J Ind Microbiol Biotechnol (2006) 33: 261–268
DOI 10.1007/s10295-005-0063-2

ORIGINAL PAPER

Mari Raulio · Viljami Pore · Sami Areva
Mikko Ritala · Markku Leskelä · Mika Lindén
Jarl B. Rosenholm · Kari Lounatmaa
Mirja Salkinoja-Salonen

Destruction of *Deinococcus geothermalis* biofilm by photocatalytic ALD and sol-gel TiO_2 surfaces

Received: 15 August 2005 / Accepted: 28 October 2005 / Published online: 14 December 2005

Abstract The aim of the present work was to explore possibilities of photocatalytic TiO_2 coating for reducing biofilms on non-living surfaces. The model organism, *Deinococcus geothermalis*, known to initiate growth of durable, colored biofilms on machine surfaces in the paper industry, was allowed to form biofilms on stainless steel, glass and TiO_2 film coated glass or titanium. Field emission electron microscopy revealed that the cells in the biofilm formed at 45°C under vigorous shaking were connected to the surface by means of numerous adhesion threads of 0.1–0.3 μm in length. Adjacent cells were connected to one another by threads of 0.5–1 μm in length. An ultrastructural analysis gave no indication for the involvement of amorphous extracellular materials (e.g., slime) in the biofilm. When biofilms on photocatalytic TiO_2 surfaces, submerged in water, were exposed to 20 W h m^{-2} of 360 nm light, both kinds of adhesion threads were completely destroyed and the *D. geothermalis* cells were extensively removed (from $>10^7$ down to below 10^6 cells cm^{-2}). TiO_2 films prepared by the sol-gel technique were slightly more effective than those prepared by the ALD technique. Doping of the TiO_2 with sulfur did not enhance its biofilm-destroying capacity. The results show that photocatalytic TiO_2 surfaces have potential as a self-cleaning technology for warm water using industries.

Keywords *Deinococcus geothermalis* · Ultrastructure · Photocatalysis · Adhesion threads · Field emission scanning electron microscope (FESEM)

M. Raulio (✉) · M. Salkinoja-Salonen
Department of Applied Chemistry and Microbiology,
University of Helsinki, P. O. Box 56, 00014 Helsinki, Finland
E-mail: mari.raulio@helsinki.fi
Tel.: +358-9-19159328
Fax: +358-9-19159301

V. Pore · M. Ritala · M. Leskelä
Laboratory of Inorganic Chemistry, Department of Chemistry,
University of Helsinki, P. O. Box 55, Helsinki, Finland

S. Areva · M. Lindén · J. B. Rosenholm
Department of Physical Chemistry, Åbo Akademi University,
Porthaninkatu 3-5, 20500 Turku, Finland

K. Lounatmaa
Laboratory of Electronics Production Technology,
Helsinki University of Technology,
02015 HUT Helsinki, Finland

Introduction

Biofilms are a source of product contamination and defects in water using industries, causing energy transfer loss in heat exchangers, inducing failure of medical devices placed in humans, and causing equipment damage and end product contamination and defects [9]. In many cases the use of chemical cleaning agents or biocides have proven ineffective or their use is restricted by regulations, e.g., in the food industry and in products to be used in food contact [7, 31, 41]. Engineering surfaces to repel microbial adhesion, or using physical methods rather than chemical for antifouling is therefore of great interest. Semiconductors like TiO_2 and SnO_2 are known to generate microbicidal, reactive oxygen species upon illumination with UVA [3, 8, 17].

The aim of the present work was to explore possibilities of photocatalytic TiO_2 film coating for reducing biofilms in warm water using industrial processes like papermaking. We used as the model organism *Deinococcus geothermalis*, which is a primary biofilm former, capable of forming durable biofilms on abiotic surfaces in the paper industry [18, 19, 39]. We present results obtained by field emission scanning electron microscopy (FESEM) on the details of *D. geothermalis* adhesion onto the surface, and on the impact of illumination at 360 nm on biofilms grown on various coated and non-coated surfaces. Data on biofilm density and its changes were obtained by scanning fluorometry. The results demonstrate a high potential of photocatalytic TiO_2 surfaces for controlling biofilms on metal surfaces.

Materials and methods

The surfaces and substrata used are listed in Table 1. The model bacterium was *D. geothermalis* E50051 (HAMBI 2411) [39]. All coupons (Table 1, ~1 cm^2) were cleaned with detergent (Nelli soap, Farmos, Turku, Finland), sonicated for 5 min in acetone and in 96% ethanol, disinfected (70% ethanol) and mounted in the wells of a polystyrene plate. The wells were filled with 3 ml of culture medium (oligotrophic medium R2 [2]) and inoculated with 5 vol-% of *D. geothermalis* -strain E50051 grown for 1 day with shaking in R2 broth at + 45°C. The plate was covered with a lid and incubated with shaking (160 rpm) for 2 day at + 45°C in the dark. The plates holding coupons with pre-grown biofilms, submerged in the R2 medium (3 mm liquid above the biofilm), were illuminated (Sylvania 18 W Blacklight Blue) or not illuminated (control) with 360 nm, 1 W/m^2, for 20 h, with shaking at + 45°C.

