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## Disinfection of surfaces by photocatalytic oxidation with titanium dioxide and UVA light

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### Abstract

Particularly in microbiological laboratories and areas in intensive medical use, regular and thorough disinfection of surfaces is required in order to reduce the numbers of bacteria and to prevent bacterial transmission. The conventional methods of disinfection with wiping are not effective in the longer term, cannot be standardized, are time- and staff-intensive and use aggressive chemicals. Disinfection with hard ultraviolet C (UVC) light is usually not satisfactory, as the depth of penetration is inadequate and there are occupational medicine risks. Photocatalytic oxidation on surfaces coated with titanium dioxide (TiO<sub>2</sub>) might offer a possible alternative. In the presence of water and oxygen, highly reactive OH-radicals are generated by TiO<sub>2</sub> and mild ultraviolet A (UVA). These radicals are able to destroy bacteria, and may therefore be effective in reducing bacterial contamination. Direct irradiation with UVC however can produce areas of shadow in which bacteria are not inactivated. Using targeted light guidance and a light-guiding sheet (out of a UVA-transmittant, Plexiglas®, for example), as in the method described in the present study, bacterial inactivation over the entire area is possible. The effectiveness of the method was demonstrated using bacteria relevant to hygiene such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus faecium*. For these bacteria, a reduction efficiency (RE) more than 6 log<sub>10</sub> steps in 60 min was observed. Using *Candida albicans*, a RE of 2 log<sub>10</sub> steps in 60 min was seen. Light and scanning electron microscopic examinations suggest that the germ destruction achieved takes place through direct damage to cell walls caused by OH-radicals.

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**Keywords:** Antibacterial effects; Free radicals; Kinetic; UV-irradiation; Light-guiding materials; Photocatalysis

### 1. Introduction

Particularly in microbiological laboratories and areas of intensive medical use, regular and thorough disinfection of surfaces is required in order to reduce the numbers of bacteria and to prevent bacterial transmission. Conventional methods of manual disinfection with wiping are not effective in the longer term, cannot be standardized, and are time-intensive and staff-intensive. In addition, there are problems associated with the use of aggressive chemicals (Hahn et al., 1997).

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Direct irradiation with ultraviolet C (UVC) rays (254 nm) is a possible alternative method of disinfection. However, since this type of radiation is injurious to health, it involves occupational medicine risks. In addition, this type of radiation is effective only when applied directly; fields obscured e.g. by instruments or other obstacles in the area irradiated—e.g., in pits in the materials used—remain untreated.

A potential alternative may be provided by substrates made of light-guiding materials, coated with specific semiconductors and stimulated by indirect mild ultraviolet A (UVA) light (320–400 nm). This method shows oxidative and disinfectant activity. The semiconducting materials about which most information is available is titanium dioxide (TiO<sub>2</sub>). A recent review article (Mills and Le Hunte, 1998) provides a comprehensive report of the mechanisms involved and the potential fields of application. There is now wide agreement regarding the mechanism: in TiO<sub>2</sub>, an electron is transferred from the valence band to the conduction band by absorption of a photon, and the resulting electron hole pair reacts with molecules on the surface of the semiconductor. Various reactive oxygen radicals caused by reactions of the hole have been identified in aqueous solution, mainly the OH radical (Jaeger and Bard, 1979; Harbour and Hair, 1986; Ireland and Valinieks, 1992). The free electron simultaneously created reacts with dissolved oxygen, to produce among other things hydrogen peroxide (Cai et al., 1992b,c). These reactive species can oxidize organic material up to complete mineralization, depending on the experimental conditions (Mills and Le Hunte, 1998). Overall, the organic molecules react with dissolved oxygen to produce CO<sub>2</sub> and H<sub>2</sub>O. During photocatalytic oxidation of *Escherichia coli*, Jacoby et al. (1998) measured the CO<sub>2</sub> released and found 54% mineralization within 75 h.

A suggested alternative explanation of the mechanism, involving direct oxidation of microorganisms through the holes (Matsunaga et al., 1985, 1988), now appears to have been ruled out (Jaeger and Bard, 1979; Harbour and Hair, 1986; Cai et al., 1992b,c; Ireland and Valinieks, 1992; Saito et al., 1992; Ireland et al., 1993; Wamer et al., 1997).

