Visible light inactivation of bacteria and fungi by modified titanium dioxide

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Received 22nd November 2006, Accepted 9th March 2007
First published as an Advance Article on the web 2nd April 2007
DOI: 10.1039/b617043a

Visible light induced photocatalytic inactivation of bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*) and fungi (*Candida albicans*, *Aspergillus niger*) was tested. Carbon-doped titanium dioxide and TiO_2 modified with platinum(IV) chloride complexes were used as suspension or immobilised at the surface of plastic plates. A biocidal effect was observed under visible light irradiation in the case of *E. coli* in the presence of both photocatalysts. The platinum(IV) modified titania exhibited a higher inactivation effect, also in the absence of light. The mechanism of visible light induced photoinactivation is briefly discussed. The observed detrimental effect of photocatalysts on various microorganism groups decreases in the order: *E. coli* > *S. aureus* ≈ *E. faecalis* ≫ *C. albicans* ≈ *A. niger*. This sequence results most probably from differences in cell wall or cell membrane structures in these microorganisms and is not related to the ability of catalase production.

Introduction

The environmental disinfection plays a crucial role in the prevention of infectious diseases. Health benefits from disinfection have been established through studies of applications such as critical instrument sterilization, water treatment and food production. The aim of disinfection is to reduce number of pathogenic microorganisms on an object or in environment. The disinfectants commonly used are of chemical origin, with the accompanying risk of allergic reactions and toxicity. Increase of microorganisms resistance to commonly applied chemotherapeutics and more rigorous hygienic standards in hospitals constrain development of new agents to be used in disinfection. The need for appropriate disinfection procedures is highlighted by the multitude of outbreaks resulting from improperly decontaminated patient-care items.

An alternative approach to disinfection may be heterogeneous (semiconductor) photocatalysis, suitable for detoxification from both industrial and biological pollutants. The photocatalysis is based on redox properties of surface trapped photogenerated charges and formed reactive oxygen species (ROS; $OH^{\bullet}$, $O_2^{\bullet-}$, H_2O_2, $^1O_2$ *etc.*).[1–4] Formation of ROS is especially efficient in the presence of irradiated titanium dioxide. Principles of the photocatalysis are described in several excellent reviews.[5–7] The redox activity of photoexcited titanium dioxide may have a significant biological impact as exemplified by its bactericidal activity *in vivo*.[5] There are many reports on cytotoxicity of reactive oxygen species photogenerated at TiO_2 surface upon UV-light irradiation.[8–10] One of the most toxic for microorganisms is the $OH^{\bullet}$ radical because of its ability to oxidise many organic substrates like carbohydrates, lipids, proteins and nucleic acids, *etc.*[11] It promotes peroxidation of polyunsaturated phospholipid components of the lipid membrane and induces disorder in the cell membrane.[12]

Since 1985, when Matsunaga *et al.* reported photocatalytic inactivation of *Saccharomyces cerevisiae* in the presence of TiO_2 irradiated with UV light,[13] the heterogeneous photocatalysis has become a promising tool allowing detoxification and disinfection. Matsunaga and his co-workers made also the first attempt to elucidate the mechanism of titania photocytotoxicity.[13] They have demonstrated that oxidation of coenzyme A (CoA) may be a possible mechanism of photoinduced cell death. However, the reactivity of ROS was thought to be non-selective. A possible role of ROS was oxidation of the cell membrane prior to the oxidation of CoA.[13,14] In 1992, Saito *et al.* reported that decomposition of the cell membrane and loss of its permeability are the main factors responsible for the bacteria cell death.[15] In 1997 Kikuchi and co-workers suggested that the lethal bactericidal agent is H_2O_2 produced from photogenerated superoxide anions. A cooperative effect of various oxidative species was postulated as an explanation of *E. coli* inactivation mechanism.[8] In 1999 Maness *et al.* presented results showing that irradiated TiO_2 induces peroxidation of polyunsaturated phospholipid components of the lipid membrane.[12] It causes major disorder in the *E. coli* cell membrane leading to inhibition of fundamental vital processes of the cell and in consequence to its death. Sunada *et al.* demonstrated that decomposition of the outer membrane caused by peroxidation process results in cytoplasmatic membrane disorder and afterwards in cellular death.[9] The cell damage initiated by photocatalytic processes may be continued by consecutive post-irradiation reactions due to a relatively high concentration of ROS.[11,12] A linear correlation between the amount of photogenerated $OH^{\bullet}$ radical and the extent of *E. coli* inactivation in TiO_2 photocatalytic disinfection has been found by Cho *et al.*[16] Their further study clearly showed a dependence of biocidal inactivation on efficiency of surface or bulk hydroxyl radical generation as well as on a type of microorganism (MS-2 phage or *E. coli*).[17]