Quantifying density of the biofilms

The method for quantifying the density of biofilms was modified from that described by Mattila et al. [28] by replacing ATP content for calibration by microscopic counting. Briefly, the coupons were rinsed with municipal tap water and stained with SYTO9 (Molecular Probes, Leiden, The Netherlands) nucleic acid stain (3.34 μmol l^{-1}) for 5 min. The coupons were rinsed with water and the emitted fluorescence from an area of 245–315 mm^2 was measured using a scanning fluorometer (Fluoroskan Ascent, Labsystems, Helsinki, Finland) at excitation wavelength of 478–492 (±2) nm and emission wavelength of 525–551 (±2) nm). Fluorescence intensity was translated into cell number using a calibration curve prepared by microscopic counting. The fluorescence intensity was proportional to cell number up to a biofilm density of 5×10^8 cm^{-2}. Stained biofilms were viewed with an epifluorescent microscope (Nikon Eclipse E800, Tokyo, Japan)

Preparation of samples for FESEM

Coupons with biofilms were rinsed with water, fixed in phosphate (0.1 M, pH 7.2) buffered 2.5% glutaraldehyde for 3 h, and rinsed with phosphate buffer three times. Dehydration was carried out with an ethanol series from 50% to 75% to 96% and absolute, followed by hexamethyldisilazane (Fluka, Buchs, Switzerland). Biofilms were coated with chromium (5 nm, 208 HR High Resolution Sputter Coater, Cressington Scientific Instruments Inc, Cranberry, PA, USA) and microscoped with FESEM of JEOL JSM-6330F (Tokyo, Japan), operated at 15 kV (Fig. 3) or Hitachi S-4800 (Tokyo, Japan) operated at 1 kV (Figs 1, 2, 4, 5).

Preparation of TiO_2 coated surfaces

Films ALD_1 and ALD_2 were grown by atomic layer deposition (ALD) on a borosilicate glass substrate. ALD is a gas phase deposition method, which produces uniform and high quality thin films [21, 35]. Film ALD_1 was prepared at 350°C using $Ti(OCH_3)_4$ and water as precursors. This process has been shown to produce anatase thin films with good photocatalytic activities [32]. Film ALD_2 was prepared at 500°C using $TiCl_4$, water and H_2S as precursors. The role of H_2S was to enhance the formation of a rutile phase and to introduce sulfur doping. Both films were approximately 100 nm in thickness.

The titanium dioxide coating on titanium substrates was prepared by the sol-gel technique according to [14, 15]. Commercially available tetraisopropyl orthotitanate, $Ti((CH_3)_2CHO)_4$ (Merck, Whitehouse Station, NJ, USA) was dissolved in half of the prescribed amount of absolute ethanol (solution I). Deionised water, and nitric acid (HNO_3, 65%) were mixed with the other half of the prescribed amount of absolute ethanol (solution II). Solution II was then slowly added to solution I under constant stirring at 0°C. Poly(ethylene glycol) (PEG; average molecular weight of 2,000) was used as the macropore forming agent. The temperature of the sols was then raised in order to dissolve the added organic templates. The sols were aged for at least 2 weeks before dip-coating. The coatings were prepared on titanium substrates by dip-coating at a withdrawal speed of 0.48 mm/s. The coated Sol-gel_1 was heat-treated at a heating ramp of 1°C/min until temperature reached 500°C, maintained at 500°C for 1 h and allowed to cool in the oven. The coated Sol-gel_2 was heat-treated at 500°C for 1 h and cooled at room temperature. The prepared films approx. 250 nm in thickness (as observed

Table 1 The studied surfaces

	Surface code					
	ALD_1	ALD_2	Sol-gel_1	Sol-gel_2	Noncoated	
Preparing method	ALD	ALD	Sol-gel	Sol-gel	–	As delivered
Active substance	TiO_2, Anatase	TiO2:S, Rutile	TiO_2, Anatase macropores	TiO_2, Anatase	–	–
Substratum	Borosilicate glass	Borosilicate glass	Titanium	Titanium	Borosilicate glass	Stainless steel AISI316

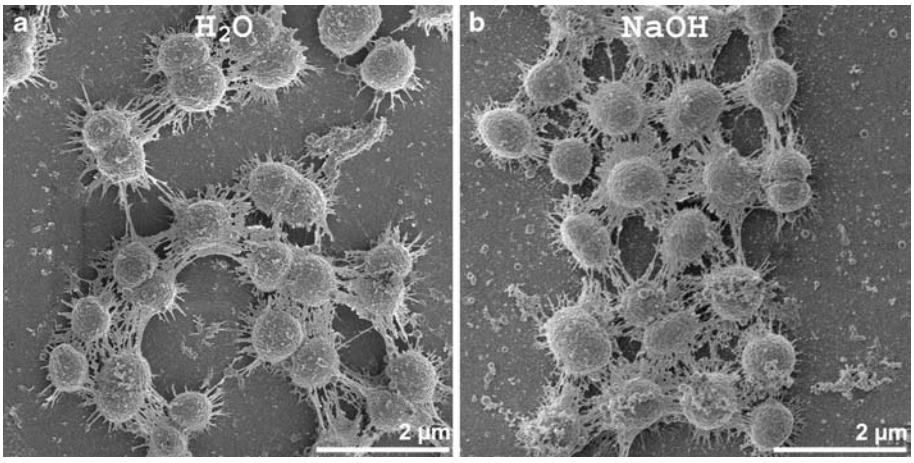

Fig. 1 Resistance of *Deinococcus geothermalis* biofilm towards washing with alkali. *D. geothermalis* E50051 was grown on glass surface in R2 medium at 45°C for 2 day under shaking at 160 rpm. The field emission scanning electron micrographs were taken after the biofilms were washed for 1 h at 45°C with drinking water (**a**) or with 0.1% (w/v) aqueous sodium hydroxide (**b**). Biofilm subjected to no washing (not included in the Figure) appeared identical to *panel a*

by cross-sectional high-resolution scanning electron microscope) were of anatase crystal structure (as observed by Thin-Film X-ray Diffraction).

