The effect of anatase and rutile crystallites isolated from titania P25 photocatalyst on growth of selected mould fungi

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Abstract

Antifungal properties of anatase and rutile crystallites isolated from commercial titania P25 photocatalyst were investigated by mycelium growth in the dark and under indoor light. Investigated fungi, i.e., *P. boydii*, *S. apiospermum*, *P. ellipsoidea*, *S. aurantiacum*, *A. versicolor*, *A. flavus*, *S. chartarum*, *P. chrysogenum*, *A. melleus*, were isolated from air and from moisture condensed on walls. Anatase and rutile were isolated from homogenized P25 (homo-P25) by chemical dissolution, and then purified by washing and thermal treatment. For comparison, homo-P25 was also thermally treated at 200°C and 500°C. Titania samples were characterized by X-ray diffractometry (XRD), X-ray photoelectron spectroscopy (XPS), diffuse reflectance spectroscopy (DRS) and scanning transmission electron microscopy (STEM). It was found that properties of titania, i.e., band-gap energy, impurities adsorbed on the surface, nanoparticle aggregation, and kind of fungal structure, highly influenced resultant antifungal activities. It is proposed that some fungi could uptake necessary water and nutrient from titania surface. It was also found that even when differences in mycelium growth were not significant, the sporulation and mycotoxin generation were highly inhibited by light and presence of titania.

Keywords: antifungal properties, water activity, anatase, rutile, P25, photocatalysis, pathogenic fungi

1. Introduction

The current lifestyle is responsible for long human staying at indoor environment, i.e., more than 80% of the day, and thus it is considered as a reason of various health problems. There are various chemical contaminants originated from the interior of buildings, e.g., building materials, consumer products and heating systems, and from outside of buildings, e.g., traffic load, and often indoor air is much more polluted than outdoor [1]. Examples include volatile organic compounds, VOCs (e.g., benzene, toluene, ethyl benzene, xylene,

formaldehyde; from glues, fabric treatments, stains and varnishes), volatile organic peroxides, VOP (from residual peroxides in synthetic materials used in rooms) [2], pentachlorophenol, PCP (from wood preservation) [3], general air pollutants (oxides of sulphur and nitrogen, ozone), inorganic and organic compounds emitted from stored supplies (solvents, toners, ammonia, chlorine) and housekeeping activities (ammonia, chlorine, and other cleaning agents such as detergent, carpet shampoo, disinfectants). Furthermore, it was estimated that common contaminants found in office and home environment are microorganism, which represent approximately 70% of the dust [4, 5]. The main group of organisms presents in bioaerosol are filamentous fungi. They might be a direct threat to the health (e.g., causing allergic reactions and upper respiratory track infections) or an indirect threat by their secrete hazardous metabolites – mycotoxins [6, 7]. Therefore if the sources of fungal contamination are not controlled, the problems connected with indoor environmental quality (IEQ) can arise, even if the buildings' ventilation systems are properly designed and well maintained.

Reducing exposure to chemicals and bioaerosols at home, school and in the workplace as a preventative action can lead to improvement of IEQ and simultaneously to better people health. In order to improve the air quality, the producers of building materials are obligated to develop new technological solutions, i.e., novel materials with reduced content of hazardous chemical compounds, and with chemical compounds possessing antimicrobial properties. The semiconductor photocatalysts, e.g., titanium(IV) oxide (TiO_2 , titanium dioxide, titania), are considered as such materials which at the same time are safe for human and may improve IEQ. Titania is one of the most widely used heterogeneous photocatalysts, due to its high chemical and thermal stability, non-toxicity, and high photocatalytic activity [8-11]. It should be clarified that the meaning of “non-toxicity” of titania originates from its chemical properties, but not from its physical structure, and as such nanoparticles of titania (and all other nanomaterials) are considered as potentially toxic, due to ability to go through the biological barriers causing damage to various organs [12]. Three polymorphic forms of titania are known, i.e., anatase, rutile and brookite. Anatase and brookite are metastable, while rutile is thermodynamically stable. Brookite has been seldom studied, due to more complicated synthetic methods of its preparation, which often need application of highly concentrated acids or high temperature and pressure [13]. Titania in rutile form is widely used for whitening of commercial products such as paints, lacquers, plastic, paper and gum. While, anatase is mainly used as photoactive coating material, due to its self-cleaning and superhydrophilicity properties [8, 14, 15]. These properties are induced by UV light irradiation of the photocatalyst and subsequent generation of reactive oxygen species (ROS)

