete bac-

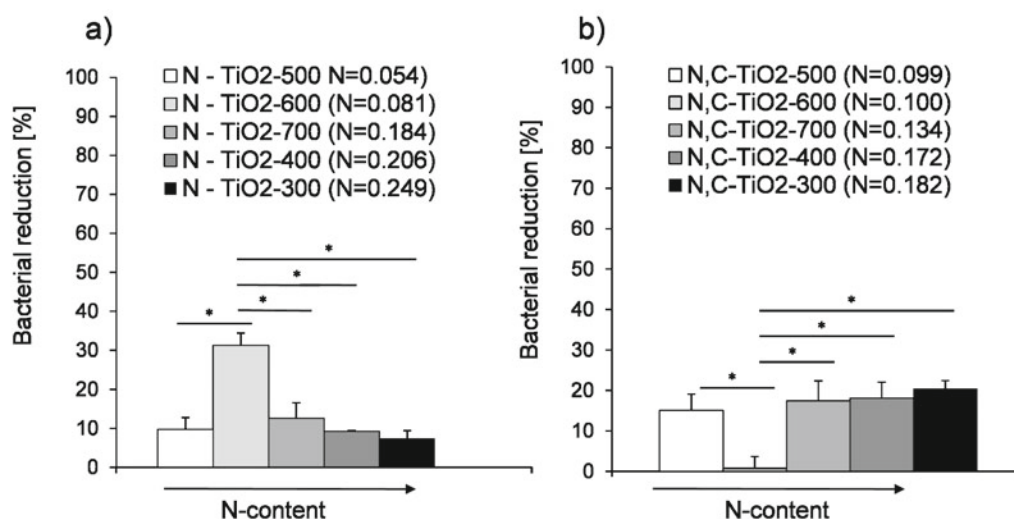


Figure 7. Photocatalytic bacteria reduction obtained for N and N,C-TiO₂ catalysts calcined at different temperatures after 15 min under UV irradiation. *Statistical significance of differences between means obtained for catalysts calcined at different N content (given in parentheses) at $P \leq 0.05$ (Tukey's test)

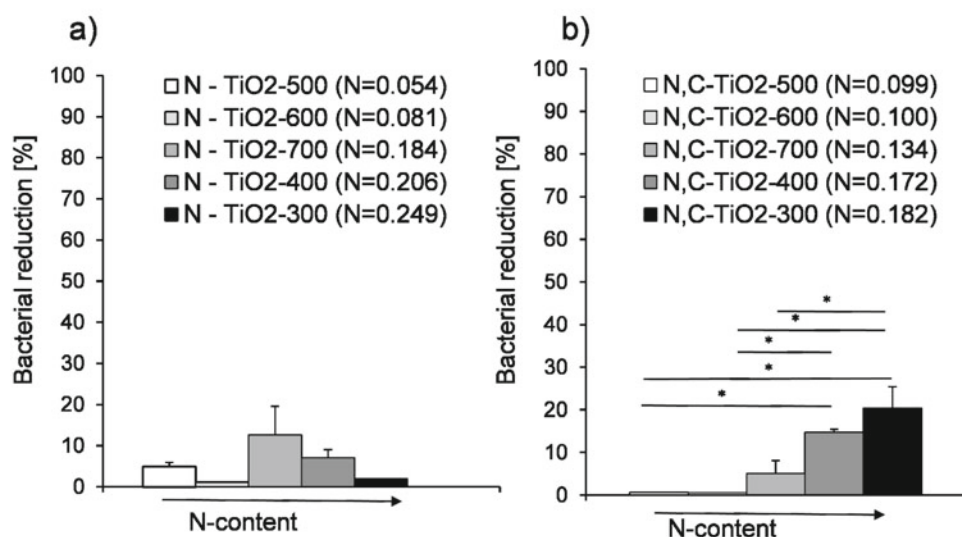


Figure 8. Photocatalytic bacteria reduction obtained for N and N,C-TiO₂ catalysts calcined at different temperatures after 15 min under artificial sunlight irradiation. *Statistical significance of differences between means obtained for catalysts calcined at different N content (given in parentheses) at $P \leq 0.05$ (Tukey's test)

teria reduction after first 15 min. The carbon content in N-TiO₂, and (N,C-TiO₂) titanias was generally lower than that in C-TiO₂ regardless of the temperature used for calcination. For that reason, influence of carbon content in N, C co-modified titania was less important than in C-modified TiO₂ (Fig. 10b).