Results

Field emission scanning electron microscope was used to establish whether adhesion of *D. geothermalis* to non-living surfaces is mediated by slime, by specific adhesion organelles like fimbria or by the sticky surface of the cell body, and how the adhesion organelles respond to alkaline washing used in industrial cleaning. *D. geothermalis* strain E50051 was allowed to form biofilm on glass coupons. The biofilms persisting after subsequent washing were then investigated by FESEM (Fig. 1).

Figure 1 reveals the dense network of thread-like organelles of *D. geothermalis* E50051, connecting the cells to the glass surface as well as the adjacent cells to one another. Each cell was connected to the glass by 20–50 threads of 0.3–0.6 µm in length. Adjacent cells were linked to one another by two to ten threads of up to

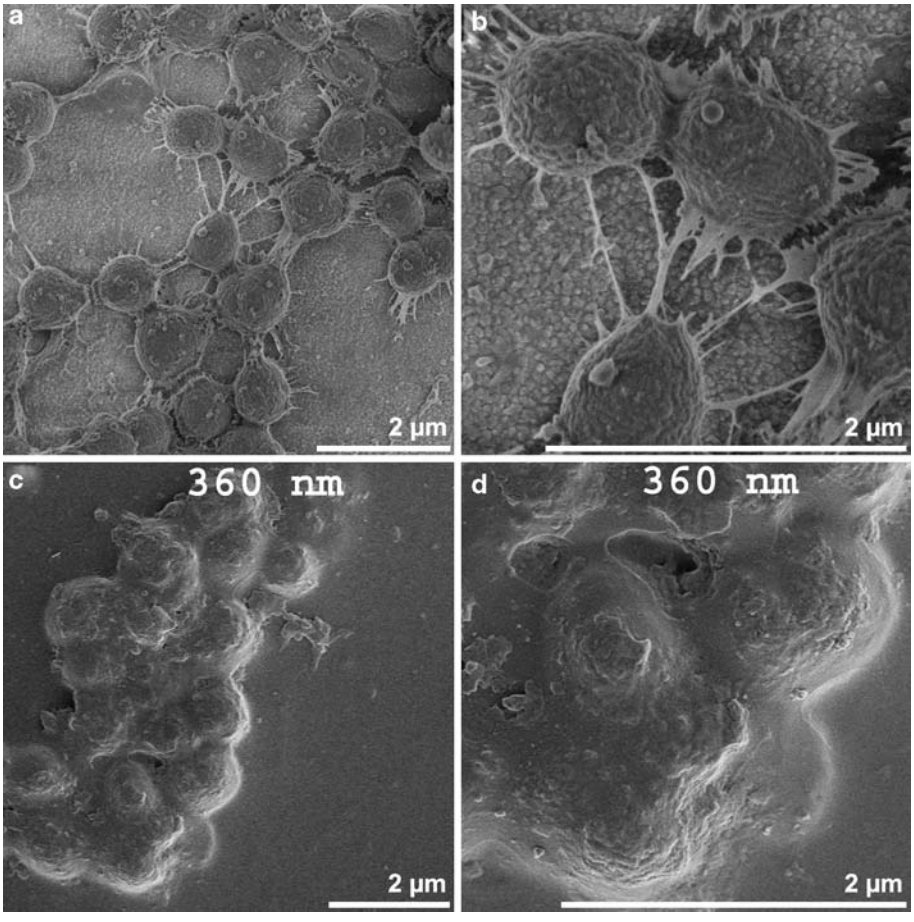

Fig. 2 Photocatalytically induced change in morphology of *Deinococcus geothermalis* growing on TiO_2 coated glass substratum prepared by the ALD method (surface ALD_1 in Table 1). Field emission scanning electron micrographs show 2 day old biofilms of the strain E50051 not illuminated (**a**, **b**) or illuminated (**c**, **d**) for 20 h at 360 nm