oxidizing adsorbed pollutants [16]. Superhydrophilicity causes also enhancement of cleaning effect, due to easily removing of dirt with rainwater [17, 18]. Since the first report by Matsunaga *et al.* in 1985 on the application of TiO₂ photocatalysis for destruction of *Lactobacillus acidophilus*, *Escherichia coli* and *Saccharomyces cerevisiae* [19] much interest in use of heterogeneous photocatalysis for water and air disinfection has been noticed. The majority of studies on the antimicrobial activity of titanium dioxide were performed with simple microorganisms, such as bacteria, viruses and prions [20-24]. Up to now extensive research has been done in the area of antibacterial action of TiO₂, i.e., on explanation of the mechanism of action and on the influence of various factors, such as irradiation (type, range, intensity), titania (amount, surface properties, content of dopants/modifiers), photocatalyst immobilisation, process conditions (temperature, water turbidity), on the efficiency of photocatalytic process [25-27]. Whereas the antifungal capability of titania photocatalyst was confirmed only for few species of yeast and mould fungi [28-31]. Our recent report on inactivation of pathogenic fungi indicated that irradiated titania P25 highly inhibited growth of fungi *Pseudallescheria-Scedosporium*, while the strains of *Aspergillus versicolor* were mainly resistant [32]. The antifungal activities have been usually tested on anatase or anatase-rutile mixture (e.g., P25) and there are no reports on the effect of pure rutile on growth of fungi. Thus, the aim of the present study was to examine the influence of polymorphs of titania, rutile and anatase, on growth of a few species of mould fungi. Titania P25 originated from Evonik company (previously Degussa) and its isolated polymorphs, i.e., anatase and rutile, have been used for the study. In order to reflect natural conditions of fungal life the experiments were performed under indoor light irradiation.

2. Materials and Methods

2.1. Antifungal tests

The antifungal activity of TiO₂ was investigated by agar plate method on standard Czapek Yeast Agar (CYA; BTL). The basal medium was supplemented with 20 g × dm⁻³ of titania samples: original P25 from Evonik (Germany), and isolated polymorphic forms (anatase and rutile) from P25.

The antifungal activities were tested for the following fungi strains isolated from air, *Penicillium chrysogenum*, strain ZUT1; *Aspergillus versicolor*, strains: ZUT130, ZUT110; *Aspergillus flavus*, strain ZUT2; *Aspergillus melleus*, strain ZUT3; *Pseudallescheria boydii*, strain ZUT25; *Pseudallescheria ellipsoidea*, ZUT12; *Scedosporium apiospermum*, strain ZUT11; *Scedosporium aurantiacum*, strain ZUT4, and from damp walls: *Stachybotrys*