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A huge majority of studies deals with neat TiO_2 activated with UV light. There are only a few reports on biocidal inactivation with visible light-sensitised titania. In 2003, Yu *et al.* showed bactericidal effect of metalphthalocyanine-sensitised titanium dioxide films under visible light irradiation.[18] In 2006, Shieh *et al.* reached 99.99% deactivation of *E. coli* on thin films fabricated from nitrogen-doped TiO_2 under visible light irradiation.[19]

A possible application of the visible light active TiO_2-coated surfaces might be a permanent reduction in the number of pathogenic microorganisms on surfaces which usually may be heavily contaminated by them. The photocatalytic bacteria-reducing process induced by ambient light would be useful in hospitals, microbiological laboratories, food processing plants, pharmaceutical industry, pharmacies, and wherever there is a strong requirement for clean and sterile surfaces. In this work we have tried to evaluate visible light active titania-based photocatalysts in killing of bacteria and fungi. The selected materials showing photocatalytic activity in mineralisation of organic pollutants under visible light irradiation were: titanium dioxide doped with highly unsaturated organic matter (carbon-doped titania; C/TiO_2)[20] and TiO_2 modified at surface with the platinum(IV) chloride complex (4% $H_2[PtCl_6]$/TH-0).[21] Photoinactivation studies were performed using the following bacterial and fungal strains: *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Candida albicans*, *Aspergillus niger.*

Experimental

Photocatalyst preparation

Commercially available TiO_2 materials, P25 (Degussa, 79 : 21% anatase : rutile; specific surface area *ca.* 50 m^2 g^{-1}) and TH-0 (Kerr-McGee, anatase; specific surface area *ca.* 330 m^2 g^{-1}) were employed. The surface modified TiO_2 photocatalyst with platinum(IV) chloride complex (4% $H_2[PtCl_6]$/TH-0) and carbon-doped TiO_2 (C/TiO_2) were prepared according to the procedures described elsewhere.[20,22]

Culture of bacteria and fungi

The following bacterial strains were used: *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) and *Enterococcus faecalis* (PCM 896). The bacteria strains were grown overnight in a TSA medium (Tryptic Soybean Agar, Difco). Aliquots of these cultures were inoculated into fresh medium (Tryptic Soy Broth, Difco) and incubated under aerobic conditions at 37 °C until the exponential growth phase was reached.

Two fungal strains: *Candida albicans* (ATCC 10231) and *Aspergillus niger* (IPFII-A.n./1) were chosen for tests. *Candida albicans* was cultured in liquid Sabouraud agar (BioMerieux) and incubated under aerobic conditions at 37 °C overnight. The slow-growing *Aspergillus niger* was grown on Czapek Dox medium (Biocorp) and incubated for seven days at 27 °C.

The standard suspensions of bacteria and fungi (approximately 10^6 CFU mL^{-1}) were obtained by serial diluting of these cultures.

Antimicrobial activity

C/TiO_2 and 4% $H_2[PtCl_6]$/TH-0 were used in immobilised form and in suspension. Immobilisation was achieved by casting the aqueous suspension of photocatalyst (total 6 mg) onto plastic plates (diameter = 6.5 cm; Nunc) followed by water evaporation. In the case of suspensions the photocatalyst concentration of 1 g L^{-1} was selected. The suspensions and solutions were freshly prepared prior to experiments using sterile water.