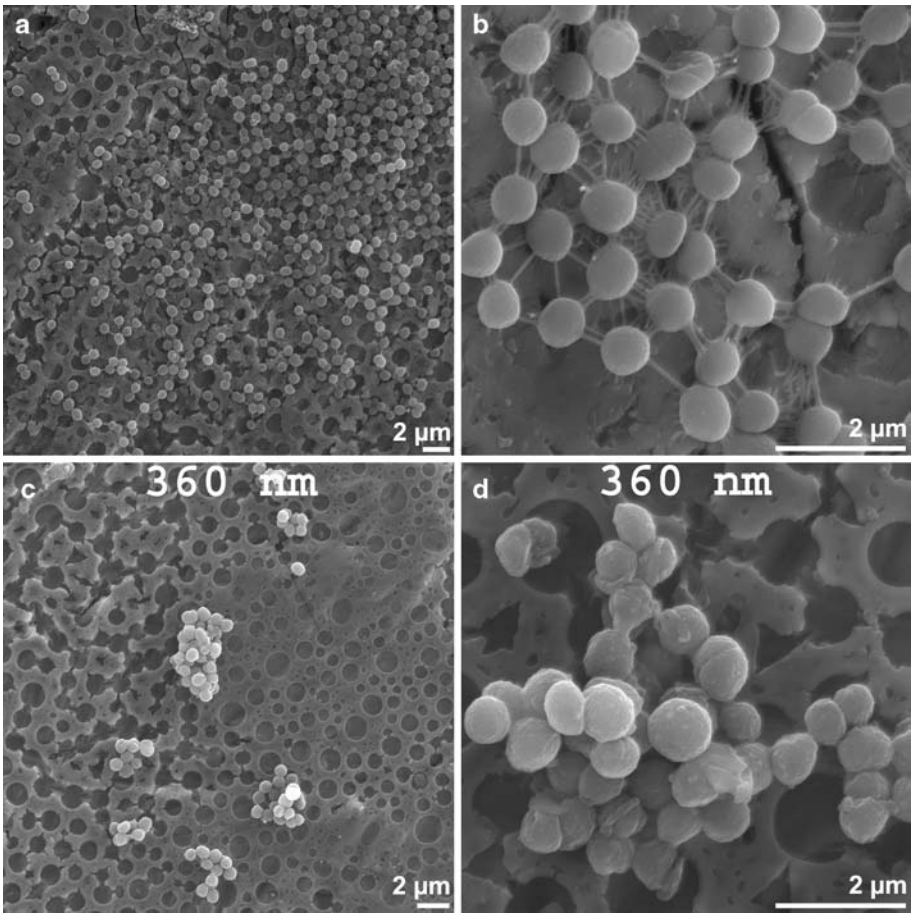

Fig. 3 Photocatalytically induced morphological change in *Deinococcus geothermalis* growing on titanium substrate coated with TiO_2 by the sol-gel method (surface Sol-gel_1 in Table 1). Field emission scanning electron micrographs show 2 day old biofilms of the strain E50051 not illuminated (**a**, **b**) or illuminated (**c**, **d**) for 20 h at 360 nm

1 μm in length. The morphology of the cells and their adhesion organelles seemed unaffected by washing with strong alkali under agitation (pH > 12, 45°C, Fig. 1b). The amount of adhered cells remained 10^7 cm^{-2} (within one log unit), irrespective of the washing.

The impact of photocatalysis on the adhesion apparatus of *D. geothermalis* was investigated using glass coupons coated with TiO_2 by the ALD method. *D. geothermalis* biofilm was grown on the surface and then illuminated or not illuminated at 360 nm (Fig. 2).

The cells adhering to TiO_2 possessed threads of 0.5–1 μm in length and ~50 nm in diameter connecting adjacent cells. The short appendices, 0.1–0.3 μm in length connecting the bacterial cell body to the TiO_2 surface, were fewer in number (5–20 $cell^{-1}$) than those connecting the same strain onto plain glass (Fig. 1).

Illumination at 360 nm of the biofilm on the TiO_2 (ALD) coated glass surface resulted in massive detachment (89 ± 5%) of cells. The cells remaining on the surface appeared collapsed and the adhesion threads destroyed (Fig. 2c, d). Parallel coupons of TiO_2 coated surface incubated for the same period (20 h) in the dark, showed no deterioration of the *D. geothermalis* biofilm.

To answer the question of whether the massive collapse of the biofilm on TiO_2 (ALD) surface in response to 360 nm irradiation was linked to the photochemistry of the TiO_2 film or to other properties of the substratum, TiO_2 coatings were prepared on titanium substrate using a different technology; sol-gel was used. *D. geothermalis* biofilm was grown and the illumination executed as for Fig. 2. It was found (Fig. 3) that the *D. geothermalis* biofilm was removed by photocatalysis from the sol-gel TiO_2 coated titanium surface at least as effectively (> 95%) as from the ALD TiO_2 surface on glass. Figure 3 shows that the cells remaining on the surface had lost all adhesion threads, even if some of the cells looked otherwise intact. Many of the cells looked lysed (Fig. 3c, d).

We conclude that *D. geothermalis* E50051 biofilms grown on TiO_2 coated surfaces were destroyed and removed by photocatalytic activity independent of the method of preparation (ALD or sol-gel) or the substratum (glass or titanium).

To distinguish between the roles of 360 nm illumination as such and of TiO_2 mediated photocatalytic activity, the effects of light exposure were inspected with non-photocatalytic stainless steel. To do this, *D. geothermalis* was allowed to grow on coupons of unpolished stainless steel and then illuminated or not illuminated. It is seen from Fig. 4a, b that *D. geothermalis* growing on stainless steel possessed attachment structures similar in morphology and number to those connecting *D. geothermalis* onto TiO_2 coated surface on glass or on titanium (Figs 2 b, 3 b). The attachment structures differed from those on uncoated glass surface (Fig. 1a) by their lower density.

