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BIOCATALYSIS

Photocatalysts for Reagentless Disinfection on the Basis of Titanium Dioxide Films Modified by Silver Nanoparticles

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Abstract—Using the test cultures of *Pseudomonas fluorescens* and *Lactococcus lactis* bacteria, photoinduced pathophysiological properties of film titanium dioxide photocatalysts modified by silver nanoparticles are investigated. It is shown that the deposition of silver leads to an increase in the adsorption of microorganisms from the solution and to a rise in the efficiency of photogeneration of hydroxyl radicals and superoxide ions, which provides the attainment of a high level of antimicrobial activity. In particular, the survival rate of *P. fluorescens* eubacteria under the UV irradiation in contact with the TiO_2/Ag catalyst decreases by a factor of more than 70 compared with irradiation in the absence of the photocatalyst. The developed photocatalysts allow the manifold increase in the efficiency of the disinfection of aqueous media with the use of ultraviolet.

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INTRODUCTION

Increased interest in photocatalysts on the basis of wide-gap semiconductors observed in recent years (first of all, titanium dioxide [1–3]) is largely associated with the possibility of the development of new highly effective technologies of the destruction of toxic organic and inorganic substances, as well as photobiocide systems, able to provide the reagentless destruction of pathogenic microorganisms, namely, bacteria, viruses, and fungi [1, 4–6]. In contrast with the direct bactericidal effect of the UV irradiation, which is caused by blocking the DNA replication as a result of the photoinduced dimerization of thymine nucleotides [7], the photoinduced pathophysiological activity of semiconductor photocatalysts is the result of the fact that the cellular structures are affected by various forms of active oxygen (radical particles, $^{\bullet}OH$, $O_2^{\bullet-}$, $^{\bullet}O_2H$, and H_2O_2), which are generated on the semiconductor surface with the participation of both photoholes and photoelectrons [1, 5, 8]. The detailed mechanism of the photocatalytic bactericidal effect remains unclear in many respects, but it is doubtless that it is based on the damage of cellular membranes, which causes the violation of breathing capacity [5, 9–11]. In the case of film photocatalysts, the attainment of a high level of antimicrobial activity implies that not only a high yield of photogenerated charge carriers but also the specific adsorption properties are provided primarily due to going to heterogeneous photocatalytic surfaces.

The goal of this study is to develop the highly active film photocatalysts based on nanostructured TiO_2 modified with silver nanodimensional particles and to investigate their photoinduced bactericidal activity with respect to environmentally widespread eubacteria *Pseudomonas fluorescens* B-22 (as model gram-negative bacteria) and *Lactococcus lactis* ssp. lactis 411 (as an example of gram-positive bacteria).

EXPERIMENTAL

Nanostructured TiO_2 films used as photocatalysts were obtained by the pulverization of aqueous TiO_2 colloids on a glazed ceramic substrate with subsequent heating at 450°C (the details of the procedure of obtaining the photocatalyst are considered in detail in [12]). According to the data of the Rutherford backscattering spectroscopy of the $^4He^+$ ions, the thickness of the TiO_2 films was ~0.2 μm. In all cases, before the deposition of the TiO_2 film on the substrate surface, we deposited the SiO_2 sublayer via pulverization of the aqueous SiO_2 sol. Investigations showed that the mentioned sublayer, which prevents the diffusion of metal ions (specifically, sodium) from the substrate at the heating stage, prevents the formation of recombination centers in the photocatalytic layer and determines high photoelectric activity [13].

To evaluate the photocatalytic activity of the films under study, we used photooxidation of a Rodamin 6Zh dye in a thin-layer photocatalytic cell (the thickness of the layer of the aqueous solution of the dye contacting with the photocatalyst surface was 1 mm, and the initial dye concentration was 5 μM). As the radiation source, we used the line 365 nm of a high-pressure mercury lamp (intensity of irradiation was ~15 mW/cm^2). We observed the photodecomposition of the dye periodically detecting the absorption spectra of the solution in the cell. As the measure of the photocatalytic activity (Y_{ph}), we used a relative decrease in the concentration

Fig. 1. Surface of the film TiO_2 photocatalyst (SEM).

of the Rodamin 6Zh dye after the first 5 min of irradiation:

$$Y_{ph} = 1 - C_5/C_0, \quad (1)$$

where C_0 and C_5 are the dye concentrations before and after irradiation.

