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DISINFECTION OF *E. COLI* BY CARBON MODIFIED TiO_2 PHOTOCATALYSTS

Photocatalytic disinfection of *Escherichia coli* by carbon modified TiO_2 photocatalysts was tested under UV and visible light irradiation. Carbon modification of TiO_2 in a pressure reactor was conducted at 120 °C for 4 h. For modification purposes, five alcohols were used (methanol, ethanol, *n*-butanol, 2-butanol, and *tert*-butanol). The amount of carbon in photocatalysts was calculated with a termogravimetric analyser. It was found that photocatalysts with low content of carbon have better antibacterial ability under visible light irradiation and photocatalysts with higher content of carbon have better antibacterial ability under UV light irradiation.

1. INTRODUCTION

Conventional methods of disinfection of water are not effective and eliminate only alive forms of microorganisms. In addition there are problems associated with the use of very expensive instruments (e.g. for UV-radiation) and aggressive chemicals, e.g. NaOCl [1]. Researchers still try to find new ways for deactivation of *E. coli*, one of which uses CuO pretreated cotton (RF plasma) or Cu pretreated cotton (DC magnetron sputtering) [2, 3]. These modified cottons inactivated *E. coli* under visible light irradiation.

Photocatalysis has recently emerged as an alternative technology for water disinfection [4–7]. The method shows very high oxidative activity. Organic matter and microorganisms from water are mineralized to carbon dioxide and water. Titanium dioxide is the most widely used photocatalyst because of its most efficient photoactivity, the highest stability and the lowest cost and non-toxicity for humans and animals [8, 9]. The photocatalyst activity tests were conducted under UVA irradiation in the range from 315 to 400 nm. As is known from literature [10, 11], ultraviolet light UVC ranging from 200 nm to 280 nm is strongly bactericidal for *E. coli*. Carp et al. [12]

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showed that antiseptic activity of light depends on time and intensity of irradiation. The strongest bactericidal capacity has the radiation of the wavelengths from 230 nm to 275 nm being absorbed by nucleic acids and proteins.

General agreement exists regarding the mechanism of the photocatalytic process [13, 14]. Antibacterial properties of photocatalysis with TiO_2 were reported fot the first time by Matsunaga et al. in 1985 [15]. Recent reviews bring an explanation of photo killing mechanisms for microorganisms [16–20]. A suggested explanations involves direct oxidation of plasma membrane lipids through holes, reduction of intracellular coenzyme A (CoA) and respiratory activity and DNA damage. Kiwi and Nadtochenko [21] investigated photocatalytic peroxidation of *E. coli* membrane wall, lipo-polysaccharide (LPS), phosphatidyl-ethanolcholine(PE), and peptidoglycan (PGN) on TiO_2 porous films by ATR-FTIR spectroscopy.

In recent years, modifications of TiO_2 to increase its antibacterial activity aroused much interest. Sayilkan et al. found that Sn^{4+}-doped TiO_2 has high antibacterial effect against gram negative *E. coli* and gram positive *Staphylococcus aureus* [22]. Wu et al. [23] found that titanium dioxide nanoparticles co-doped with nitrogen and silver are an effective visible light driven photocatalyst with strong bactericidal activity against *E. coli*. They found that the primary role of Ag ions in enhancing the bactericidal activity of Ag_2O/TiON under visible light illumination was photocatalytic in promoting the production of hydroxyl radicals rather than acting as the bactericidal agent themselves. The prevalent mechanisms for the photocatalytic killing of *E. coli* consisted of oxidative damage on the cell wall and the cell membrane, and alterations of internal DNA molecules. Cheng et al. [24] showed that different TiO_2 crystal surfaces have different affinities towards cellular protein fibronectin. Using scanning electron microscopy and confocal Raman mapping techniques, they demonstrated that better bacterial interaction is associated with better pathogen killing performance when carbon modified TiO_2 samples were tested in bactericidal experiments.

The carbon modified photocatalysts are widely tested because of their activity under visible light irradiation. Also inactivation of bacteria by that type of photocatalysts was studied, nanocarbon/TiO_2 composite photocatalyst [25], multi-wall carbon nanotubes coated by titanium dioxide [26] or titania/carbon nanotubes [27], antibacterial activity was tested under UV and visible light irradiation.