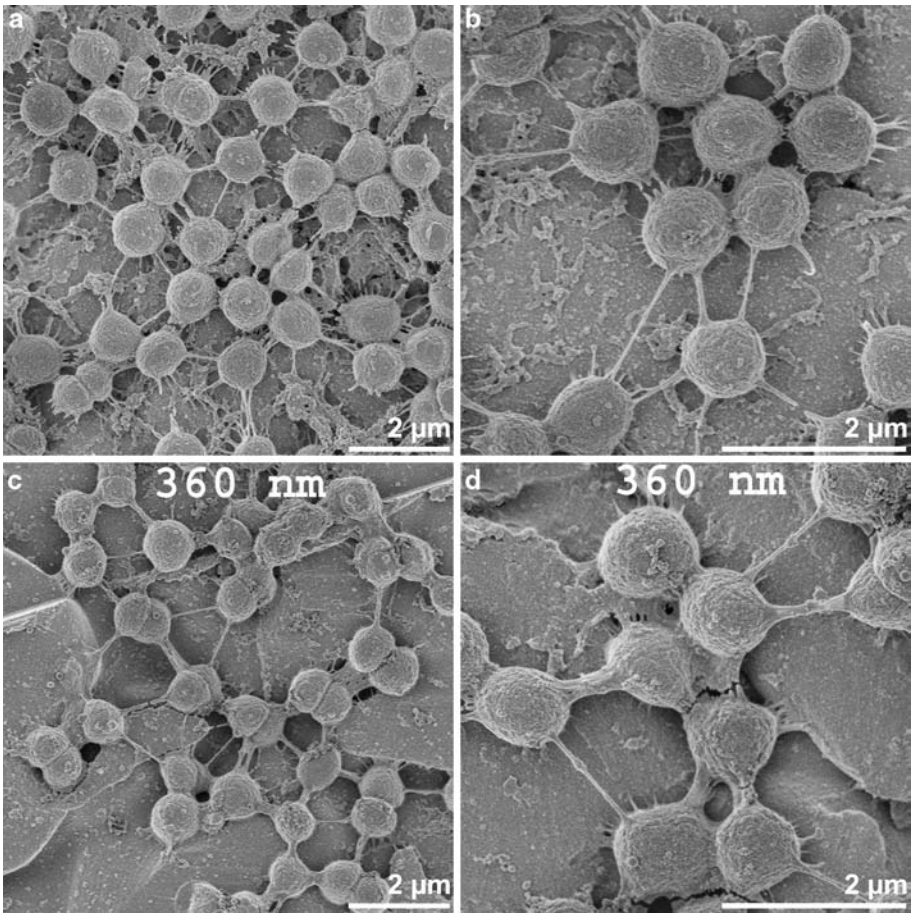

Fig. 4 Effect of 360 nm illumination on *Deinococcus geothermalis* growing on stainless steel, investigated with field emission scanning electron microscope. The micrographs show *D. geothermalis* E50051 grown for 2 day on stainless steel not illuminated (**a**, **b**), or illuminated (**c**, **d**) for 20 h at 360 nm

When *D. geothermalis* E50051 grown on stainless steel was illuminated at 360 nm, no ($<<$ 1 log) reduction was observed in cell density, and the adhesion threads looked similar in density and dimensions independent of the exposure to illumination (Fig. 4a–d). Therefore, the effects displayed in Figs. 2c, d and 3c, d must have been due to TiO_2 induced photocatalysis.

Ultrastructural details of the cell surface and adhesion organelles of *D. geothermalis* are shown in high magnification (×100,000) FESEM micrographs in Fig. 5. It shows that the cell surface (Fig. 5a) of *D. geothermalis* is not smooth but is composed of many differently shaped structures. The adhesion threads are non-uniform in shape, thickness and length (Fig. 5b). Several threads expand to a foot-like form at the site where the thread touches the steel surface (arrows in Fig. 5b).

To collect quantitative data on the effects of the substratum and the treatments on biofilm density, a method is needed for counting of cells independent of the possible presence of extracellular materials like

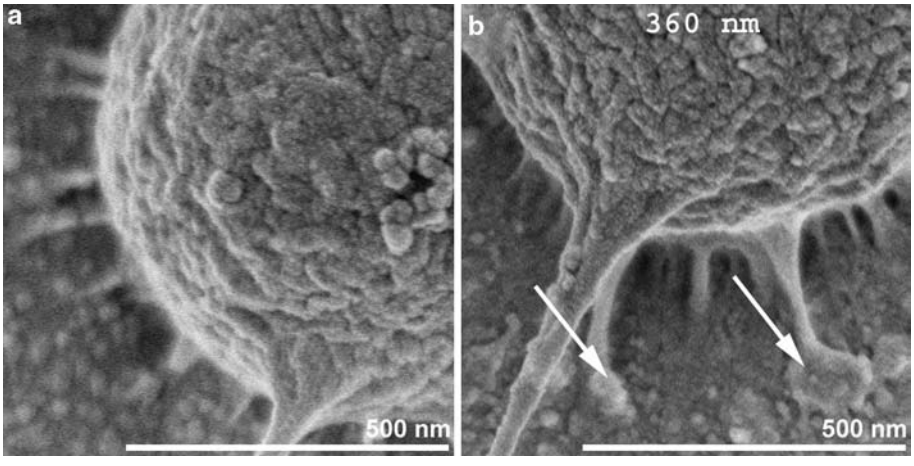

Fig. 5 Ultrastructural details of *Deinococcus geothermalis* cells attached on stainless steel coupons. The images were taken with field emission scanning electron microscope applying ×100,000 magnification. *D. geothermalis* was grown and illuminated as in Fig. 4. Not illuminated (**a**), or illuminated (**b**) for 20 h at 360 nm

slime. We developed a method based on optical fluorescence reading to quantitate biofilms. The fluorescence emitted by bacterial biomass stained with a nucleic acid specific dye, SYTO9, is quantified using a scanning fluorometer. Figure 6 shows examples of microscopic views of steel coupons prepared for fluorescent readout. It shows surface attached deinococcal cells of diameter of 1 μm occuring singly, in pairs, in small microcolonies where individual cells (3–10) can be distinguished and as larger intensively fluorescing microcolonies where the individual cells can no longer be distinguished. All cell aggregate sizes were approximately equally distributed on the un-coated steel surface whether illuminated (Fig. 6b) or not illuminated (Fig. 6a).

Figure 7 shows data obtained by the fluorescent reading technique of *D. geothermalis* biofilms on different substrates and their responses to photocatalytic activity. It shows that *D. geothermalis* E50051 adhered approximately 1 log more effectively to steel and to TiO_2 coated surfaces than to glass. Photocatalytic TiO_2 ALD surfaces were effective in destroying the adhered biofilms in response to irradiation, reducing the biofilm quantity by 1 log value and those prepared by the sol-gel method, by 2 log values. Doping of ALD-TiO_2 with sulfur (ALD_2, Fig. 7) did not enhance its biofilm destroying activity.