Photoinactivation activity was evaluated applying two methodologies depending on the form of the photocatalyst-suspended or immobilised. In the first case the photocatalyst and microorganisms suspensions (10^6 CFU mL^{-1}; 30 mL) were irradiated under continuous stirring. In the tests involving immobilised photocatalysts 2 mL of microbial suspension (10^6 CFU mL^{-1}) was pipetted onto the photocatalyst-coated plastic plate and irradiated. In both methods, after irradiation, the aliquots of 100 μL were collected, serially diluted and spread onto the nutrient agar medium. In order to determine the number of viable cells (counted as colony-forming units, CFU mL^{-1}) the samples were incubated at 37 °C for 24 h and thereafter bacteria colonies were counted. The results are given as survival fractions, S/S_0.

All experiments were repeated at least two times. Estimated errors did not exceed 20%. Control tests involving unmodified photocatalysts (P25, TH-0), UV or UV-vis light alone and photocatalysts kept in the dark were carried out for all experiments described in this paper applying adequate procedures.

Light source and irradiation conditions

The suspensions were irradiated with high pressure mercury lamp (HBO-500, Osram) through the 0.1 M $CuSO_4$ aqueous solution filter (10 cm) and optical cut-off filter ($\lambda > 385$ nm; UV-vis; or $\lambda > 455$ nm; vis), Fig. 1. Measured light intensities were 1.8 W cm^{-2} and 1.0 W cm^{-2} for 385 and 455 nm cut-off filters, respectively. The samples were irradiated in a cylindrical photoreactor (volume 30 mL, 3 cm optical path) upon continuous stirring. Immobilised photocatalysts were irradiated with a halogen lamp (1000 W) through the water filter (14 cm) at a light intensity of 0.2 W cm^{-2}. The light intensity was measured with a radiant power meter (Nova II, OPHIR) equipped with a PD 300UV head.

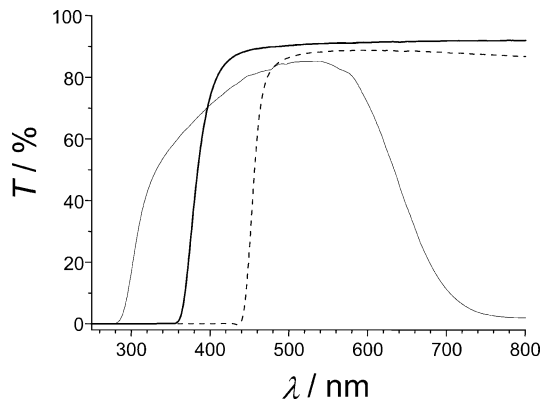

Fig. 1 Transmission spectra of applied optical filters: cut-off 385 nm (thick line), 455 nm (dashed line) and $CuSO_4$ solution filter (thin line).

Hydroxyl radical scavenging

Suspensions of the studied photocatalysts (0.5 g L^{-1}) in aqueous solutions of benzoic acid (10^{-2} M) were irradiated with high pressure mercury lamp (HBO-500, Osram, *vide supra*) through

selected cut-off filters. Samples were withdrawn, filtered through the Millipore filters and the formed salicylic acid was determined monitoring its fluorescence at 400–420 nm when excited at 300 nm. Fluorescence spectra were recorded using a Perkin Elmer LS 50B luminescence spectrometer.

Results and discussion

Bactericidal effect in titania suspension

Transformed diffuse reflectance spectra of studied photocatalysts, C/TiO_2 and 4% $H_2[PtCl_6]/TH\text{-}0$, are presented in Fig. 2. In addition to the UV-absorption characteristic for neat TiO_2 both photocatalysts show broad absorption shoulders extending up to *ca.* 700 nm.