To determine the efficiency of generation of the superoxide ions during the functioning of the photocatalyst (I_s), we used the reaction of their selective capture by tetranitromethane [14]. The photocatalyst was irradiated under a thin water layer, after which an aqueous solution of tetranitromethane was introduced into the cell, and the amount of forming nitroform $C(NO_2)_3^-$ was determined photometrically (the value of the extinction of nitroform at a wavelength of 350 nm is 14800 mol/(l cm) [14]).

Photoelectrochemical investigations were performed under potentiostatic conditions with the use of a 0.25 M Na_2SO_4 solution with the addition of 0.1 M CH_3COONa as the hole acceptor. The working electrodes were the films of photocatalysts deposited on ITO (mixed indium–tin oxide). The potentials are given with respect to the Ag/AgCl reference electrode.

The images of the surface of photocatalysts were obtained using a LEO-1420 scanning electron microscope. The morphology of a silver nanophase deposited on the TiO_2 surface was investigated by transmission electron microscopy with the use of a LEO 906E microscope; the samples were prepared by the method of carbon replicas with extraction.

The hydrophilic properties of film photocatalysts were investigated on a Krüss G10 setup for contact angle measurements. A drop of distilled water was deposited on the surface of the photocatalyst, and the contact angle was measured by the projection method.

The photoinduced bactericidal properties of photocatalytically active coatings were evaluated by a decrease in the survival rate of *P. fluorescens* and *L. lactis* bacteria in the presence of the photocatalyst under the effect of UV irradiation. For this purpose, the one-day bacterial culture, after dilution in a sterile 0.15 M NaCl solution to a necessary concentration, was placed into Petri dishes with the samples of ceramic tile of 2 × 2 cm with a photocatalytic coating placed on their bottom or without coating in the case of the reference sample. The suspensions were subjected to 10-min UV irradiation, after which the cellular material was seeded on a nutrient agar (in the case of *P. fluorescens*) or on a peptone–yeast medium (in the case of *L. lactis*). The seedings were incubated for 48 h at 30°C, and by the results of the count on the number of formed colonies, the concentration of survived bacteria was determined. The measure of the bactericidal activity was the reduction factor:

$$RF = C_c/C, \quad (2)$$

where C is the cell concentration in the suspension after the UV irradiation, and C_c is the cell concentration in the suspension after the UV irradiation in the case of the reference sample (ceramic substrate).

The absence of manifestations of significant dark pathophysiological activity of the samples under study with respect to the studied bacteria was confirmed in corresponding reference experiments.

The efficiency of the adsorption of bacteria was evaluated by the value of a decrease in the bacteria concentration in the cellular suspension in contact with the photocatalyst surface. The volume of the cellular suspension was 900 μl with the area of the adsorption surface of 0.75 cm^2. The suspension was kept in contact with the photocatalyst for 10 min (preliminary studies showed that this time is sufficient to establish the adsorption equilibrium), after which, by the seeding method, the bacteria concentration in the suspension and then the efficiency of adsorption, %, were determined:

$$N_a = 100(1 - C_a/C_0), \quad (3)$$

where C_a is the concentration of freely suspended cells upon finishing the adsorption process, and C_0 is the starting cell concentration in the suspension. The fluorescent images of bacteria adsorbed on the surface of the photocatalysts were obtained using a Leica TCS SP confocal microscope; the bacteria were preliminary colored by Rodamin 6Zh. To obtain the mentioned images, the photocatalysts after bacteria adsorption were rinsed and transported into a physiological solution.

RESULTS AND DISCUSSION

The film titanium dioxide photocatalysts obtained by the sputtering of colloids had a developed surface (Fig. 1) and were characterized by high adhesion to the substrate. Silver was deposited on their surface by photocatalytic deposition under the UV irradiation of the TiO_2 film in contact with a 10 μM Ag_2SO_4 solution.