Most of the reactions tested experimentally have been conducted using a suspension of TiO<sub>2</sub> with environmental chemicals, deoxyribonucleic acid (DNA), endotoxins, viruses, microorganisms and mammalian cells (Mills and Le Hunte, 1998). In some approaches, attempts have been made to produce a self-disinfecting effect on surfaces by adding TiO<sub>2</sub> to them—e.g., in tiles and glass (Jacoby et al., 1998; Mills and Le Hunte, 1998; Sunada et al., 1998). However, the same difficulty applies here as to direct ultraviolet irradiation—that areas lying in shadow cannot be effectively disinfected. This approach is therefore of limited practicability.

The present study investigated the disinfection of bacteria relevant to hygiene during irradiation of a photocatalytically active surface from below and from above. Coupling of light through the lateral surface is also possible, and leads to comparable reduction of germs. This report describes the inactivation of *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecium* and *Candida albicans*, as well as presenting the results of microscopic techniques examining the structure of the inactivated germs.

## 2. Materials and methods

### 2.1. Photocatalyst

The photocatalysts used in the study were provided by UV-Systeme GmbH, Heidelberg, Germany. Slices (30 × 30 mm) of UVA-transparent Plexiglas® (Plexiglas® is a registered trademark of Roehm GmbH, Darmstadt, Germany) were coated with TiO<sub>2</sub> (P25, Degussa-Hüls AG). The heterogeneity of this coating is high, leading to experimental results showing a considerable range of standard deviation. Uncoated samples of the same material were used as controls.

Plexiglas® decomposes when the conditions required to prepare the samples for scanning electron microscopy (SEM) are applied. For this examination, TiO<sub>2</sub> was therefore coated on glass specimen.

### 2.2. Microorganisms

*E. coli* (ATCC 11229), *P. aeruginosa* (ATCC 15442), *S. aureus* (ATCC 6538), *E. faecium* (ATCC 6057) and *C. albicans* (ATCC 10231) were cultured in broth for 16 h, centrifuged, and washed in NaCl solution (0.9%) twice. The required bacterial concentration was adjusted by dilution with the NaCl solution (0.9%).

### 2.3. Photocatalysis without time resolution

Two hundred microlitre of the bacterial solutions was pipetted onto the coated substrates and illuminated from above or from below with UVA light (Philips, 2 × 15 W, white light 356 nm peak emission). A tub of ice-cold water was placed between the samples and the spot (distance 7 cm) as an infrared filter (Fig. 1). The temperature increase in the samples was 4 °C at most after 60 min of irradiation. After irradiation, each sample was placed in a sterile cup with 9.8 ml of NaCl solution (0.9%), shaken for 10 min (GFL, 200 rpm) and plated after appropriate dilutions using a spiral plater (Don Whitley Scientific Ltd.) onto CASO plates (for *E. coli*, *P. aeruginosa*, *S. aureus*, *E. faecium*) or SPS plates (for *C. albicans*). The plates were incubated for 16 h at 37 °C, and the colony-forming units (CFUs) were then

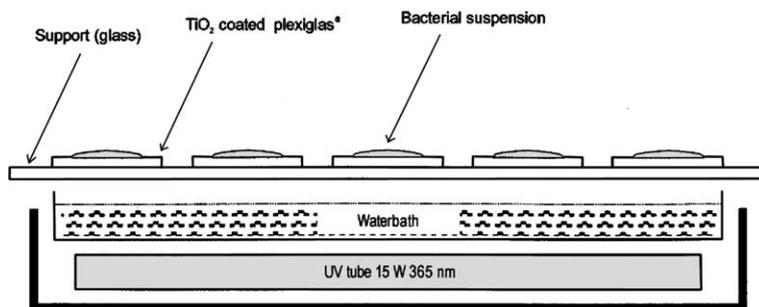


Fig. 1. Experimental set-up.

counted. In the case of the slow-growing *C. albicans*, the count was checked after a further 32 h. If no CFUs were observed, the experiment was repeated and the inoculum (200  $\mu$ l) was incubated for 24 h in CASO bouillon. If no turbidity occurred, the bacterial content was set as <5 CFU/ml.

**Controls:** the effect of UVA light alone on the microorganisms was determined on uncoated samples. The stability of the microorganisms during the experiment was measured in the dark on uncoated and TiO<sub>2</sub>-coated samples. In addition, sterile and positive controls were performed.