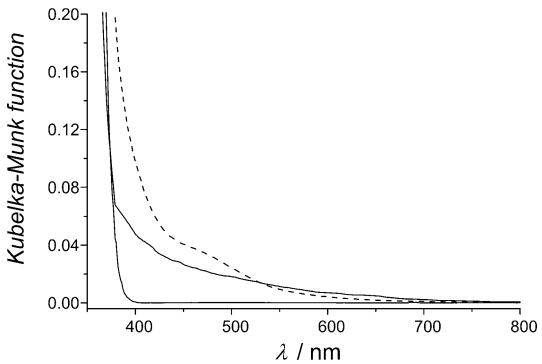

Fig. 2 Electronic spectra of studied photocatalysts: neat TiO_2 (TH-0, thin line), C/TiO_2 (thick line) and 4% $H_2[PtCl_6]/TH\text{-}0$ (dashed line).

Suspensions of *E. coli* and C/TiO_2 were irradiated with the HBO lamp equipped with appropriate cut-off filters transmitting UV-vis ($\lambda > 385$ nm) or visible light ($\lambda > 455$ nm). The viability of the treated cells was determined by colony counting after 24 h of incubation.

UV-vis light treatment resulted in a complete inactivation of *E. coli* regardless whether titania (neat or modified) was present or not (Fig. 3). A relatively high light intensity in our experimental set-up assured photoinactivation in a narrow range of applied UV light ($\lambda > 385$ nm; 1.8 W cm^{-2}). A slight decrease of the inactivation rate in the case of C/TiO_2 | UV-vis and P25 | UV-vis systems in comparison to UV-vis treatment alone is probably caused by the inner filter effect – absorption of the harmful UV-light by suspended titanium dioxide which protects microorganisms to some extent. Also light penetration in the case of suspensions is not as deep as in the case of the photocatalyst-free system. A similar inner filter effect was reported for the UV-induced photoinactivation at high light intensities which was slower in the presence of titania than in its absence.[23] This effect is not observed when a harmless visible light is applied (*vide infra*).

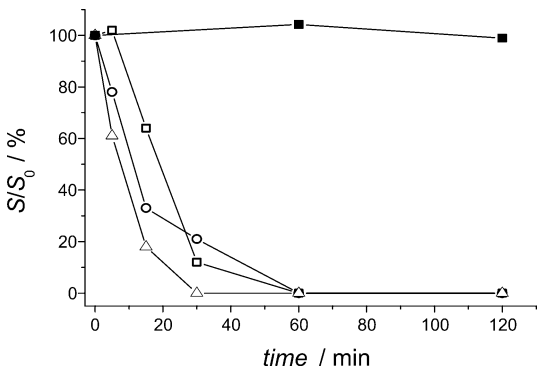

Fig. 3 Survival fraction of *E. coli* under UV-vis light irradiation ($\lambda >$ 385 nm): irradiation in the absence of any photocatalyst (△), in the presence of neat titania P25 (○), in the presence of C/TiO_2 (□), and in the presence of C/TiO_2 kept in the dark (■).

For experiments under visible light irradiation the 455 nm cut-off filter was selected (compare Fig. 1 and 2). These conditions did not allow either a direct titania excitation or a direct bacteria inactivation. Therefore irradiation of the neat TiO_2 suspension did not result in any significant *E. coli* photoinactivation (Fig. 4), although a relatively high light intensity was assured ($\lambda > 455$ nm; 1.0 W cm^{-2}). However, in the presence of C/TiO_2 the S/S_0 fraction decreased by 81 ± 4% after 2 h of irradiation (Fig. 4). This observation points at a beneficial role of the titanium dioxide photosensitization toward visible light.

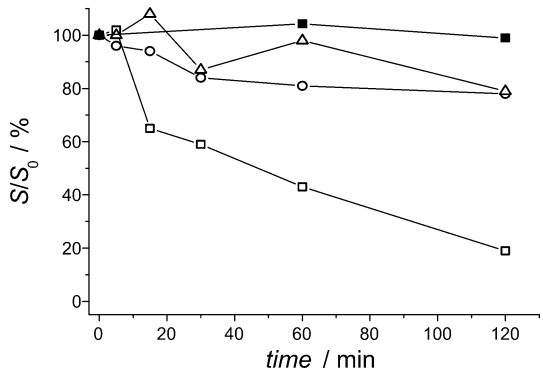

Fig. 4 Survival fraction of *E. coli* under visible light irradiation ($\lambda >$ 455 nm): irradiation in the absence of any photocatalyst (△), in the presence of neat titania P25 (○), in the presence of C/TiO_2 (□), and in the presence of C/TiO_2 kept in the dark (■).