DISCUSSION

The analysis of the results has shown, that temperature of calcination greatly affected on antibacterial properties of N-TiO₂, C-TiO₂ and co-modified N,C-TiO₂. The observed differences could arise from the fact that titania phase transformed during heat treatment. At the calcination temperature higher than 600°C rutile phase appearance increased. It has been known that anatase particles possess a larger band-gap and a smaller electron effective mass, resulting in the higher mobility of the charge carriers. It caused the increase of reactive oxygen species (ROS) formation. From a toxicological point of view greater generation of ROS and other important

characteristics of photocatalyst as its size, surface area, crystallinity, shape, solubility and agglomeration/aggregation state are important. However, it is also believed that the co-presence of both anatase and rutile phases is important for photocatalytic efficiency^{19,21}. Better bacterial reduction was achieved for photocatalysts, which consisted from 3.0 to 4.1% of rutile (from 97 to 95.9 anatase phase respectively) than for single-phase titania. The rutile content over 16.4 is considered to be the cause of reduced antibacterial properties demonstrated for samples prepared at 700°C.

No clear correlation between increasing calcinations temperature and antibacterial properties was observed for unmodified photocatalyst (Ar-TiO₂). These observation was made after 15 minutes from start of photocatalytic process, which indicated the existence of an induction period. It has been known that during the first minutes bacteria use self-defence and auto-repair mechanisms to protect against the active species generated under irradiation. Oxidation of outer membrane begins gradually but not sufficiently to cause serious damage to the

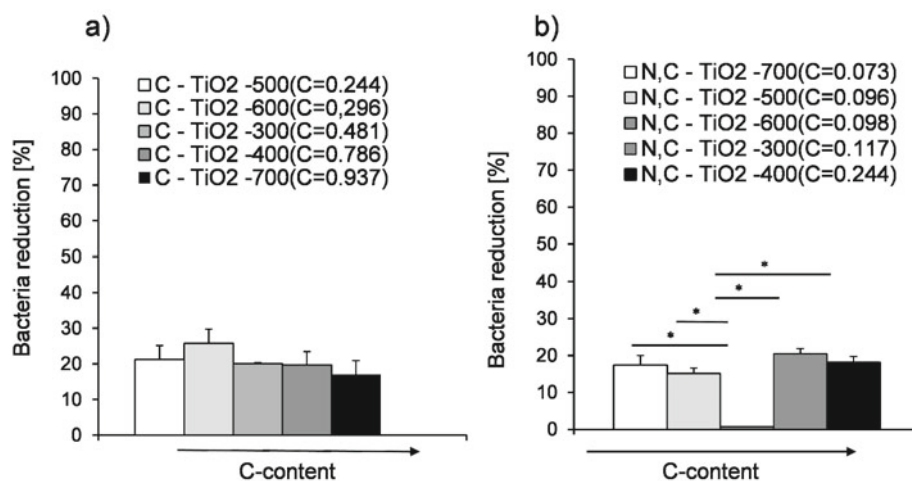


Figure 9. Photocatalytic bacteria reduction obtained for C and N,C-TiO₂ catalysts calcined at different temperatures after 15 min under UV irradiation. *Statistical significance of differences between means obtained for catalysts calcined at different C content (given in parentheses) at $P \leq 0.05$ (Tukey's test)