chartarum, strain ZUTST. Ten-day old fungal cultures on MEA slants at 25°C were used for preparation of spore suspensions. 5 ml of sterile physiological saline were added to each slant. The slants were then vigorously shaken by a vortex for 3 minutes. Each plate was centrally inoculated with 5 µl of spore suspension. Due to limited amount of photocatalysts for experiments carried out on *P. chrysogenum* and *A. mellus* three symmetrical places of each plate were inoculated with spore suspension (as shown for 4-day incubated samples in Fig. 5c). It must be pointed that even for these experiments at least 2 g of photocatalyst had to be prepared for tests of one fungal strain in the dark and light conditions. One part of the plates was incubated in the dark, while another part was exposed to natural indoor light (intensity of ca. 120 W m⁻²). The irradiation was daily intensified by 8-hour exposition to halogen light (intensity of ca. 1300 W m⁻²) from three halogen lamps (50 W/each) mounted ca. 50 cm above plates. The UV light intensity was negligible (ca. 0.3 W m⁻²). The incubation was carried out for 10 days at 25°C. The colony diameters were measured after 2, 4, 6, 8 and 10 days of incubation. The daily growth rates (mm/day) were calculated from the linear regression equation:

$$r = a \cdot t + b,$$

where “r” is colony radius (mm); “t” is incubation time (day), “a” is daily growth rate, and “b” is growth retardation time (lag phase; λ).

According to Dantigny *et al.* [33] the lag time calculated from macroscopic observations of mycelium has no biological significance, and thus the analysis of b values has been abandoned in present study.

2.2. Separation of polymorphic forms from titania P25

Commercial titania photocatalysts, P25, composed of a mixture of two crystalline phases: anatase (73-85%) and rutile (14-17%), and amorphous titania (0-13%) [34] was supplied by Evonik. Experiments were performed for a few titania samples obtained from P25, i.e., i) homogenized P25 (homo-P25), ii) thermally post-treated homo-P25 at 200 and 500°C (homo-P25-200, homo-P25-500), iii) isolated anatase (A), iv) isolated rutile (R), v) isolated anatase thermally post-treated at 200°C (A-200), vi) isolated rutile washed with NaOH and thermally post-treated at 200°C (R-200), and vii) isolated anatase washed with NaOH (A_{Na}). Since commercial P25 is highly heterogeneous [34], and even samples taken from the same package differ in composition (anatase/rutile/amorphous), P25 was first homogenized. The P25 sample of 20 g was suspended in 0.2-L Milli-Q water for 12 h under 500 rpm and then freeze-dried at -80°C and 10 Pa for 24 h (homo-P25). Anatase particles (A)

were obtained from homo-P25 (6 g) by selective dissolution of rutile phase in a mixture of hydrogen peroxide (30 wt%, 200 mL) and ammonia (25 wt%, 6 mL) under magnetic stirring for 15 h at 25°C [35]. The residual anatase titania was washed with Milli-Q water and centrifuged under 15,000 rpm several times, and freeze-dried. Thermal post-treatment at 200°C for 2.5 h was applied for sample purification and the resultant sample was labelled as A-200. Rutile particles were isolated from P25 by selective dissolution of anatase phase from homo-P25 (9 g) in a diluted hydrofluoric acid (10 wt%, 200 mL) under magnetic stirring for 24 h at room temperature [36]. The residual titania was washed several times with Milli-Q water, centrifuged under 4,000 rpm, dried by the freeze-drying process and labelled as R. For further sample purification, the sample was additionally washed with aqueous sodium hydroxide (to remove HF), freeze-dried and thermally post-treated at 200°C for 2.5 h. The resultant sample was labelled as R_{Na}-200. To study the possible influence of thermal treatment on surface properties of titania P25, e.g., aggregation and sintering, and thus on resultant antifungal properties, homo-P25 was also thermally treated at 200 and 500°C and obtained samples were labelled as homo-P25-200 and homo-P25-500. In addition, the possible influence of sodium on antifungal properties was also investigated for anatase sample (A) washed with aqueous sodium hydroxide, freeze-dried and labelled as A_{Na}.