An analysis of the shape of the survival fraction curve may provide useful information on the mechanism of the photoinactivation process. In the case of C/TiO_2 | vis system three steps of bacteria killing may be observed: incubation, fast and slow inactivation (Fig. 5). The kinetics of the last two steps can be described by a two exponential decay equation. For this model

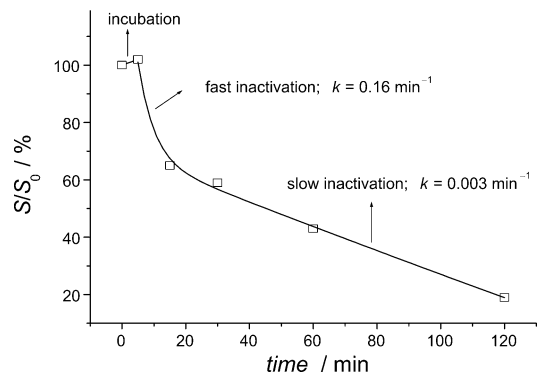

Fig. 5 Steps of *E. coli* inactivation in the suspension of C/TiO_2 irradiated with visible light.

the rate constants k are equal to 0.16 min^{-1} and 0.003 min^{-1} for the fast (time between 5 and 15 min) and slow (time between 15 and 120 min) inactivation, respectively. In the first step, when the bacteria concentration remains unchanged, the concentration of photogenerated ROS increases until it reaches a level which is harmful to bacteria. Below this concentration the self-defence mechanisms of bacteria involving enzymes, catalase and superoxide dismutase (SOD), protect the cell from the oxidative stress:

$$2H_2O_2 \xrightarrow{\text{catalase}} O_2 + 2H_2O$$

$$2O_2^{-\cdot} + 2H^+ \xrightarrow{\text{SOD}} O_2 + H_2O_2$$

These mechanisms are insufficient, when the concentration of ROS is higher. The oxidation of cell wall and membrane components leads to bacteria death. The decreasing inactivation rate in the last step is probably caused by the ROS consumption not only by living cells, but also by products of bacterial lysis.[24] The system can also achieve the dynamic equilibrium when the photocatalytic inactivation of *E. coli* compensates with the growth of the bacteria cells.[24]

Tests of photoinactivation of *E. faecalis* and *S. aureus* in the suspensions of C/TiO_2 irradiated with visible light have shown that these bacteria are more resistant to photoinduced stress than *E. coli* (data not shown). *E. faecalis* does not produce catalase and therefore enzymatic self-defence mechanisms should be less efficient in this case.[25] *S. aureus*, similarly to *E. coli*, uses catalase for self-protection. A comparison of the surviving abilities of *E. faecalis*, *S. aureus* and *E. coli* shows that the enzymatic mechanism of bacteria defence from oxidative stress may not be the most important one. Perhaps the cell wall properties (its structure, thickness, *etc.*) rather than production of antioxidating enzymes have more decisive influence on the survival abilities of bacteria. Nevertheless our results are in agreement with usually reported order of bacteria resistance in UV photoinactivation processes in the presence of titania: *E. coli* < *S. aureus* ≈ *Enterococcus sp.*[24,26,27]