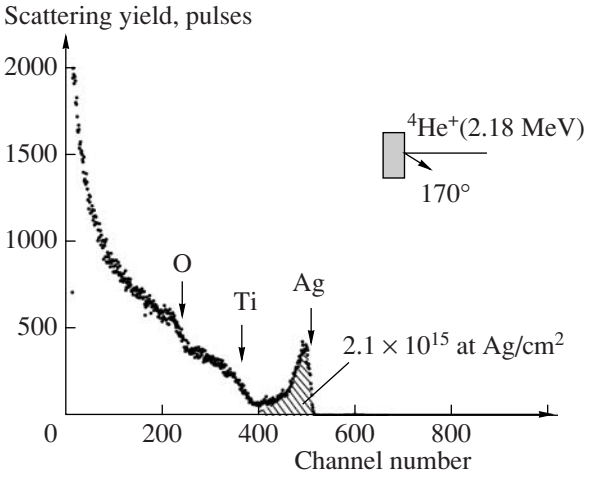

Fig. 2. Spectrum of Rutherford backscattering for the TiO_2/Ag photocatalyst. The surface concentration, atoms/cm^2, of the deposited silver is determined by the area of the peak of scattering on the Ag atoms (marked by shading).

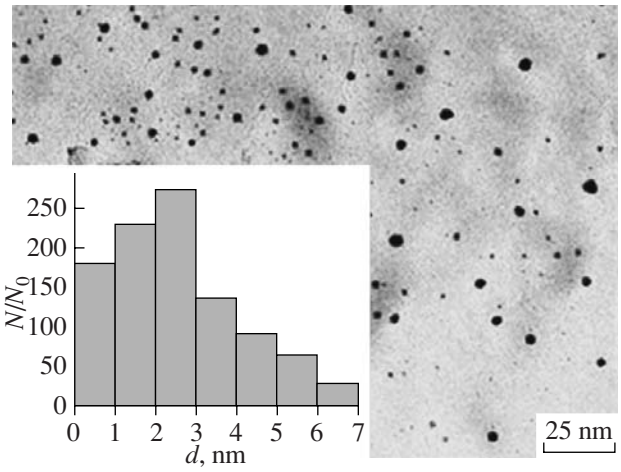

Fig. 3. Electron microscopy image of Ag particles on the surface of the TiO_2/Ag photocatalyst (TEM) and their size distribution (in inset).

We varied the amount of deposited silver by varying the duration of photocatalytic deposition in order to provide the maximum photoactivity of the obtained photocatalyst in the test reaction of the photodecomposition of Rodamin 6Zh. According to the data of Rutherford backscattering spectroscopy (Fig. 2), the surface concentration of silver is ~2×10^{15} at/cm^2. The average particle size of the silver nanophase was ~2.5 nm; the evaluation is obtained by the results of transmission electron microscopy (Fig. 3).

The results of the investigation of the pathophysiological effect of the photocatalysts under study in the UV irradiation conditions are generalized in Table 1. The represented data indicate a decrease in the survival rate of the test culture under irradiation in the presence of the photocatalysts and, consequently, the expressed bacterial activity inherent to latter ones.

For example, under irradiation of the cellular suspension in the presence of the TiO_2 photocatalyst, the concentration of survived *P. fluorescens* bacteria decreases by a factor of 33, and that of *L. lactis* decreases by a factor of 16 compared with the case of the irradiation of the same suspension in contact with the photocatalytically inactive surface. Modifying the surface of titanium dioxide with silver nanoparticles leads to a further increase in efficiency of the deactivation of bacteria. For example, in the case of *P. fluorescens*, the reduction factor with the use of photocatalytic TiO_2/Ag reaches a value of 71; the same tendency is also retained in the case of *L. lactis*, but an increase in the reduction factor is less considerable (~15%). The efficiency of photocatalytic disinfection is vividly represented in Fig. 4.

Taking into account the short lifetime of most of the photogenerated forms of active oxygen (first of all, OH radicals, which possess the highest pathophysiological activity [5, 11, 15]), the efficiency of the photodeactivation of microorganisms in contact with the film photocatalyst is to a large extent determined by the adsorption properties of the photocatalytic surface.

It is evident from the confocal microscopic images of the surface of photocatalysts TiO_2 and TiO_2/Ag, the amount of *P. fluorescens* and *L. lactis* bacteria adsorbed on the TiO_2 surface modified by silver particles is higher than in the case of starting TiO_2 (Fig. 5).