The objective of the study was to evaluate the activity of five alcohol modified TiO_2 photocatalyst in disinfection processes. Water disinfection tests were conducted by inactivation of gram-negative cells of *Escherichia coli* under UVA and visible light irradiation.

2. MATERIAL AND METHODS

Pulp TiO_2 is commercially available (Police company, Poland). P25 (Evonik, Germany) with BET surface area of 55.5 $m^2 \cdot g^{-1}$ was used as a reference in comparison

with carbon-modified TiO_2 photocatalysts. New groups of carbon-modified TiO_2 photocatalysts were prepared using modification under elevated pressure. 4 g pristine TiO_2 and 5 cm^3 of appropriate organic solvent – alcohol (1:1 weight ratio) were placed inside a pressure reactor and heated up to programmed temperature (120 °C) for 4 h. After that time the reactor was cooled down to room temperature and the prepared materials were dried at 105 °C for 24 h. Various alcohols (methanol, ethanol, *n*-butanol, 2-butanol and *tert*-butanol) of high purity were used as sources of carbon. The carbon content in photocatalysts was analysed using a thermogravimetric analyser (Netzsch, STA 449 C, Germany). In Table 1 the carbon content in used photocatalysts is presented.

Table 1. The carbon content in photocatalysts

Alcohol used for preparation	Carbon in a photocatalyst [wt. %]
Methanol	0.07
Ethanol	0.08
n-Butanol	0.9
2-Butanol	0.5
tert-Butanol	0.27

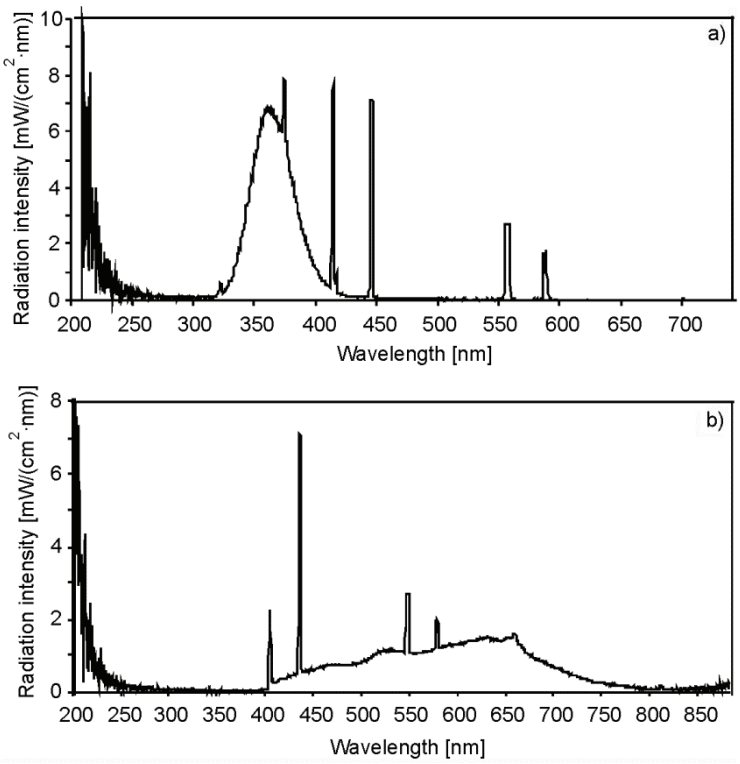

Fig. 1. Emission spectra of light sources: a) UV, b) visible

Escherichia coli ACCT 25922 were cultured in broth for 24 h at 37 °C. The required bacterial concentration was adjusted by serial dilution of NaCl solution (0.9%). Glass beakers of 0.5 dm^3 were used as batch reactors, mixing was applied during disinfection process. Concentration of photocatalysts amounted to 0.2 $g \cdot dm^{-3}$. Control contained 0.5 dm^3 of demineralised water. The emission spectra of light sources: UV (6×20 W, Philips) and visible light (4×18W, Philips, TL-D 18W/33-640) are presented in Fig. 1.