Bactericidal effect at immobilised titania

By introducing immobilisation of the photocatalyst several factors influencing efficiency of the photocatalytic inactivation of microbes may be changed: the access of light and oxygen to the photocatalyst surface, the distance between microbes and photocatalyst, possible penetration of the photocatalyst nanoparticles into microbe cells.[11] Although photoinactivation of bacteria should be more efficient in suspensions of a photocatalyst as compared to its immobilised form, the latter has a particular advantage—immobilised photocatalyst may form a self-disinfecting and self-cleaning surface. Therefore in our further experiments the photoinactivation of bacteria was tested in the presence of immobilised visible light active titania. Searching for surfaces easily self-disinfectable upon illumination with typical light sources we have decided to use a halogen lamp instead of a high pressure mercury lamp. The spectrum of the light emitted by a halogen lamp resembles the spectrum of diffuse daylight more than that emitted by a high pressure mercury lamp. It comprises only a very small part of UV-light necessary for direct excitation of unmodified titania. Unfortunately, the photoinactivation effect tested in this set-up (compare Experimental) in the presence of visible light active C/TiO_2 was not observed neither in the case of *E. coli* nor *S. aureus* (data not shown). Apparently, under the experimental conditions of the experiment (immobilised form of the photocatalyst instead of suspension, significantly lower light intensities) the induced oxidative stress appeared to be insufficient for an effective bacteria inactivation. Therefore another photocatalyst was applied—the platinum(IV) surface modified TiO_2, 4% $H_2[PtCl_6]$/TH-0, a material known for its high activity towards *p*-chlorophenol oxidation induced by visible light .[22,28–31]

The toxic effect in the presence of illuminated 4% $H_2[PtCl_6]$/TH-0 was reached after 30 min (98 ± 8% of bacteria reduction; Fig. 6). For comparison, in the control experiments a complete reduction was achieved after 60 min of irradiation in the presence of unmodified titania (TH-0) and after 90 min of bacteria incubation in the presence of 4% $H_2[PtCl_6]$/TH-0 kept in the dark (Fig. 6). The total reduction of the surviving fraction observed in the dark can be explained by the toxic properties of Pt^{IV} complexes,[32,33] whereas the reduction of *E. coli* populations on illuminated immobilised TH-0 (complete inactivation after 60 min of irradiation) is a result of ROS photogeneration at the photocatalyst surface.

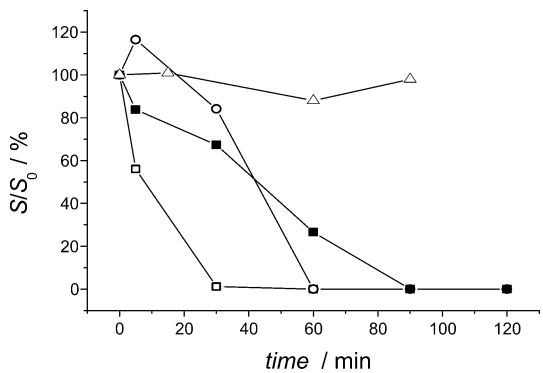

Fig. 6 Survival fraction of *E. coli* at immobilised 4% $H_2[PtCl_6]$/TH-0 under halogen lamp irradiation: 4% $H_2[PtCl_6]$/TH-0 (□), TH-0 (○), irradiation in the absence of any photocatalyst (△), and the dark experiment in the presence of 4% $H_2[PtCl_6]$/TH-0 (■).

Toxicity of 4% $H_2[PtCl_6]$/TH-0 in the dark together with no toxic effect of TH-0 without irradiation point at cytotoxic properties of used platinum(IV) photosensitiser which can desorb slowly from the photocatalyst surface.[31] Moreover, in the presence of some reducing agents the platinum species can be partially reduced to platinum(II).[22] Both, platinum(II) and platinum(IV) complexes are known inhibitors of bacterial DNA, RNA and protein synthesis.[33,34]

Although the immobilised 4% $H_2[PtCl_6]$/TH-0 inactivates bacteria already in the dark, illumination of this material enhances this effect. Under tested conditions the rate of total *E. coli* inactivation was approximately three times higher than in the dark.

Inactivation of fungi

Fungal cells are other disinfection targets. Therefore photoinactivation tests on selected strains of fungi, *Candida albicans* and *Aspergillus niger*, were also performed in the presence of C/TiO_2 suspensions.