Quantitatively, the adsorption efficiency was evaluated by a decrease in the concentration of microorganisms in the suspension after carrying it in contact with

Table 1. Survival rate of *P. fluorescens* and *L. lactis* bacteria under UV irradiation in the presence and absence of film photocatalysts. The initial concentration of the cells: 700 ml^{-1} (*P. fluorescens*) and 3000 ml^{-1} (*L. lactis*)

Photocatalytic surface	*P. fluorescens*		*L. lactis*	
	VCAI*/ml	RF	VCAI/ml	RF
TiO_2	3.6×10	33.3	2.8×10^2	16.1
TiO_2/Ag	1.7×10	70.6	2.4×10^2	18.8
Ceramic substrate	1.2×10^3	1.0	4.5×10^3	1.0

* The number of viable cells after irradiation.

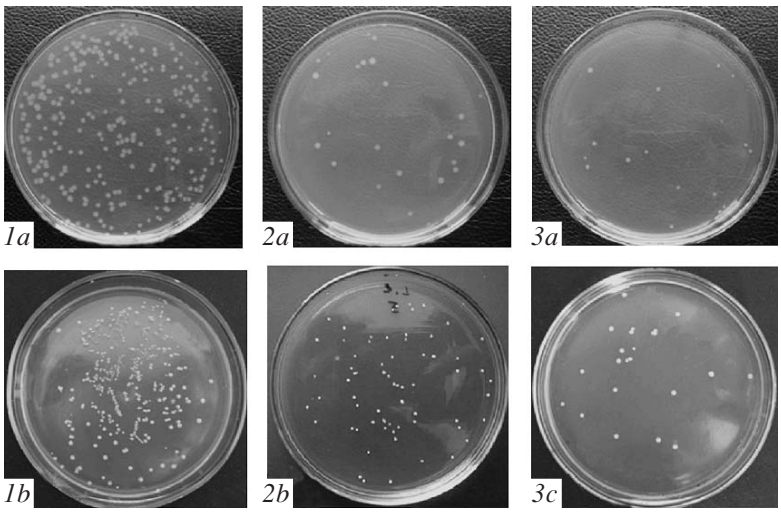

Fig. 4. Petri dishes with a nutrient medium, in which bacteria (*a*) *P. fluorescens* and (*b*) *L. lactis* were seeded after the UV irradiation of the cell suspension in contact with (*1*) the ceramic substrate, (*2*) TiO_2 films, and (*3*) TiO_2/Ag.

the photocatalyst surface. The amount of adsorbed bacteria on the titanium dioxide photocatalyst modified with silver increases the number of bacteria (N_a) adsorbed on titanium dioxide by a factor of 3–6:

Bacteria	*P. fluorescens*	*L. lactis*
N_a:		
TiO_2	5.6	10.6
TiO_2/Ag	32.6	31.5

As one of the factors responsible for an increase in the adsorption ability of the photocatalytic surface with respect to the microorganisms under study in the case of the TiO_2/Ag photocatalyst, we can consider an abrupt increase in the wetting ability of titanium dioxide as a result of its modification with silver nanoparticles. In particular, the measurements with the use of a water drop (see inset in Fig. 5) show that the contact angle decreases from 72° for TiO_2 down to 49° in the case of TiO_2/Ag. A high efficiency of deactivation of microorganisms in the case of the TiO_2/Ag photocatalyst cannot be attributed exclusively to the factor of increasing the wetting ability since, as was shown in [15], the antimicrobial activity of the films of the superhydrophilic TiO_2 + In_2O_3 composite is considerably lower than for the films of lesser hydrophilic TiO_2.

Another factor responsible for the observed increase in the photoinduced bactericidal activity of TiO_2 as a result of modification with silver nanoparticles is a total increase in photocatalytic activity on going from the TiO_2 films to the TiO_2/Ag films (the rate of the photooxidation of Rodamin 6Zh increases by a factor of two in this case (Table 2).