5 μL of the bacterial solution was pipetted into the reactor and illuminated from above with UVA or Vis light for 45 min. Samples were taken every 5 minutes. The irradiation experiments were performed at room temperature (30 °C) reaching 37 °C during irradiation. The experiment was verified three times. Control experiments of photocatalytic processes were conducted with addition of 0.2 $g \cdot dm^{-3}$ catalysts to bacterial suspension but without irradiation (in dark). Samples were taken every 10 min. Serial dilutions were prepared if necessary in NaCl solution (0.9%) and the samples plated on TTC – tergitol agar (Biocorp, Poland). The plates were incubated for 24 h at 37 °C and the colony-forming units (CFUs) were then counted.

The photocatalysts were characterized by UV-Vis/DR using a spectrophotometer (Jasco, Japan) equipped with an integrating sphere accessory for diffuse reflectance spectra ($BaSO_4$ was used as a reference). Diffuse reflectance FTIR/DRS spectra of photocatalysts were recorded using FTIR spectrometer (Jasco, Japan) equipped with DR accessory of Harrick Company (USA).

3. RESULTS AND DISCUSSION

After carbon modification of TiO_2, the character of UV-Vis/DR spectra changed. In Figure 2, typical UV-Vis/DR spectra of *n*-butanol TiO_2 and TiO_2 pulp are presented. A decrease in intensity of the reflectance spectra in the range 400–800 nm is observed.

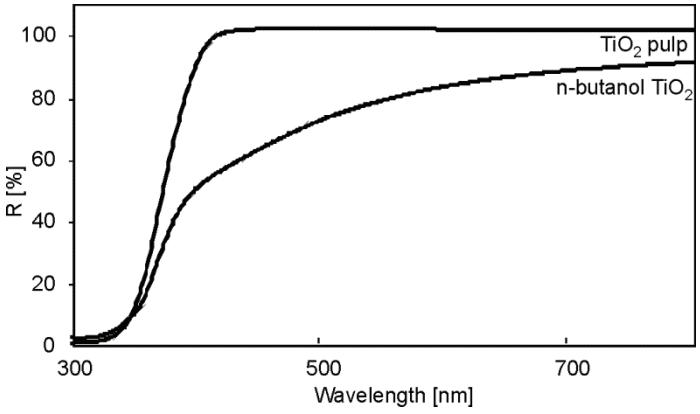

Fig. 2. UV-Vis/DR spectra of pulp and *n*-butanol TiO_2

FTIR/DRS spectra of *n*-butanol TiO_2 and TiO_2 pulp are presented in Fig. 3. Characteristic bands originating from hydroxyl groups assigned to both dissociated water and molecularly adsorbed water were found at 2800–3700 and 1550–1800 cm^{-1}. At 1623 cm^{-1} H–O–H bending vibration of hydroxyl group of molecular water [28].

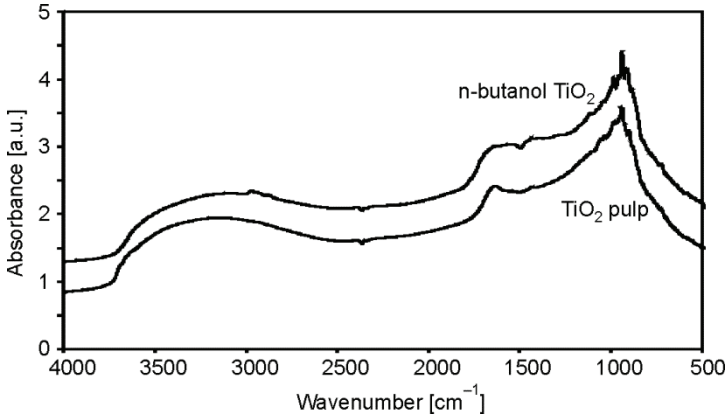

Fig. 3. FTIR/DRS spectra of pulp and *n*-butanol TiO_2

Pressure modification led to appearance of carbon groups on photocatalyst surface however without reduction of the number of surface hydroxyl groups.

E. coli are commonly used as index in microbiological contamination of water [12]. The disinfection ability of titanium dioxide photocatalysts is often tested on *E. coli* [26–31]. In this study, antibacterial capacity of five carbon modified photocatalysts with various amount of carbon (Table 1) was tested under UVA and visible light irradiation. In Figure 4, the disinfection ability of carbon modified TiO_2 under UV light irradiation is shown. The presented results show that carbon modified TiO_2 photocatalysts have strong disinfection ability.