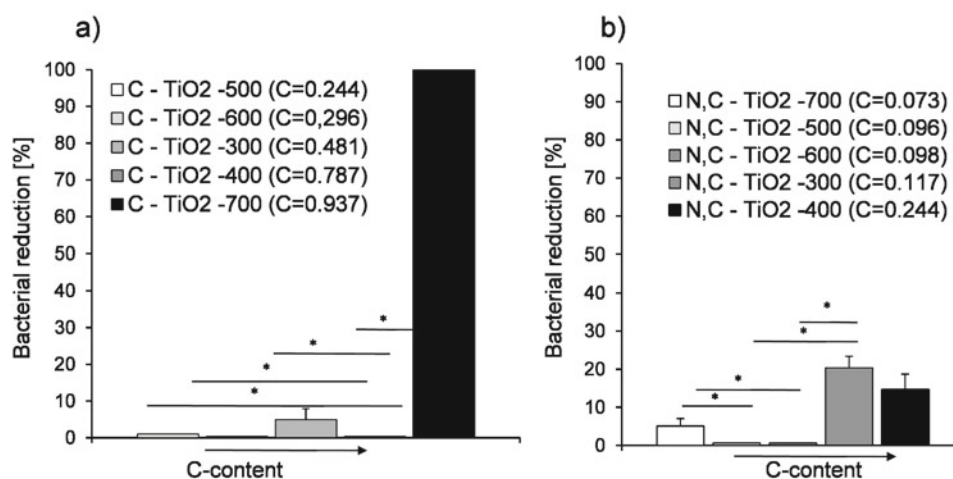


Figure 10. Photocatalytic bacteria reduction obtained for C and N,C-TiO₂ catalysts calcined at different temperatures after 15 min under artificial sunlight irradiation. *Statistical significance of differences between means obtained for catalysts calcined at different C content (given in parentheses) at $P \leq 0.05$ (Tukey's test)

bacterial cells²². It was assumed that better antibacterial properties presented photocatalyst characterized by similar anatase and rutile crystallite sizes. The optimal ratio between rutile and anatase crystallites should be from 1.1 (Ar-TiO₂-600°C) to 2.0 (Ar-TiO₂-700°C).

Our results indicated that visible light was sufficient to activate the photocatalytic process. Under artificial sunlight irradiation the antibacterial efficacy of examined N-TiO₂, C-TiO₂ and co-modified N,C-TiO₂ photocatalysts was high. Some of examined photocatalyst (C-TiO₂, N,C-TiO₂ calcined at 300 and 400°C, C-TiO₂ calcined at 600°C and N-TiO₂ calcined at 700°C) presented almost the same germ-destroying impact as under UVA irradiation. It means that modification of titania contributed the expected results. It is worth to notice that time required to complete inactivation of *E. coli* was the same as for Ag-doped photocatalyst. According to Hu et al.²³ and Shi et al.²⁴, the complete elimination of bacteria occurred after 40 to 60 min of their contact with Ag/AgBr/TiO₂ and Ag/AgX-CNTs under artificial sunlight. However, the excellent disinfection activity of Ag modified titania is caused mainly by Ag itself instead of photocatalytic activity. Due this reason, Ag modified titania very often presents antibacterial activity also in dark conditions. That

way the use of silver modified titania photocatalysts have been extensively explored disinfection methods for the potential environmental applications²⁵. However, there is lack of consistency in research results concerning the silver self-toxicity²⁶. Another very important aspect which should be taken into account is bacterial resistance to silver. Starting to use silver NPs in many products such as: cosmetics (e.g., toothpaste, cloths and pharmaceutical products (e.g. bandage, plaster)) to combat bacteria was presented as a safety and effective alternative to antibiotics, also against multi antibiotic resistant bacteria (e.g. Methicillin-resistant *Staphylococcus aureus*, MRSA). It is now turning out that sil genes, encoding silver resistance, occurred at a high frequency and were found in e.g. *Enterobacter* spp., *Klebsiella* spp., human *Escherichia coli*. Moreover silver resistant bacteria appear rapidly most frequently in human isolates. For example silver-resistant *Enterobacter cloacae* strain was isolated from a chronic leg ulcer after only three weeks of treatment with silver-based dressings²⁷. For that reason use of silver modified nanomaterials, e.g., titania may affect the bacterial resistance, thereby, can be a limiting factor in development of disinfections technologies. On the contrary, there are no such problems with regard