The results of photoelectric measurements show that the deposition of silver on the TiO_2 surface leads to a substantial increase in the anode photocurrent (Fig. 6a). This in turn indicates an increase in the yield of photogenerated charge carriers in the case of TiO_2/Ag and a decrease in the level of surface recombination. The obtained results are in good agreement with the previously established fact of the reconstruction of surface states during the deposition on TiO_2 of small silver particles (less than 3–4 nm in diameter), which leads to the shift of the maxima of the distribution density of the mentioned states to the edges of the bands of semiconductors [16] and the destruction of the surface recombination channel (Fig. 6b). In this case, it was established that the deposition of large particles, which is accompanied by the recrystallization of the silver nanophase, leads to a decrease in the modifying effect of the deposited metal on the structure of surface states [16]. In our case, an increase in the amount of deposited silver (and, consequently, in the particle size) is accompanied by an abrupt decrease in the photoinduced pathophysiological activity of the TiO_2/Ag photocatalyst.

The pathophysiological activity of the photocatalytic systems is caused not only by the sorption proper-

Table 2. Photocatalytic activity and efficiency of photogeneration of O_2^- for the photocatalytic systems under consideration

Photocatalytic surface	Y_{ph}	I_s^*
TiO_2	0.19	1.5×10^{15}
TiO_2/Ag	0.33	2.0×10^{15}
Ceramic substrate	0.10	0

* The number of superoxide ions formed on 1 cm^2 of the photocatalyst for 10 min of UV irradiation in contact with an aqueous medium

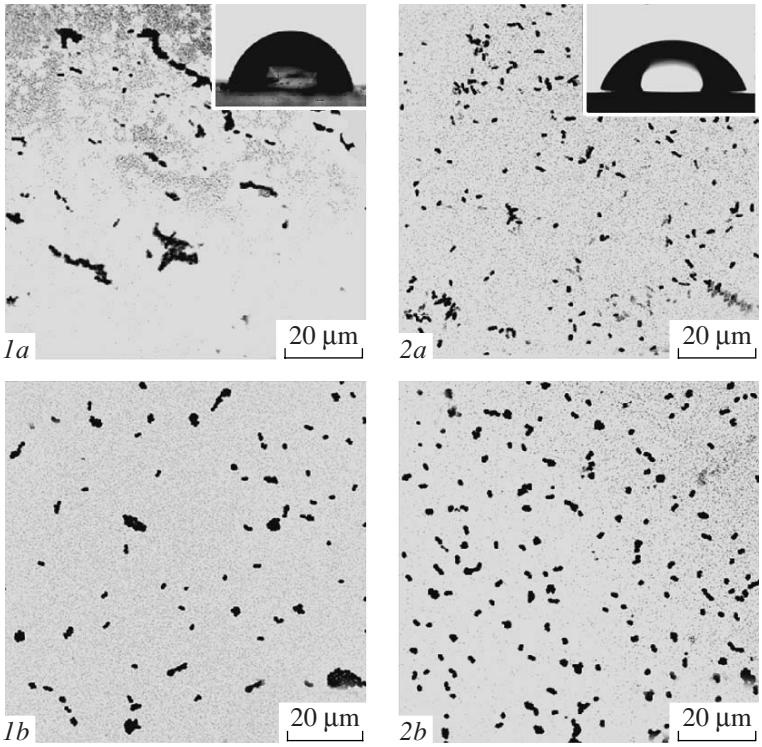

Fig. 5. Confocal microscopic images of bacteria (*a*) *P. fluorescens* and (*b*) *L. lactis* adsorbed on the surface of (*1*) TiO_2 and (*2*) TiO_2/Ag. Dark segments in images correspond to luminescent colored microorganisms. The images of the water drop on the surface of TiO_2 and TiO_2/Ag are in insets.