2.3. Characterization of titania samples

Properties and purity of titania samples were characterized by X-ray diffractometry (XRD), X-ray photoelectron spectroscopy (XPS), diffuse reflectance spectroscopy (DRS) and scanning transmission electron microscopy (STEM). XRD patterns were recorded on SmartLab diffractometer (Rigaku) equipped with: i) X-ray generator (a copper target; operated at 30 mA and 40 kV), ii) D/teX high-speed position-sensitive detector system, and iii) ASC-10 automatic sample changer. XRD measurements were performed for 2θ of 10–90°, scan speed of 1° min⁻¹ and scan step of 0.008°. XRD data were analysed by Rigaku PDXL, a crystal structure analysis package. Crystalline sizes were evaluated from corrected width of the most intensive anatase and rutile diffractions peaks ({101} and {110}) using Scherrer equation. Non crystalline (NC) content was evaluated using highly crystalline nickel oxide as an internal standard by its braying (20 wt%) with a sample (80 wt%) in an agate mortar before XRD analysis.

The purity of sample was examined on XPS (JEOL, JPC-9010MC). 20 scans were carried out and average data were taken for determination of titanium, oxygen and carbon

content. Considering the low amount of fluorine (used for anatase dissolution) and sodium (used for sample washing), 50 scans were performed for evaluation of their content.

The morphology of titania samples was observed by scanning transmission electron microscopy (STEM; HITACHI HD2000) with the accelerating voltage and emission current of 200 kV and 30 μ A, respectively. Powders were dispersed in ethanol in an ultrasonic bath for a few minutes, and then droplet of suspension was deposited on a carbon-covered copper microgrid, which was dried under vacuum overnight. Images were acquired at a wide range of magnifications (50,000–800,000) in normal resolution at working distance of 3 mm as secondary electron (SE), Z-contrast (ZC), and bright-field (BF) modes.

3. Results

3.1. Characterization of photocatalysts

The experiments were performed on commercial titania P25 possessing one of the highest photocatalytic activity among other titanias for various reactions systems [37]. P25 is composed of two polymorphic forms of titania, i.e., anatase and rutile, and non-crystalline (NC) part in the amount of 73-85, 14-17 and 0-13%, respectively [34]. In this regard, it is impossible to judge which part of P25 or if the interaction between its components is responsible for antifungal properties. Thus, isolated polymorphic forms, i.e., A (anatase) and R (rutile), were used for detailed studies. It must be pointed that P25 is highly heterogeneous, and thus homogenized samples of P25 were used as starting material (homo-P25). The composition of titania samples, i.e., homo-P25 and isolated phases without and with thermal post-treatment (homo-P25, homo-P25-200, homo-P25-500, A, A_{Na}, A-200, R and R_{Na}-200) is shown in Table 1 and exemplary XRD patterns are presented in the left part of Fig. 1.

Table 1. Phase composition of titania samples

Sample code name		Composition (%)			Crystalline size (nm)	
		anatase	rutile	NC phase	anatase	rutile
homo-P25	sample 1	77.6	14.1	8.3	25.3	39.6
	sample 2	77.6	14.4	8.0	28.7	33.9
	sample 3	77.1	14.0	8.9	30.2	36.1
	average	77.4	14.2	8.4	28.1	36.5
homo-P25-200		74.8	13.6	11.6	30.5	35.2
homo-P25-500		71.3	18.8	9.9	34.1	52.4
A		86.6	0	13.4	25.9	-
A _{Na}		80.5	0	19.5	22.3	-
A-200		85.9	0	14.1	23.7	-
R		0	88.6	11.4	-	38.6
R _{Na} -200		0	97.1	2.9	-	39.5