to carbon and nitrogen photocatalyst. Although in the presented studies only survival tests were performed, there is no evidence that nitrogen, carbon and nitrogen/carbon co-modified titanias are harmful for bacteria in dark conditions. The antibacterial activity only under irradiation brought about by desirable modification is beneficial to disinfection processes, as it facilitates controls which mainly occurs in contact with photocatalyst and depends on the source of light. Using carbon and nitrogen for titania surface modification appears to be more safety due to lack of evidence of toxicity induced by mentioned above modifiers. However, the question remains: What is the underlying mechanism of their antibacterial activity? It seems that both temperature treatment and modifier can influence on antibacterial properties of modified titania. Due to different surface coatings, photocatalysts have a preferential interaction with bacterial membrane components. By confocal Raman mapping techniques, Cheng et al.²⁷ proved that better bacterial interaction was associated with better pathogen-killing performance. For this reason antibacterial activity of modified titania might be significantly enhanced by carbon. Our results only partially confirmed this hypothesis. When the carbon content is high ($C = 0.937\%$) and there is no nitrogen (C-TiO₂-700), the complete bacteria reduction is possible after 15 minutes of UV irradiation. On the other hand, this photocatalyst showed considerably stronger antibacterial effects than it might have resulted from anatase crystallite size (relatively big crystallites approx. 48.1 nm), active surface area (relatively small approx. 31.0 m²/g). It can be explained by zeta potential, which is changed by carbon or nitrogen modification. Our previous study showed that zeta potentials of the nitrogen modified TiO₂ films were negative (≥ -32 mV), whereas the zeta potential of carbon-modified TiO₂ photocatalysts was remarkably higher and amounted to -1.5 mV or equaled to zero²⁸⁻²⁹. The zeta potential of TiO₂ surfaces do not only play a crucial role in the controlling the adsorption properties of aqueous contaminants, but also can influence on adhesion of the bacteria. Due to the negatively charged *E. coli* outer membrane³⁰ particles of photocatalyst are repelled each other that enhanced antibacterial effect, which results in appearance of induction period. On the other hand, the generation of reactive oxygen species (ROS) – the main factor caused oxidative stress in bacteria cells depends on crystal structure and surface area. According to Jiang et al.³¹, ROS generation is associated with the number of defect sites per surface area, and an S-shaped curve was observed as a function of particle size. The ability of titania nanoparticles to generate ROS grows as follows: amorphous > anatase > anatase/rutile mixtures > rutile. Our results indicated that nitrogen and carbon content most strongly influenced on antibacterial properties of titania photocatalyst under artificial sunlight irradiation.

Further studies on relationships between structure and antibacterial activity are needed to explain all possible mechanisms. It was shown that there is lack of correlation between nitrogen, carbon and nitrogen-carbon content and calcination temperature of titania. For this reason a linear correlation between temperature and antibacterial performance was not established. Different results were presented by Cheng et al., who examined the

property of N-doped TiO₂ films annealed in the range 400°C–600°C²⁷. Authors suggested the antimicrobial activity of N-doped TiO₂ increased slightly with increasing of annealing temperatures.

Unexpectedly, carbon and nitrogen co-modified photocatalyst did not present enhanced antibacterial activity. It was not in accordance with Chen et al. findings³² that binary oxide photocatalysts often exhibited higher catalytic activity than predicted from the properties of their components. The difference in the energy level of their conduction bands and valence bands provides rapid separation of the photogenerated electrons and protects against recombination, thus enhancing the generation of photogenerated ⁺OH radicals. The most effective method for increasing the photocatalytic activity under visible irradiation is co-doping with non-metal and metal³³. In present study two non-metals were used, which is likely the main cause lowering the photocatalytic activity. A second reason of low antibacterial performance of N,C-TiO₂ photocatalysts can originate from lower nitrogen and carbon content in those samples than in single modified photocatalysts (C-TiO₂ or N-TiO₂). The photocatalyst affinity to bacterial membrane, derived from banning site existing on the photocatalyst surface, is probably reduced.

In conclusion, we found that majority of obtained C-TiO₂, and (N,C-TiO₂) photocatalysts can be used in the sun light settings to reduce the transmission of *E. coli* bacteria from water.

CONCLUSIONS

Carbon and nitrogen single and dual modified titania photocatalysts obtained during calcination at 300–400°C demonstrated satisfactory antibacterial properties towards *Escherichia coli* bacteria under artificial sunlight irradiation. It was their unique structures (small anatase crystallites) and large active surface area the main factors influenced on antibacterial properties.

ACKNOWLEDGEMENT

This work was financially supported by project Maestro 3 No. DEC-2012/06/A/ST5/00226 from the National Science Centre (Poland).

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