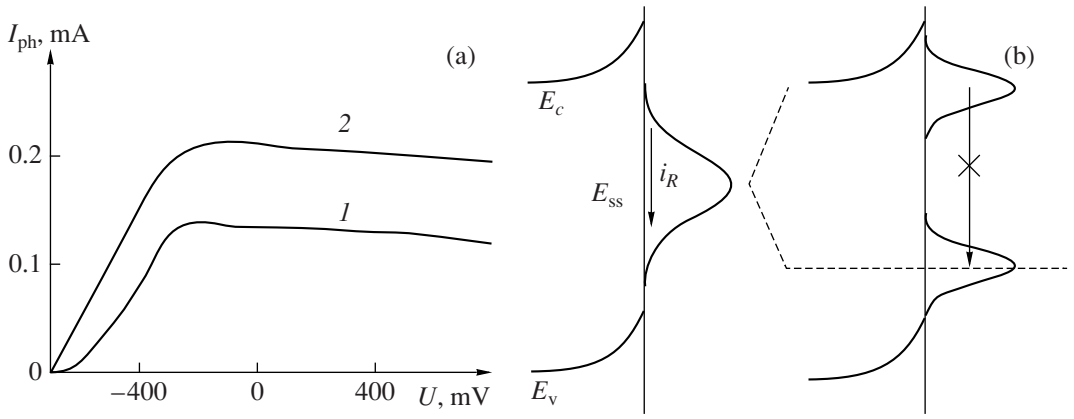

Fig. 6. (a) Polarization dependences of the photocurrent for film photocatalysts (*1*) TiO_2 and (*2*) TiO_2/Ag. (b) Schematic image of varying the distribution character of the surface states in the band gap of TiO_2 as a result of the deposition of silver nanoparticles [16]. The surface of TiO_2 is depicted in contact with an aqueous solution: to the left is before the deposition of silver and to the right is after the deposition, E_c and E_v are the energies of the edges of the conduction band and valence band, E_{ss} is the energy of surface states, and i_R is the recombination process with the involvement of surface states in the band gap.

ties of the photocatalysts and the efficiency of the generation of nonequilibrium charge carriers (photoelectrons and photoholes) under the conditions of actinic radiation but also by the efficiency of the conversion of photogenerated carriers with the formation of chemically active agents upon proceeding the surface oxidation–reduction reactions, as well as by the sensitivity of test cellular cultures with respect to different photogenerated forms of active oxygen. The results of photoelectrochemical measurements indicate an increase in the yield of such strong oxidizing agents as photoholes and hydroxyl radicals in the case of TiO_2/Ag, which is

the consequence of a decrease in the level of recombination losses. In turn, the chemoluminescent measurements indicate that modifying the TiO_2 surface by silver particles is accompanied by the yield of superoxide ions, which are formed as a result of the capture of photoelectrons by molecular oxygen (see Table 2).

The observed distinctions in the photobiocide activity of the TiO_2 and TiO_2/Ag catalysts with respect to gram-negative and gram-positive bacteria (see Table 1) can be explained by the specific features of the structure of mentioned microorganisms. The mechanism of the antimicrobial effect of film photocatalysts on the basis of titanium dioxide is first of all caused by the effect of active forms of oxygen of the cells; the cause of the death of the latter is the damage of cell envelopes [17, 18]. The dynamics of the process of the death of bacteria on the surface of the TiO_2 films under the UV irradiation, as a rule, includes two stages, namely, the "slow" one, which is associated with the accumulation of damages in the cell wall, and the accompanying "rapid" one, which corresponds to the violation of the plasmatic membrane [11, 19]. In the case of bacteria *L. lactis*, there is a strong murein skeleton over the plasmatic membrane. This skeleton includes no less than 40 murein layers, which are strengthened by interchain peptide bridges and molecules of teichoic acids; the total thickness of the cell envelope reaches 40 nm. However, the cell wall of *P. fluorescens* includes only a thin (~2 nm) murein layer and the outer membrane consisting of lipopolysaccharides, phospholipids, and proteins. We can assume that the peptidoglycan skeleton of lactococcus is less vulnerable for active forms of oxygen than the outer membrane of pseudomonades, which are based on polar lipids. Therefore, the penetration of active forms of oxygen through cell walls of bacteria to the main target—plasmatic membrane—should be simpler and more rapid for gram-negative *P. fluorescens* bacteria and more complex and prolonged for gram-positive *L. lactis* bacteria.