Homogenization resulted in preparation of uniform titania samples of ca. 77% of anatase, 14% of rutile and 8% of NC, as confirmed by three XRD analyses (samples 1-3 in Table 1). Thermal treatment of homo-P25 at 500°C resulted in slight increase of rutile content up to 18.8%, due to thermally induced crystallization of amorphous titania and phase transition from anatase to rutile. Isolated samples (A, A_{Na}, A-200, R, R_{Na}-200) contained only one crystalline phase of anatase and rutile with minority of NC phase, as shown in the left part of Fig. 1. It is suspected that NC part contained small amount of adsorbed water (<3% based on thermogravimetry measurements [38]) and amorphous titania. XRD patterns proved crystalline purity of isolated samples, i.e., disappearance of anatase and rutile peaks for R/R_{Na}-200 and A/A_{Na}/A-200 samples, respectively. The size of anatase and rutile crystallites equalled to 28.1 and 36.5 nm, respectively, in homo-P25. Isolation process only slightly reduced crystalline size of anatase, probably due to chemical etching. Crystalline sizes increased by thermal treatment, especially at higher temperature of 500°C reaching 34.1 and 52.4 nm for anatase and rutile phases, respectively.

Fig. 1. (left) XRD patterns and (right) DRS spectra of homo-P25, A-200 and R_{Na}-200: ♦ anatase, * rutile

The absorption properties of samples were studied by DRS, and exemplary data are shown in the right part of Fig. 1. The observed hypsochromic and bathochromic shifts of photoabsorption for A-200 and R_{Na}-200 samples, respectively, in comparison with homo-P25, were expected, due to narrower band-gap of rutile (ca. 3.0 eV) than anatase (ca. 3.2 eV). The surface purity of rutile samples was also examined by XPS analysis, i.e., the efficiency of post-treatment operation during rutile photocatalysts washing. Five main elements were analyzed in details by narrow XPS scanning, i.e., titanium, oxygen, carbon, fluorine and sodium, and obtained data are summarized in Table 2. It was found that isolated rutile sample

unwashed with NaOH (R) possessed large content of fluorine on the surface reaching 8.35 mol% (in relation to titanium). Whereas R-200 sample, R washed with NaOH and thermally treated at 200°C, contained much lower amount of fluorine on the surface of 2.26 mol%. Both samples possessed excess of carbon on the surface, i.e., 26.1 and 50.5 mol% for R_{Na}-200 and R, respectively. The atomic ratio of oxygen to titania for R sample slightly exceeded stoichiometric value of 2.0 reaching 2.2. Whereas for R_{Na}-200 the lack of oxygen was notice since 1.72% of O/Ti ratio was obtained. It is thought that sample washing with NaOH removed adsorbed fluorine, carbon and oxygen (in the form of hydroxyl groups). It must be pointed that though some adsorbents were washed out from titania surface, washing with NaOH caused adsorption of sodium on the surface. After deconvolution of titanium and oxygen peaks (Fig. 2), it was found that titanium in R and R_{Na}-200 samples existed mainly in oxidized form of Ti⁺⁴ (97.2% and 95.7%, respectively), as shown in Table 3. O 1s region could be deconvoluted for three peaks at BE of 529.3-529.5 eV, 531.3-531.8 eV and 533.4-533.8 eV. The first peak can be attributed to oxygen in crystalline lattice of TiO₂, the second peak to OH group binding with two Ti atoms or Ti₂O₃, while the third peak to surface oxygen in Ti-OH [39, 40]. Oxygen mainly occurred in the form of TiO₂, reaching 65% and 79% for R and R-200, which confirmed that alkali washing resulted in desorption of hydroxyl groups from the surface.

Fig. 2. XPS spectra of (left) Ti 2p and (right) O 1s regions for rutile samples: R (a) and R_{Na}-200 (b).

Table 2. Chemical composition of rutile photocatalysts based on XPS analysis

Sample	Ti mol%	O mol%	O:Ti	C mol%	C:Ti	F mol%	F:Ti	Na mol%	Na:Ti
R	15.09	33.16	2.2	50.49	3.3	1.26	0.08	-	-
R _{Na} -200	22.59	38.94	1.7	26.1	1.2	0.51	0.02	11.86	0.5

Table 3. Oxidation state of titanium and oxygen in rutile samples based on XPS analysis