The following terms and abbreviations are used:

- CCE germ count of control [CFU/ml] after irradiation  
 CE germ count of the sample [CFU/ml] after irradiation  
 RE reduction efficiency =  $\log_{10}(\text{CCE} - \text{CE})$

#### 2.4. Photocatalysis with time resolution

Reaction chambers were formed with silicone spacer gaskets (SIGMA Z36.588-2) on the photocatalyst and the control samples, filled with 500  $\mu$ l of the bacterial suspension and covered. After corresponding times of exposure, 10  $\mu$ l were taken from the samples and processed as above. The measurements were carried out in triplicate. Uncoated samples (threefold) served as controls in light again. The remaining controls, not time-resolved, were handled as above. The data were evaluated as survival curves ( $\log_{10}(N_t/N_0)$  against time  $t$ ,  $N_t$  = germ number at time  $t$ ,  $N_0$  = germ number at time 0).

#### 2.5. Light microscopy

*C. albicans* was used as the subject for light-microscopy techniques. Solutions with *C. albicans* were either taken from the samples and stained, or directly processed on the samples. Staining was carried out using the standard Gram procedure. Trypan blue served as an indicator of viability (Sigma Cataloge, 2000). Dead cells

stain strongly blue, while viable cells have only a light blue appearance.

#### 2.6. Scanning electron microscopy

*C. albicans* with 10<sup>6</sup> CFU/ml deposited on TiO<sub>2</sub>-coated glass slides (Plexiglas® swells and slowly dissolves in ethanol/water solutions) was illuminated for 1 h (controls were uncoated glass slides). The samples were subjected to the standard procedure for sample preparation: after being fixed with glutaraldehyde and osmium tetroxide, the samples were drained with ethanol/water in increasing concentrations of ethanol. The absolute ethanol was replaced by dimethoxymethane, and the samples underwent critical point drying with CO<sub>2</sub>. The glass slides were glued onto stages with conductive silver and metallized with gold. The samples were microscopied and photographed with a scanning electron microscope (Philips SEM 505). In the same experiment, it was confirmed that the coated glass slides were photocatalytically active.

### 3. Results and discussion

The bacteria *E. coli*, *P. aeruginosa*, *S. aureus*, *E. faecium* and *C. albicans*, which are relevant for hygiene, were illuminated on the sample specimen in different concentrations for 1 h. The reduction efficiencies (REs) achieved, and the corresponding experimental conditions are shown in Table 1.

The REs were found to decrease in the following order: *E. coli* > *P. aeruginosa* > *S. aureus* > *E. faecium* > *C. albicans*. The complexity and density of the cell wall increased in the same order of precedence: *E. coli* and *P. aeruginosa* have thin and slack cell walls (Gram-negative), *S. aureus* and *E. faecium* have thicker and denser cell walls (Gram-positive), and *C. albicans* has a thick eukaryotic cell wall. This order of precedence appears reasonable if it is assumed that the primary step in photocatalytic decomposition consists of an attack by OH radicals on the cell wall, leading to punctures

Table 1  
Inactivation of various bacteria by photocatalysis on TiO<sub>2</sub>-coated Plexiglas® with UVA light (60 min)

Germ	Multiplicity	Initial germ count ± error (CFU/ml)	Final germ count ± error (CE in CFU/ml)	Control final germ count ± error (CCE in CFU/ml)	Reduction efficiency
<i>E. coli</i>	3	$1.2 \times 10^7 \pm 0.09 \times 10^7$	<5	$1.1 \times 10^7 \pm 0.13 \times 10^7$	>6.3
<i>P. aeruginosa</i>	4	$1.5 \times 10^6 \pm 0.12 \times 10^6$	<5	$1.2 \times 10^6 \pm 0.22 \times 10^6$	>5.4
<i>S. aureus</i>	2	$0.8 \times 10^5 \pm 0.15 \times 10^5$	<5	$4.3 \times 10^4 \pm 2.6 \times 10^4$	>3.9
<i>E. faecium</i>	4	$3.6 \times 10^7 \pm 0.36 \times 10^7$	$1.3 \times 10^4$	$1.6 \times 10^7 \pm 0.51 \times 10^7$	3.1
<i>C. albicans</i>	3	$1.1 \times 10^5 \pm 0.08 \times 10^5$	$4.1 \times 10^3$	$6.2 \times 10^4 \pm 1.1 \times 10^4$	1.2

Reduction efficiencies (RE) =  $\log_{10}(\text{CCE} - \text{CE})$ ; CE = number of viable cells in CFU/ml after irradiation; CCE = number of viable cells of the control in CFU/ml after irradiation; error: at multiplicity  $n \geq 3$ : standard deviation of the mean; at  $n < 3$ : error =  $1/2 \times (\text{value 1} - \text{value 2})$ .