CONCLUSIONS

Modifying the surface of the films of nanostructured titanium dioxide with small amounts of silver (one or two calculation monolayers) provides a substantial increase in their bactericidal activity under the conditions of UV irradiation with respect to both gram-negative and gram-positive bacteria. The observed increase in the rate of the photodeactivation of microorganisms in the case of the TiO_2/Ag photocatalyst is the result of the effect of the complex of factors including the increased adsorption ability of the TiO_2 surface modified with silver particles, the high generation efficiency of electron–hole pairs under the conditions of UV irradiation, and the high yield of superoxide ions. A high photoinduced bactericidal activity of the TiO_2/Ag film photocatalysts makes it possible to develop effective photocatalytic systems of the reagentless disinfection of aqueous media on their basis.

REFERENCES

1. Fujishima, A., Rao, N., and Tryk, D., *J. Photochem Photobiol. C: Photochem. Rev.*, 2000, vol. 1, no. 1, p. 1.
2. Hoffmann, M.R., Martin, S.T., Choi, W., and Bahnemann, D.W., *Chem. Rev.*, 1995, vol. 95, no. 1, p. 69.
3. Shchukin, D.G. and Sviridov, D.V., *J. Photochem. Photobiol. C: Photochem. Rev.*, 2006, vol. 7, no. 1, p. 23.
4. Kikuchi, Y., Sunada, K., Iyoda, T., Hashimoto, K., and Fujishima, A., *J. Photochem. Photobiol. A: Chem.*, 1997, vol. 106, no. 1, p. 51.
5. Blake, D.M., Maness, P.-C., Huang, Z., Wolfrum, E.J., Huang, J., and Jacoby, W.A., *Separat. Purif. Methods*, 1999, vol. 28, no. 1, p. 1.
6. Lee, S.-H., Pumprueg, S., Moudgil, B., and Sigmund, W., *Colloids Surf., B: Biointerfaces*, 2005, vol. 40, no. 2, p. 93.
7. Harris, G.D., Dean, V.A., Darwin, L.S., and Curtis, M.S., *Water Res.*, 1987, vol. 21, no. 6, p. 687.
8. Cho, M., Chung, H., Choi, W., and Yoon, J., *Appl. Environ. Microbiol,* 2005, vol. 71, no. 1, p. 270.
9. Saito, T., Iwase, T., and Morioka, T., *J. Photochem. Photobiol. B: Biol.*, 1992, vol. 14, no. 1, p. 369.
10. Huang, Z., Maness, P.-C., Blake, D.M., Wolfrum, E.J., Smolinski, S.L., and Jacoby, W.A., *J. Photochem. Photobiol., A: Chem.*, 2000, vol. 130, nos. 2–3, p. 163.
11. Sunada, K., Kikuchi, Y., Hashimoto, K., and Fujishima, A., *Environ. Sci. Technol.*, 1998, vol. 32, no. 1, p. 726.
12. Skorb, E.V., Ustinovich, E.A., Kulak, A.I., and Sviridov, D.V., *J. Photochem. Photobiol. A: Chem.,* 2008, vol. 193, nos. 2–3, p. 97.
13. Shchukin, D.G., Kulak, A.I., and Sviridov, D.V., *Photochem. Photobiol. Sci.*, 2002, vol. 1, no. 10, p. 742.
14. Rabani, J., Mulac, W.A., and Maseson, M.S., *J. Phys. Chem.*, 1965, vol. 69, no. 1, p. 53.
15. Skorb, E.V., Antonovskaya, L.I., Belyasova, N.A., and Sviridov, D.V., *Dokl. Nats. Akad. Nauk Belarusi*, 2007, vol. 51, no. 3, p. 62.
16. Kulak, A.I., Kokorin, A.I., and Sviridov, D.V., *J. Mater. Res.*, 2001, vol. 16, no. 8, p. 2357.
17. Ireland, J.C., Klostermann, P., Rice, E.W., and Clark, R.M., *Appl. Environ. Microbiol.*, 1993, vol. 59, no. 5, p. 1668.
18. Jacoby, W.A., Maness, P.C., Wolfrum, E.J., Blake, D.M., and Fennell, J.A., *Environ. Sci. Technol.*, 1998, vol. 32, no. 17, p. 2650.
19. Lu, Z.X., Zhou, L., Zhang, Z.L., Shi, W.L., Xie, Z.X., Xie, H.Y., Pang, D.W., and Shen, P., *Langmuir*, 2003, vol. 19, no. 21, p. 8765.