Sample	Ti 2p (%)		O 1s (%)		
	Ti ⁺⁺⁴	Ti ⁺⁺³	-OH	(-OH) ₂ , Ti ₂ O ₃	TiO ₂
R	97.2	2.8	8.0	27.5	64.5
R _{Na} -200	95.7	4.3	3.9	16.7	79.4

Morphology of samples was studied by STEM and exemplary images of A, R and homo-P25 are shown in Fig. 3. Similar to XRD data, slightly smaller NPs were detected for anatase (Fig. 3, bottom, right) than for rutile (Fig. 3, bottom, left) samples of 20-40 nm and 30-60 nm, respectively. Therefore, it is proposed that larger and smaller NPs detected in homo-P25 (Fig. 3, top) correspond to rutile and anatase crystallites, respectively. Large aggregates of 100-200 nm were noticed only in isolated rutile and mixed sample indicating that mainly rutile crystallites formed these aggregates.

Fig. 3. STEM images of homo-P25 (top), R (bottom, left) and A (bottom, right).

3.2. Antifungal properties of photocatalysts

3.2.1. Antifungal properties of isolated phases (A and R) against selected fungi

Raw titania (A and R) samples (without purification)

At first antifungal properties were tested for following fungi: *Pseudallescheria boydii* (ZUT25), *Scedosporium apiospermum* (ZUT11), *Pseudallescheria ellipsoidea* (ZUT12),

Scedosporium aurantiacum (ZUT4), *Aspergillus versicolor* (ZUT130, ZUT110), *Aspergillus flavus* (ZUT2) and *Stachybotrys chartarum* (ZUTST). The rates of fungal daily growth during experiments performed in the dark (D) and under light (L) on isolated phases (A and R) are shown in Fig. 4.

The complete inhibition of growth was achieved for only one strain *Pseudallescheria boydii* (ZUT25) on media supplemented with anatase under irradiation (Fig. 4a). The light also inhibited fungal growth on anatase for ZUT 110 and ZUT2, and slightly for ZUT12, ZUT4, ZUT130 and ZUTST. However, the stimulative effect of light on fungal growth was observed for ZUT 11. Almost all observed differences were statistically significant (ZUT25, ZUT11, ZUT12, ZUT4, ZUT110, ZUT2, ZUTST). The indoor light (photolysis, but not photocatalysis) mostly inhibited fungal growth what was observed on control media (without titania). It should be pointed that presence of anatase accelerated growth of ZUT2, ZUT4, ZUT12 and ZUTST under irradiation, and inhibited growth of all tested fungi in the dark (except ZUTST). This indicates that irradiated anatase positively changed fungal environment, possibly due to change of water accessibility (superhydrophilicity) and thus stimulated fungal growth. The free water is essential for normal activity of extracellular enzyme in a process known as absorptive nutrition. The enzymes, which are released by fungi, break down complex (polymer) substances in building materials. Then, the simple compounds are dissolved in water and absorbed by the fungi. Our recent report on antifungal properties of P25 for various water activity ($a_w=0.850-0.999$) has indicated that titania stimulated fungal growth in the lack of accessible water (however, mainly in the dark) [32]. The inhibition of fungal growth by non-irradiated anatase could be caused by its adsorption on fungal surface, which could result in inhibition of sporulation. According to Calvo *et al.* [41] interactions between sporulation and mycotoxin production in relation to environmental factors are not understood at a genetic level. It is possible, however, that some compounds have subtle effects on sporulation (decrease in asexual spore production is not detectable by the unaided eye), but exhibit a decrease mycotoxin production. For example simple sugars such as glucose, fructose, sucrose, and sorbitol support high fungal growth, sporulation, and aflatoxin production. In contrast, peptone and the more-complex sugars like galactose, xylose, mannitol, do not support aflatoxin production well [41].