Discussion

This paper describes the biofilm behavior of paper machine pink slime former *D. geothermalis* on abiotic surfaces and reversal of the adhesion by illuminated photocatalytic films. *D. geothermalis* adhered onto glass, steel and TiO_2 coated materials by an extensive network of adhesion threads. The FESEM images gave no indication of any significant contribution of extracellular materials in the biofilm. The adhesion threads in *D. geothermalis* were first observed by Kolari et al. [19]. These threads connect the cells to an abiotic substratum, leading to formation of an endurable biofilm that cannot be removed by vigorous shaking in water or by chemically aggressive cleaning formulations such as sodium dodecyl sulphate [19] or sodium hydroxide [this paper; 19] at an elevated temperature. The inertness towards chemical cleaning and shearing forces distinguishes the deinococcal adhesion apparatus from that described for other taxons like *Burkholderia cepacia* and *Staphylococcus epidermidis* [19] and *Acinetobacter* sp. [12].

It has been shown in many studies that bacteria growing as biofilms are more resistant to antimicrobial agents than the same bacteria in planktonic form [4–6, 20, 22, 36]. Kolari et al. [20] reported that in the presence of biocides, many biofilm bacteria, including *D. geothermalis*, produced even more biofilm than in the absence of biocides.

In this study, we show that biofilms of *D. geothermalis* on TiO_2 coated surfaces were effectively removed (1–2 log units) by titanium dioxide generated photocatalytic action in response to irradiation at 360 nm. This wavelength is emitted by daylight sources (fluorescent tubes) commonly used in offices and workspaces.

FESEM analysis of *D. geothermalis* biofilms after exposure to TiO_2-generated photocatalysis upon illumination at 360 nm showed that adhesion threads connecting the *D. geothermalis* cells to each other and to the abiotic surface were completely destroyed. Photocatalytic activity by TiO_2 is known to have disinfecting action [1, 11, 13, 24, 26, 37, 40]. Its effectiveness in killing water suspended microorganisms is well documented and also has practical applications.

The potential of photocatalytic TiO_2 as a tool against a biofilm mode of growth has received little attention so far. Li and Logan [23] studied the response of low density (10^5 cells cm^{-2}) adhered *Escherichia coli* (strains JM109, D21 and D21f2), *B. cepacia* (strains G4 and Env435) and *Pseudomonas aeruginosa* (strains PA01 and PDO300) on TiO_2 coated glass upon illumination at 254 (UVC) and 340 nm (UVA). They achieved up to 50% removal at 340 nm and 30–70% removal at 254 nm using irradiation doses of 28–46 W h m^{-2}. In our study, from 90% to close to 100% removal of *D. geothermalis* was observed at 360 nm irradiation upon exposure to 20 W h m^{-2}. Li and Logan [23] used a 20 nm thick

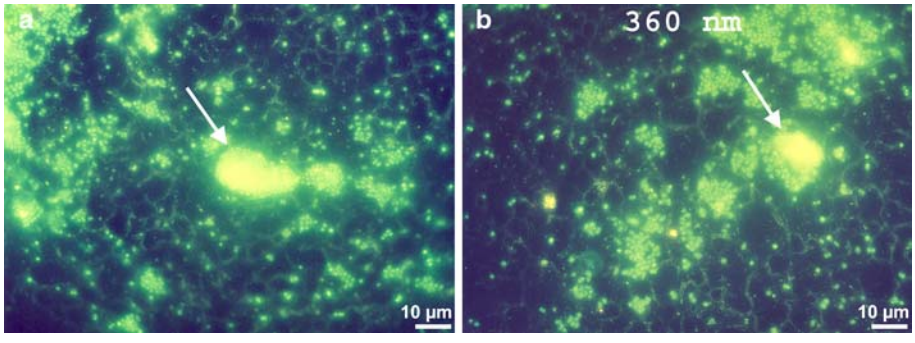

Fig. 6 Epifluorescence micrographs of SYTO9 stained 2 day grown *Deinococcus geothermalis* E50051 biofilm on steel before (**a**) and after (**b**) illumination at 360 nm (Nikon Eclipse E800; Excitation-λ 478–492 (±2) nm, emission-λ 525–551 (±2) nm). Fluorescent readout (Fig. 7) makes it possible to estimate cell numbers in larger, multi-layered, microcolonies, where microscopic counting is not possible (*arrows*)

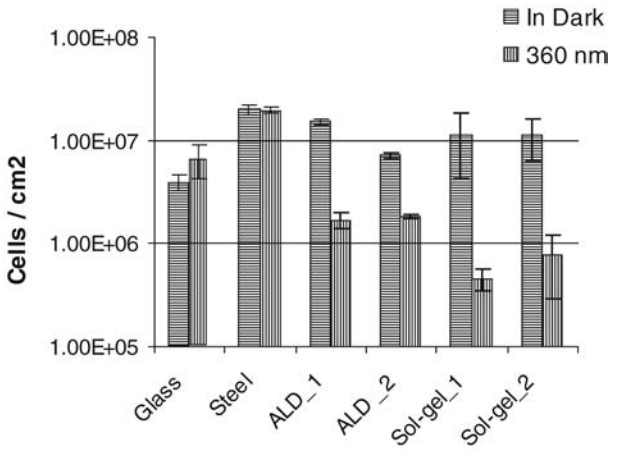

Fig. 7 Densities of *Deinococcus geothermalis* E50051 biofilms on abiotic surfaces and the effects of illumination at 360 nm. Biofilms were grown and then irradiated as described in the legend for Fig. 1. The coupons were stained with SYTO9 and intensity of the emitted fluorescence quantitated with a scanning fluorometer. Fluorescence values were translated in to cell numbers by using standard curve based on microscopic counting. *Error bars* indicate standard deviations

anatase TiO_2 film. Photocatalytic coatings used in our work were prepared by ALD [21] and sol-gel [14, 15] techniques to a thickness of 100 nm, possibly explaining the higher efficacy of biofilm removal achieved.