(Saito et al., 1992). This is supported by our finding (data not shown), that it was not possible to reduce the spores of *Bacillus subtilis* by photocatalytic treatment during 60 min. Extended photocatalytic treatment however by Greist et al. (2002) led to external changes (examined by SEM) of spores of *B. subtilis* after 11.75 h, lesions occurred after 36 h. This shows the principle susceptibility even of high condensed organic matter, the envelope of the spores. Not shown in Table 1 are the results with vegetative *B. subtilis* (ATCC 6633) and *Mycobacterium terrae* (ATCC 15755); these are strongly UVA-sensitive without titanium dioxide, and their photocatalytic inactivation could therefore not be determined (for example, vegetative *B. subtilis* was reduced by  $7 \log_{10}$  stages within 10 min with UVA alone).

Since the effectiveness of heterogeneous catalysis also depends on the adsorption of reaction partners to the TiO<sub>2</sub> (Kormann et al., 1991), it would be interesting to measure the surface charge and the hydrophobicity of the bacteria and surfaces (Gilbert et al., 1991; Lin et al., 1997) and to correlate these findings with the sensitiveness to photocatalytic oxidation.

*E. coli* and *P. aeruginosa* showed the best responses with this method. The time dependence of inactivation was recorded for these bacteria; Figs. 2 and 3 show the survival curves. The initial inactivation follows a first-order dependence ( $\log_{10}(N_t/N_0) = -kt$ , where  $k$  = rate constant); the  $k$  of *E. coli* is numerically larger than the  $k$  of *P. aeruginosa*. *P. aeruginosa* showed a smaller RE after an hour compared to Table 1, and this could be explained by the inhomogeneity of the photocatalysts used. The time dependence of photocatalytic inactivation with TiO<sub>2</sub> observed is in accordance with previous reports in the literature (Matsunaga et al., 1985; Saito et al., 1992; Watts et al., 1995). During longer exposure periods, the curve deviates from this time dependence, and the rate constant increases. The controls without a photocatalyst showed bacterial reduction after approximately 60 min (*E. coli*), or after as little as 10 min in the case of *P. aeruginosa*. This can be explained by the fact that UVA, with only relatively low energy, damages cells

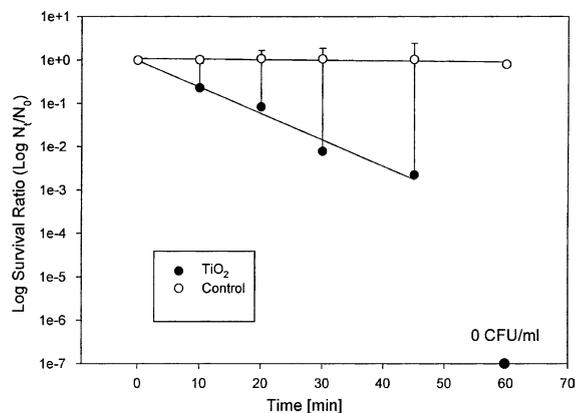


Fig. 2. Kinetics of inactivation of *E. coli*. Controls were uncoated and irradiated. The lines represent data regressions.

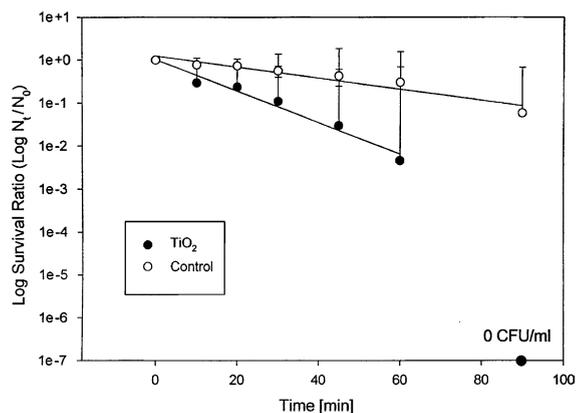


Fig. 3. Kinetics of inactivation of *P. aeruginosa*. Controls were uncoated and irradiated. The lines represent data regressions.

through the oxidative stress caused by oxygen radicals within the cells (Bock et al., 1998). Different kinds of DNA damage were detected (Bock et al., 1998), such as single-strand breaks or photomodified bases. This type of damage can be repaired by the cell to a certain extent.