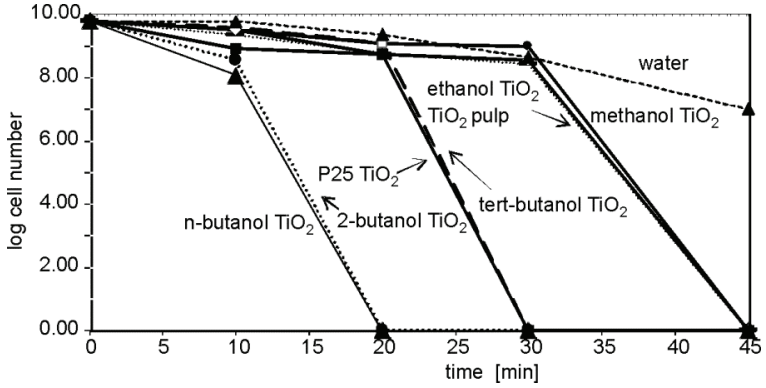

Fig. 4. Disinfection ability of carbon modified photocatalyst under UV irradiation

After 20 min of UV light irradiation in the reactor with the photocatalysts modified with 2-butanol and *n*-butanol, 100% reduction of bacteria occurred. Photocatalysts modified with *tert*-butanol reduced 100% amount of bacteria after 30 min of UV irradiation. Similar activity had commercial TiO_2 P25. After 45 min of irradiation in water with photocatalysts modified with methanol, ethanol and pulp (unmodified TiO_2) the presence of *E. coli* was not confirmed. Carbon modified photocatalysts obtained by modification with 2-butanol and *n*-butanol have even better disinfection ability than commercial TiO_2 P25. Figure 5 shows plates with *E. coli* colony in initial water sample, in water after 10 min of UV irradiation and in water with *n*-butanol TiO_2 photocatalyst after 10 min of UV irradiation. In water sample with *n*-butanol TiO_2 photocatalyst, significant reduction of initial amount of *E. coli* was observed.

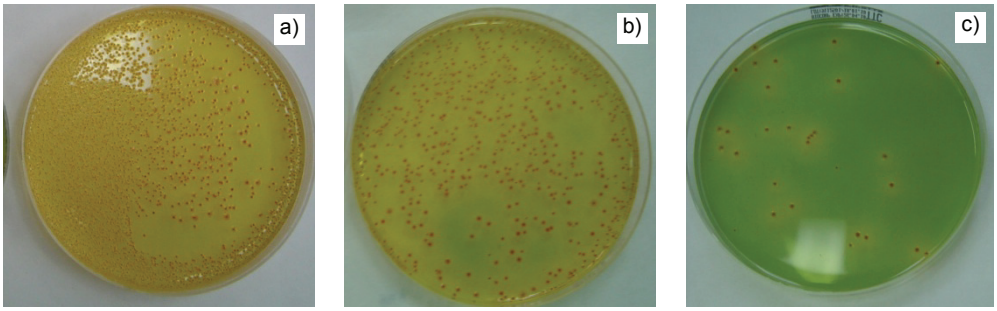

Fig. 5. Plates with *E. coli* colony: a) in initial water sample, b) in water after 10 min of UV irradiation, c) in water with *n*-butanol TiO_2 photocatalyst after 10 min of UV irradiation

Samples without photocatalyst were also irradiated with UV and visible light. After 10 min of irradiation only 2% of bacteria reduction was achieved and even after 45 min of irradiation with UV light 71.4% of *E. coli* were still present in water. After 45 min of irradiation with visible light 98.3% of *E. coli* were present in water.