The survival fractions in the case of *C. albicans* were decreasing significantly slower than those observed under analogous conditions for *E. coli* (Fig. 7, compare also Fig. 3 and 4). It should be noticed that the applied number of *C. albicans* cells was the same as in the case of experiments with bacteria (*E. coli*); 10^6 CFU mL^{-1}. After 60 min, the measured reduction of S/S_0 was 70–75% under UV-vis irradiation and only *ca.* 20% under visible light illumination. After 2 h the effect of C/TiO_2 on the survival fraction of *C. albicans* is relatively poor, however only for the C/TiO_2 | UV-vis system the S/S_0 fraction reached 50% already after *ca.* 20 min. Total inactivation of *C. albicans* was achieved only with UV light.

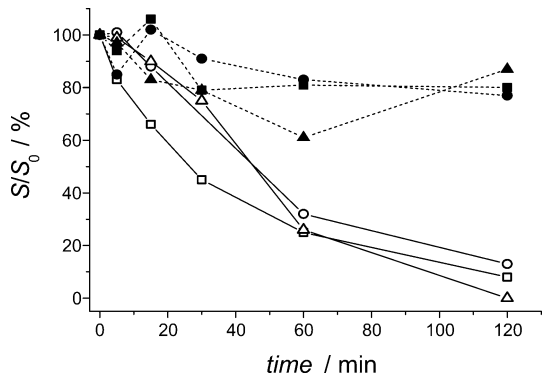

Fig. 7 Survival fraction of *Candida albicans* under visible ($\lambda > 455$ nm, full symbols) and UV-vis light irradiation ($\lambda > 385$ nm, open symbols): irradiation in the presence of C/TiO_2 (■,□), in the presence of neat titania P25 (●,○) and in the absence of any photocatalyst (▲,△).

Aspergillus niger was even more resistant than *Candida albicans*—irradiation with visible light did not result in any growth inhibition in the absence nor presence of any photocatalyst. The inhibition effect was observed when UV-vis light was applied ($\lambda > 385$ nm), however it did not depend on the presence of photocatalyst (data not shown).

Fungi are eucaryotic organisms surrounded by a rigid cell wall composed mainly of soluble and insoluble polysaccharide polymers, like chitin, β-glucans and glycoproteins. Moreover, cells of *Candida* species are approximately 25–50 times larger than those of bacteria.[35] The applied concentration of fungi (*C. albicans*) was the same as the concentration of bacteria (*E. coli*); 10^6 CFU mL^{-1}. Equal concentrations and huge differences in size of these microorganisms may explain the slower inactivation of fungi. In addition, the cell wall of *Candida* is much more resistant to oxidative agents as compared to bacterial cells.[26,27,36] These differences between bacteria and fungi may explain the slower and less evident inactivation of fungi in our experiments.

Comparison of biocidal photoinactivation

The photoinactivation effect induced in the presence of the tested photocatalysts may be ordered as follows:

E. coli > *S. aureus* ≈ *E. faecalis* ≫ *C. albicans* ≈ *A. niger*

Gram-negative bacteria > Gram-positive bacteria ≫ fungi

The complexity and density of the cell wall follows the opposite order as presented above. *E. coli*, as a representative of Gram-negative bacteria, has a rather thin peptidoglycan layer in its cell wall; *S. aureus* and *E. faecalis* have thicker and more compact cell walls, typical for Gram-positive bacteria, while *C. albicans* has a thick eukaryotic cell membrane containing sugar polymers.

Photosensitised titanium dioxide can generate hydroxyl radicals either upon UV-light or visible light irradiation. A simple indirect method of $OH^{\bullet}$ detection is its reaction with benzoic acid leading to formation of salicylic acid.[30] The latter can be determined by fluorescence spectroscopy. Fig. 8 shows the results of salicylic acid formation in the presence of irradiated photocatalysts suspended in aqueous solution of benzoic acid. Salicylic acid was also found as the result of a much slower thermal oxidation of benzoic acid with H_2O_2 in aqueous solution (data not shown). A possibility of $OH^{\bullet}$ formation *via* the so called reductive pathway, *i.e.* as a consequence of adsorbed oxygen reduction by electrons from the conduction band, can be concluded from these results. Analogous experiments and a detailed analysis of the results for 4% $H_2[PtCl_6]$/TH-0 have been described elsewhere.[30]