If the stress on the cell exceeds a certain threshold, the cell dies or becomes incapable of further division. The extent of this effect depends on the kind of bacterium and its history (growth phase, status of nutrition). In the independent experiments the results of which are shown in Table 1 and Fig. 3, the bacterial counts for the control of *P. aeruginosa* did not change after 60 min (Table 1, Fig. 3).

For the microscopic techniques, the yeast *C. albicans* was used because of its size. In addition to the intact yeast cells that were left on the photocatalytically active slides after irradiation, destroyed cells can also be seen (Fig. 5). The surfaces of the cells look grainy, dissolved, furrowed, crumbled, or dilapidated. The coarse dark grey structure below the yeast is the photocatalyst titanium dioxide. On the control slides, the structures are mainly conserved. Some of the bacteria are unstable when exposed to electron beams—they lose the smooth, tight surface usually seen at greater magnification and acquire a golfball-like structure. This type of destruction is easily distinguished from photocatalytic breakdown. In Fig. 4, an illuminated control sample shows intact cells, a yeast cell with a daughter cell, a pseudomycelium, and a golfball-like structure in the middle.

The results of the SEM examinations of *C. albicans* were supported by light-microscopy techniques (data not shown). In the Gram preparations, a high percentage of Gram-unstable behaviour was found after photo-oxidation in addition to cells with Gram-positive behaviour. When the viability stain method with trypan blue was applied to photocatalytically inactivated *C. albicans*, a large number of strongly coloured cells appeared in contrast to the control, indicating damaged yeast cells.

Fig. 5 shows *C. albicans* after photocatalytic treatment. This may represent an intermediate stage on the way to complete mineralization. Using SEM, Jacoby



Fig. 4. SEM photograph of *C. albicans* on an uncoated and irradiated control plate.

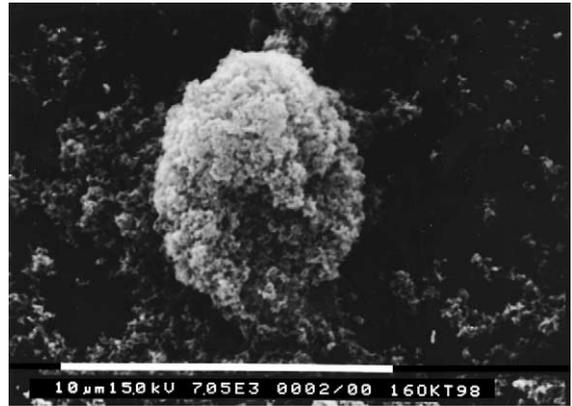


Fig. 5. SEM photograph of partially destroyed *C. albicans* on a  $\text{TiO}_2$ -coated and irradiated plate.

et al. (1998) were able to show that during irradiation of *E. coli* on  $\text{TiO}_2$ -coated glass slides for 75 h, the bacteria are completely destroyed and removed from the target. Transmission electron microscopy studies carried out by Saito et al. (1992) showed that the cell walls of *Streptococcus sobrinus* appear broken after photocatalysis. In addition, they were able to demonstrate that illuminated cells release potassium, protein and ribonucleic acid into the medium during the reaction. The increase in the concentration of these indicators of changes in the permeability of the cell envelope go parallel to inactivation, and occur as soon as the reaction commences. The explanation for this that is currently favoured in the literature (see Section 1, above) suggests that oxygen radicals and hydrogen peroxide are produced photocatalytically, and the OH radical is regarded as being the most effective agent. It has been shown (Cai et al., 1992a; Kubota et al., 1994; Dunford et al., 1997) that scavengers for hydrogen peroxide and these radicals inhibit the reaction, and this was confirmed by own observations with dimethyl sulfoxide as the scavenger (data not shown). In a primary step of the inactivation process, an attack by the highly reactive oxygen radicals on the outside of the bacterium, with damage to the cell wall and/or cell membrane, can be accepted as an explanation, in view of the results presented here and the findings reported in the literature in general. Further experiments are needed to clarify a number of other issues, including the potential of epifluorescence methods using different markers (Matsunaga et al., 1995).