The adhesion threads connecting *D. geothermalis* to the substratum were shown to be non-uniform in thickness and length, and attachment to an abiotic surface appeared to involve a foot-like structure at the contact site. No similar structure has been reported in any other bacteria until now. *D. geothermalis* is a non-motile organism, possessing the ability to glide on a surface when exposed to external pressure [19]. Active gliding motility designated as "social gliding motility" or "twitching motility" has been shown in wide range of bacteria, e.g., *Pseudomonas aeruginosa, Neisseria gonorrhoeae* and *Myxococcus xanthus* [10. 27, 29] Such motility has been shown to depend on a type IV pilus. Type IV pili are known to be involved also in adhesion of *Shewanella oneidensis* onto glass [38], of *Ralstonia solanacearum* to polyvinylchloride (PVC) plastic [16] and microcolony formation of *P. aeruginosa* on polyvinylchloride plastic [30]. Interestingly, it was shown recently that the type IV pili occurring in iron reducing *Geobacter* species are electrically conductive nanowires connecting the bacteria to an inorganic surface [33].

The complete chromosomal sequence of one deinococcal species, *D. radiodurans*, is known [25]. It contains the full set of genes needed for the type IV pilus production machinery. A preliminary sequence involving type IV pilus genes has also been reported in *D. geothermalis* DSM11300 (accession number: NZ_AAHE00000000, [34]). However, the structures of *D. geothermalis* shown in this paper by FESEM and earlier by atomic force microscopy (AFM) [19] neither resemble morphologically the type IV pili such as published by Kang et al. [16] in *R. solanacearum* nor the anchor-like adhesion organelles of *Acinetobacter* sp. [12]. In addition to the adhesion threads (Figs. 1, 2, 3, 4, 5 in this paper) *D. geothermalis* possesses a patchy cell surface responding heterogeneously to adhesion forces measured by AFM [19]. The patchy surface observed by FESEM in this study (Fig. 5) and by AFM by Kolari et al. [19] may thus have local and heterogeneous adhesiveness, possibly explaining why some of the *D. geothermalis* cells persistently adhered to TiO_2 film post irradiation in spite of the complete loss of adhesion threads.

The high efficacy of photocatalytic TiO_2 films in destroying the adhesion apparatus of the tenacious biofilm former *D. geothermalis* indicates that this technology could be used as a tool towards a biocide free process in warm water industries such as paper mills.

Acknowledgements This work is a part of the technology programme Clean Surfaces 2002–2006 supported by the Finnish National Technology Agency (TEKES) and Academy of Finland Center of Excellence grant Nr. 53305. We thank Dr Marianna Kemell and Dr Pia Niinimäki for expert advice. We also thank Helsinki University Viikki Science Library for excellent information services, the Faculty of Agriculture and Forestry Instrument Centre for technical support and Leena Steininger, Hannele Tukiainen and Tuula Suortti for many kinds of help.

References

1. Amézaga-Madrid P, Nevárez-Moorillón GV, Orrantia-Borunda E, Miki-Yoshida M (2002) Photoinduced bactericidal activity against *Pseudomonas aeruginosa* by TiO_2 based thin films. FEMS Microbiol Lett 211:183–188
2. Anonymous (1992) Heterotrophic plate count. In: Clesceri LS, Greenberg AE, Eaton AD (eds) Standard methods for the examination of water and wastewater, American Public Health Association, American Water Works Association and Water Environment Federation, 20th edn. American Public Health Association, Washington DC, pp 9–34 to 9–36
3. Cho M, Chung H, Choi W, Yoon J (2004) Linear correlation between inactivation of *E. coli* and OH radical concentration in TiO_2 photocatalytic disinfection. Water Res 38:1069–1077
4. Das JR, Bhakoo M, Jones MV, Gilbert P (1998) Changes in the biocide susceptibility of *Staphylococcus epidermidis* and *Escherichia coli* cells associated with rapid attachment to plastic surfaces. J Appl Microbiol 84:852–858
5. Donlan RM, Costerton JW (2002) Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev 15:167–193
6. Dunne WM Jr (2002) Bacterial adhesion: seen any good biofilms lately? Clin Microbiol Rev 15:155–166
7. Eagon RG (1995) Paper, pulp and food grade paper. In: Rossmoore HW (ed) Handbook of biocide and preservative use. Blackie Academic & Professional, an imprint of Chapman & Hall, Glasgow, pp 83–132
8. Fujishima A, Honda K (1972) Electrochemical photocatalysis of water at a semiconductor electrode. Nature 238:37–38
9. Geesey GG, Bryers JD (2000) Biofouling of engineered materials and systems. In: Bryers JD (ed) Biofilms II: process analysis and applications. Wiley-Liss, Wiley, New York, pp 237–279
10. Harshey RM (2003) Bacterial motility on a surface: many ways to a common goal. Annu Rev Microbiol 57:249–273
11. Huang Z, Maness P-C, Blake DM, Wolfrum EJ, Smolinski SL, Jacoby WA (2000) Bactericidal mode of titanium dioxide photocatalysis. J Photoch Photobio A 130:163–170