Rutile showed similar action to anatase for *P. boydii* (ZUT25) and *A. versicolor* (ZUT130, ZUT110), but inhibition of growth of ZUT25 and ZUT130 in the dark was 3-4 times lower than that on anatase, which could be caused by large particles of rutile and thus lower interface between titania and fungus. Whereas, activity of rutile under irradiation was

two times higher than that of anatase for *A. versicolor* (ZUT110), probably due to its broader photoabsorption (≤ 420 nm for rutile and ≤ 400 nm for anatase, Fig. 1, left), which is crucial for present studies performed under daily light (not UV lamps). In the dark and under irradiation rutile stimulated growth of fungi which growth was accelerated by irradiation of anatase, i.e., ZUT12, ZUT4, ZUT2 and ZUTST. All differences between samples tested under irradiation in the presence and absence of rutile were statistically significant (Fig. 4b). Though used amount of photocatalyst ($20 \text{ g} \times \text{dm}^{-3}$) was not sufficient for complete elimination of mould fungi, the further experiments were continued for the same conditions for clear analysis (comparison) of data.

a)

b)

Fig. 4 Mean daily radius growth of selected mould on media supplemented with $20 \text{ g} \times \text{dm}^{-3}$ of (a) anatase and (b) rutile incubated in dark (D) and light (L) conditions; A – anatase (A), R – rutile (R), C - control (without

photocatalyst), strains of fungi: ZUT25 - *P. boydii*, ZUT11 - *S. apiospermum*, ZUT12 - *P. ellipsoidea*, ZUT4 - *S. aurantiacum*, ZUT130 and ZUT 110 - *A. versicolor*, ZUT2 - *A. flavus*, ZUTST - *S. chartarum*; black bars mean standard deviations between replications, black arrows mean statistically significant differences between daily growth rate in light and dark at $p \leq 0.05$.

Summarizing, it must be pointed that though surface properties of both crystalline forms were similar (particle size), they differed significantly by resultant antifungal properties. Activity of rutile was highly accelerated by light, due to its ability of absorption of more photons than anatase (narrower band-gap). While, irradiated anatase stimulated fungal growth possibly as a result of its superhydrophilic properties which could influence water accessibility by fungi. In addition, it was found that antifungal properties of titania depended significantly on structure of examined fungi, and thus either enhancement or inhibition of fungal growth was noticed.

Purified titania samples (A-200, R_{Na}-200)

It must be pointed that isolation process was performed by chemical etching, and thus isolated anatase and rutile (A, R) possessed chemical impurities adsorbed on titania surface, e.g., nitrogen, fluorine (as has been proved by XPS data), which could influence the resultant antifungal properties. In this regard, purification of samples by alkali washing (only for rutile to remove HF) and thermal treatment (for both samples) was carried out, and antifungal properties of purified samples, i.e., A-200 and R_{Na}-200, was tested and data are presented in Fig. 5(a-b). Sample purification resulted in enhancement of antifungal properties of anatase under irradiation for four fungal strains, i.e., *S. apiospermum* (ZUT11), *P. ellipsoidea* (ZUT12), *S. aurantiacum* (ZUT4), *A. flavus* (ZUT2). However, antifungal properties of anatase for *P. boydii* (ZUT25) and *A. versicolor* (ZUT130) decreased. In the dark antifungal properties of anatase worsened for five fungal strains, i.e., *S. apiospermum* (ZUT11), *P. ellipsoidea* (ZUT12), *S. aurantiacum* (ZUT4), *A. versicolor* (ZUT130, ZUT110), which could be caused by decrease of interface between titania and fungus, due to particle aggregation under thermal treatment.