The experiments with  $\text{TiO}_2$  previously described in the literature—particularly the earlier ones—were usually carried out with suspensions of *E. coli*, and showed REs of 3 to a maximum of 5  $\log_{10}$  steps within 30–120 min (Matsunaga et al., 1985; Ireland et al., 1993; Matsunaga and Okochi, 1995; Bekbolet and Araz, 1996; Lin et al., 1997; Sunada et al., 1998). The photocatalytic surfaces described in the present study are equivalent to,

or even superior to those, used in previous experiments in terms of their inactivation power.

As shown in Table 1, the bacterial RE achieved by photocatalyzed oxidation lies in the same order of magnitude as that required by the DGHM guidelines (DGHM, 1991; Exner and Gebel, 1998) for disinfectants. In risk zones,  $5 \log_{10}$  stages need to be inactivated within an hour, and this is the case for *E. coli* and *P. aeruginosa*. The required 1 h value is not met for *E. faecium*, *S. aureus*, and *C. albicans*, but exposure times of up to 4 h for inactivating  $5 \log_{10}$  stages are satisfactory outside risk zones (Exner and Gebel, 1998). The behaviour of these bacteria during extended exposure periods is to be examined in the future.

One possible application of the photoactive  $\text{TiO}_2$  surface might be for permanent maintenance of bacteria-free conditions on surfaces that have been disinfected already, e.g. using conventional wiping with disinfectant. In this case, the requirements for the RE would not need to be so critical.

The aim of disinfection is to reduce the number of infectious bacteria in an area or an object, preventing it from providing a starting-point for future infection (Gundermann et al., 1991). The disinfectants traditionally used are of chemical origin, with the accompanying risk of allergic reactions and toxicity when staff members have a high degree of exposure (Hahn et al., 1997). Regular application of disinfection procedures is necessary in areas of the hospital requiring special antibacterial protection (e.g., the department of surgery) or in fields in which infections are likely (e.g., sepsis). Compared to the customary disinfectants,  $\text{TiO}_2$ -coated surfaces do not involve aerosol formation. Another disadvantage of conventional disinfection methods is poor validation. Even the automatic dispensers often used cannot completely exclude mistakes involving the correct concentration of the disinfectant. By contrast, the procedure presented here is easy to apply. An active concentration does not need to be prepared, and a supply of electric power is all that is needed for the procedure.

Although disinfection does require killing and inactivation of pathogenic bacteria, in most cases a reduction of around  $3\text{--}5 \log_{10}$  stages is sufficient—equivalent to 99.900–99.999%, in comparison with complete sterilization (Gundermann et al., 1991). The requirement for a reduction of  $3\text{--}5 \log_{10}$  stages is easily achieved by the procedure presented here. In an area of the laboratory in which constant bacterial reduction is required,  $\text{TiO}_2$ -coated surfaces would also be active during working procedures, and this would therefore be a good application for the technique. However, this would still require the development of specialized methods of conducting UVA light, in order to guarantee the safety of those working in the vicinity.

To allow widespread use of the method, it will certainly be necessary to validate it in difficult conditions, particularly with dried microorganisms and with high levels of protein (DGHM, 1991). To make the self-disinfectant surfaces practical, further investigations of other biological materials will be needed—e.g., DNA (plasmids), viruses, endotoxins and toxic or allergenic proteins. Experiments on methods of conducting the light, on the mechanical and chemical stability of the materials, and on possible mutagenic and toxic effects, are also indispensable.

This bacteria-reducing process would ideally be usable in fields in which flat working areas are used: in the medical field, in genetics laboratories, in food processing, in the pharmaceuticals, in microbiological laboratories, and wherever there is a strong requirement for clean surfaces. If it were possible to produce flexible plastic surfaces using these techniques, further important types of application would also become possible—such as aquiferous systems in which reliable prevention of contamination and biofilm formation is needed.

#### 4. Conclusions

In this contribution it was shown that

- it is possible to disinfect surfaces consisting of a light-guiding material coated with a specific semiconductor ( $\text{TiO}_2$ ) and stimulated by indirect UVA. This indirect irradiation is described for the first time here;
- this method has the highest kill-rates on surfaces documented in the literature;
- the assumption of an initial attack on the microbial objects by OH radicals from outside (envelope or membrane) is highly supported.

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