12. Ishii S, Koki J, Unno H, Hori K (2004) Two morphological types of cell appendages on a strongly adhesive bacterium, *Acinetobacter* sp. Strain Tol 5. Appl Environ Microbiol 70:5026–5029
13. Jacoby WA, Maness P-C, Wolfrum EJ, Blake DM, Fennell JA (1998) Mineralization of bacterial cell mass on a photocatalytic surface in air. Environ Sci Technol 32:2650–2653
14. Kajihara K, Takeshi Y (1998) Macroporous morphology of the titania films prepared by a sol-gel dip-coating method from the system containing poly(ethylene glycol). I Effect of humidity. J Sol-gel Sci Tech 12:185–192
15. Kajihara K, Takeshi Y (1998) Macroporous morphology of the titania films prepared by a sol-gel dip-coating method from the system containing poly(ethylene glycol). I Effect of solution composition. J Sol-gel Sci Tech 12:193–201
16. Kang Y, Liu H, Genin S, Schell MA, Denny TP (2002) *Ralstonia solanacearum* requires type 4 pili to adhere to multiple surfaces and for natural transformation and virulence. Mol Microbiol 2:427–437
17. Kikuchi Y, Sunada K, Iyoda T, Hashimoto K, Fujishima A (1997) Photocatalytic bactericidal effect of TiO_2 thin films: dynamic view of the active oxygen species responsible for the effect. J Photoch Photobio A 106:51–56
18. Kolari M, Nuutinen J, Salkinoja-Salonen MS (2001) Mechanisms of biofilm formation in paper machine by *Bacillus* species: the role of *Deinococcus geothermalis*. J Ind Microbiol Biot 27:343–351
19. Kolari M, Schmidt U, Kuismanen E, Salkinoja-Salonen MS (2002) Firm but slippery attachment of *Deinococcus geothermalis*. J Bacteriol 184:2473–2480
20. Kolari M, Nuutinen J, Rainey FA, Salkinoja-Salonen MS (2003) Colored moderately thermophilic bacteria in paper-machine biofilms. J Ind Microbiol Biot 30:225–238
21. Leskelä M, Ritala M (2002) Atomic layer deposition (ALD): from precursors to thin film structures. Thin Solid Films 204:138–146
22. Lewis K (2001) Riddle of biofilm resistance. Antimicrob agents ch 45:999–1007
23. Li B, Logan BE (2005) The impact of ultraviolet light on bacterial adhesion to glass and metal oxide-coated surface. Colloid Surface B 41:153–161
24. Liu H-L, Yang TC-K (2003) Photocatalytic inactivation of *Escherichia coli* and *Lactobacillus helveticus* by ZnO and TiO_2 activated with ultraviolet light. Process Biochem 39:475–481
25. Makarova KS, Aravind L, Wolf YL, Tatusov RL, Minton KW, Koonin EV, Daly MJ (2001) Genome of the extremely radiation-resistant bacterium *Deinococcus radiodurans* viewed from the perspective of comparative genomics. Microbiol Mol Biol R 65:44–79
26. Maness P-C, Smolinski S, Blake DM, Huang Z, Wolfrum EJ, Jacoby WA (1999) Bactericidal activity of photocatalytic TiO_2 reaction: toward an understanding of its killing mechanism. Appl Environ Microbiol 65:4094–4098
27. Mattick JS (2002) Type IV pili and twitching motility. Annu Rev Microbiol 56:289–314
28. Mattila K, Weber A, Salkinoja-Salonen MS (2002) Structure and on-site formation of biofilms in paper machine water flow. J Ind Microbiol Biot 28:268–279
29. McBride MJ (2001) Bacterial glidingmotility: multiple mechanisms for cell movement over surfaces. Annu Rev Microbiol 55:49–75
30. O'Toole GA, Kolter R (1998) Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development. Mol Microbiol 30:295–304
31. Ottenio D, Escabasse J-Y, Podd B (2004) Packaging materials 6. Paper and board for food packaging applications. ILSI Report Series 6, International Life Sciences Institute, ILSI Press, Brussels
32. Pore V, Rahtu A, Leskelä M, Ritala M, Sajavaara T, Keinonen J (2004) Atomic layer deposition of photocatalytic TiO2 thin films from titanium tetramethoxide and water. Chem Vapor Depos 10:143–148
33. Reguera G, McCarthy KD, Mehta T, Nicoll JS, Tuominen MT, Lovley DR (2005) Extracellular electron transfer via microbial nanowires. Nature 435:1098–1101
34. Richardson P (2005) Annotation of the draft genome assembly of Deinococcus geothermalis DSM 11300. http://genome.ornl.gov/microbial/dgeo/15apr04/dgeo_N_cogs.html
35. Ritala M, Leskelä M (2002) Atomic layer deposition, chapter 2. In: Nalwa HS (ed) Handbook of thin film materials vol 1. Academic, New York, pp 103–159
36. Stewart PS, Costerton JW (2001) Antibiotic resistance of bacteria in biofilms. Lancet 358:135–138
37. Sunada K, Watanabe T, Hashimoto K (2003) Studies on photokilling of bacteria on TiO_2 thin film. J Photoch Photobio A 156:227–233
38. Thormann KM, Saville RM, Shukla S, Pelletier DA, Spormann AM (2004) Initial phases of biofilm formation in *Shewanella oneidensis* MR-1. J Bacteriol 186:8096–8104
39. Väisänen OM, Weber A, Bennasar A, Rainey FA, Busse H-J, Salkinoja-Salonen MS (1998) Microbial communities of printing paper machines. J Appl Microbiol 84:1069–1084
40. Wolfrum EJ, Huang J, Blake DM, Maness P-C, Huang Z, Fiest J, Jacoby WA (2002) Photocatalytic oxidation of bacterial, bacterial and fungal spores, and model biofilm components to carbon dioxide on titanium dioxide-coated surfaces. Environ Sci Technol 36:3412–3419
41. Yeager CC (1995) Legislative aspects. In: Rossmoore HW (eds) Handbook of biocide and preservative use. Blackie Academic & Professional, an imprint of Chapman & Hall, Glasgow, pp 19–49