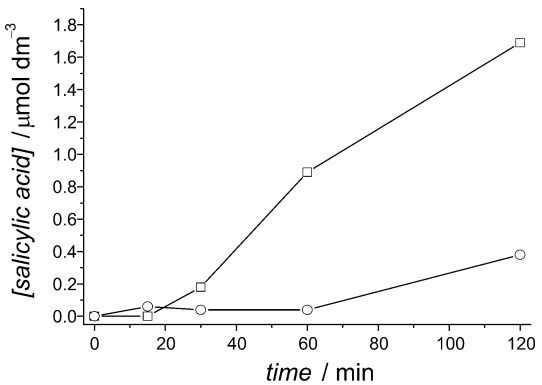

Fig. 8 Salicylic acid formation upon irradiation of C/TiO_2 (□) and 4% $H_2[PtCl_6]$/TH-0 (○) suspended in 0.01 M solution of benzoic acid. Irradiation with visible light ($\lambda > 455$ nm).

The formation of hydrogen peroxide and hydroxyl radicals should have a decisive impact on the photoinactivation effect. H_2O_2 is generated at the surface of irradiated titania (both neat and photosensitised) as a result of a two step reduction of an adsorbed oxygen molecule with superoxide radical as an intermediate (Fig. 9).[3] The electrons participating in this process come from the conduction band of photoexcited TiO_2 (e_{CB}^-).[20,31] In addition, superoxide may be reduced to hydrogen peroxide also by SOD. The formation of H_2O_2 competes with its decomposition in the presence of catalase. Hydrogen peroxide in a photocatalytic system acts as an irreversible electron acceptor which reduces significantly the electron–hole recombination and leads to the formation of $OH^{\bullet}$ radicals.[37] Highly reactive $OH^{\bullet}$ is generated either as a result of the described multistep reduction of oxygen or in the process of direct water oxidation by photogenerated holes in the valence band of titania, h_{VB}^+. The latter reaction, however, can only take place under direct TiO_2 excitation with UV light. Hydroxyl radicals together with $O_2^{\bullet -}$ are responsible for cellular wall damage, cell death and final mineralisation of organic matter (Fig. 9).[9]

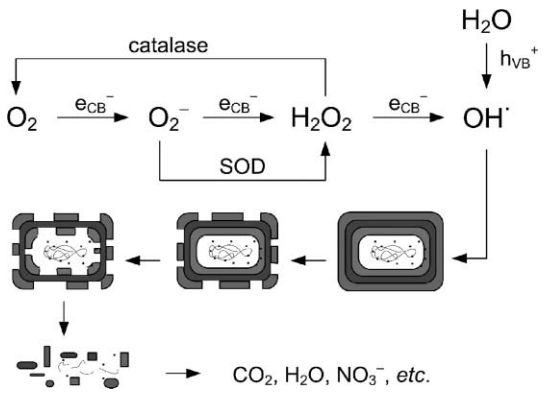

Fig. 9 Selected processes occurring during the photocatalytic inactivation of *E. coli* in the presence of neat and photosensitised titania. Schematic view of *E. coli* cell wall consisting of three layers: outer membrane, peptidoglycan and cytoplasmic membrane.

Conclusions

The present work describes visible light induced photoactivity of modified titanium dioxide in the processes of bacteria and fungi killing. A significant effect of visible light illumination was observed only in the case of carbon modified TiO_2 and Gram-negative bacteria *E. coli.* Although a complete photoinactivation requires relatively high visible light dosages, this work may be a first step in the right direction for future applications. An improvement of visible light absorption properties and efficiency of reactive oxygen species photogeneration seems to be a promising direction for further investigations.

Acknowledgements

The authors thank Ms. K. Pakosz and Ms. I. Domagała (Chair of Microbiology, Jagiellonian University Medical College) for their technical help. This work was supported by Polish Ministry of Science and Higher Education (grants no. PB1283/T09/2005/29 and PB0756/P05/2005/28).

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