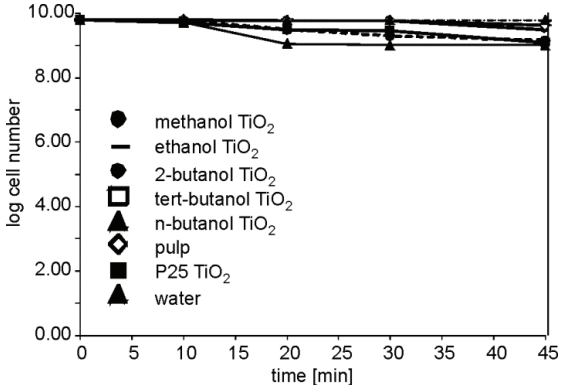

Fig. 6. Concentration of bacteria in water in the presence of photocatalysts

In Figure 6, time dependences of bacteria concentration in water in the presence of photocatalysts are shown. After 45 min insignificant adsorption of bacteria on photocatalyst surface occurs. This adsorption depends on the amount of carbon in photocatalysts. For photocatalysts with higher amount of carbon

,such as *n*-butanol TiO_2 (0.9 wt. % of carbon), 2-butanol TiO_2 (0.5 wt. % of carbon), *tert*-butanol TiO_2 (0.27 wt. % of carbon), the adsorption amounted to 8.1%, 6.6% and 7.2 %, respectively. For photocatalysts with low amount of carbon such as methanol TiO_2 (0.07 wt. % of carbon), ethanol (0.08% wt. % of carbon) the adsorption amounted to 3.3% and 0.2%. In pure water, after 45 min of stirring only 0.1% reduction of *E. coli* was achieved.

Photocatalytic activity of photocatalysts under visible light irradiation aroused interest for many years [12, 35–38]. The possibility of applying visible light photocatalysts in photocatalytic water disinfection may significantly reduce the cost of this process. Additionally solar light irradiation may also be used.

The activity of photocatalysts under visible light irradiation is generally lower than that under UV light irradiation [35, 39–40]. In Figure 7, the antibacterial capacity of photocatalysts under visible light irradiation is presented. The best antibacterial activity has photocatalyst modified in methanol and pure titanium dioxide which is the photocatalyst with the lowest amount of carbon. These photocatalysts reduced all bacteria after 45 min of irradiation with visible light. After the same time photocatalysts with higher amount of carbon: *n*-butanol TiO_2, 2-butanol TiO_2 and *tert*-butanol TiO_2 reduced 27.4%, 17.5% and 26.5% of the initial amount of bacteria, respectively.

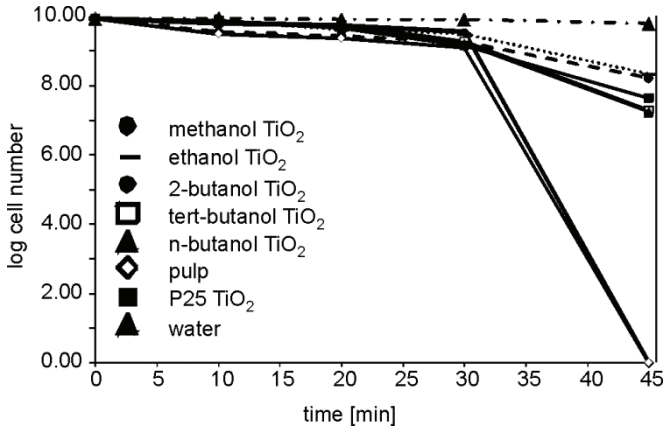

Fig. 7. Disinfection ability of carbon modified photocatalyst under visible light irradiation

Contrary to antibacterial activity of photocatalysts under visible light irradiation, the highest activity under UV light irradiation had photocatalysts with higher amount of carbon. All carbon modified photocatalysts had the same or better disinfection ability than unmodified material (pulp TiO_2). Carbon-modified photocatalysts activity

under visible light irradiation depends on the amount of carbon. Photocatalysts are active only when the amount of carbon in photocatalyst is very low, higher amount of carbon caused a decrease of their activity under visible light irradiation. The highest disinfection ability under visible light irradiation had unmodified photocatalyst (TiO_2 pulp).

4. CONCLUSIONS

Antibacterial activity of carbon modified TiO_2 was tested under UV and visible light irradiation. Five photocatalysts with various content of carbon were obtained. It was found that photocatalysts with a low amount of carbon have better antibacterial ability under visible light irradiation; photocatalysts modified with methanol reduced 100% of *E. coli* after 45 min of irradiation with visible light. The photocatalysts with a higher amount of carbon had better antibacterial ability under UVA light irradiation; photocatalysts modified with 2-butanol and *n*-butanol reduced 100% of bacteria after 20 min of irradiation with UV light.

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