Due to limited amount of purified rutile, tests were conducted only for two strains of fungi: *P. boydii* (ZUT25) and *S. chartarum* (ZUTST). In comparison with non-purified rutile (Fig. 4b), the daily growth rates of examined fungi by purified rutile increased slightly in the dark and increased highly under irradiation. It is suggested that removal of HF by washing with NaOH resulted in change of rutile surface (XPS data shown in Tables 2 and 3), and adsorbed sodium on titania surface could:

- i) (under irradiation) work as recombination centers for photogenerated charge carriers (e^-/h^+) resulting in decrease of photocatalytic activity [42, 43]; it is also possible that during thermal treatment photocatalytically inactive sodium titanate could be formed [44])

a)

b)

Fig. 5 (a and b) Mean daily radius growth of selected mould on media supplemented with 20 g x dm^{-3} of purified (a) anatase (A-200) and (b) rutile (R_{Na} -200) incubated in dark (D) and light (L) conditions; A – anatase (A-200), R – rutile (R_{Na} -200), C - control (without photocatalyst), strains of fungi: ZUT25 - *P. boydii*, ZUT11 - *S. apiospermum*, ZUT12 - *P. ellipsoidea*, ZUT4 - *S. aurantiacum*, ZUT130 and ZUT 110 - *A. versicolor*, ZUT2 - *A. flavus*, ZUTST - *S. chartarum*; black bars mean standard deviations between replications, black arrows mean statistically significant differences between daily growth rate in light and dark at $p \leq 0.05$; (c) photograph of mycelium growth of *P. chrysogenum* ZUT1 after 4-day incubation in the dark on reference (without titania) and on titania samples: P25-500, P25-200, A_{Na} , R (antifungal data shown in Fig. 6).

- ii) (in the dark) accelerate fungal growth as nutrient which was also suggested by already mentioned decrease in antifungal properties in the dark. There is partial evidence from the literature of significant influence of NaCl and CaCl_2 on the fungal growth [45].

It is clear that fungal growth rates are dependent on both the purity of photocatalysts and a strain of fungi.

3.2.2. Experiments for *Penicillium chrysogenum* and *Aspergillus melleus*

To further investigate an influence of thermal treatment and presence of impurities on titania surface antifungal properties for two additional strains of fungi: *Penicillium chrysogenum* (ZUT1) and *Aspergillus melleus* (ZUT21) were examined on P25 thermally treated at 200°C (P25-200) and 500°C (P25-500) and on isolated anatase washed with NaOH (A_{Na}) and isolated rutile (R). It was found that growth of fungi was strongly inhibited by presence of titania both under irradiation and in the dark, as shown in Figs. 5c and 6. It is suggested that rutile phase is mainly responsible for titania activity under irradiation, due to narrower band-gap, and thus ability of visible light absorption. In addition, presence of adsorbed fluoride on rutile surface could both i) activate titania towards visible light by e.g., generation of localized state in the band-gap creating surface oxygen vacancies, and ii) inhibit recombination of charge carriers [46]. In the case of *P. chrysogenum*, the same antifungal activity in the dark for P25-200 and A_{Na} was not surprised since P25 was mainly composed of anatase. Slight decrease in antifungal activity for P25-500 could be caused by sintering and aggregation of titania nanoparticles at higher temperature and thus due to decrease of interface between titania and fungus. The highest antifungal activity of rutile in the dark could be caused by the presence of fluoride on titania surface. Similarly to inhibition of fungal growth in the dark on non-purified rutile sample as is evident by comparison of data from Figs. 4b (R) and 5b (R_{Na} -200). Much worse activity of anatase than rutile under irradiation and in the dark could be caused by sodium presence, similarly as was proposed for alkali washed rutile sample (R_{Na} -200), i.e., enhanced recombination of charge carriers and nutrient promoted fungal growth, respectively. According to recent studies of Boumaaza *et al.* [45] sodium presence in various concentrations can promote or reduce the in vitro mycelial growth and sporulation. In contradistinction to *P. chrysogenum*, P25-200 exhibited the highest antifungal properties under irradiation against *A. melleus*. It is possible that synergism between anatase and rutile, i.e., transfer of charge carriers between two polymorphs, could result in higher photocatalytic activity as was previously proposed for decomposition of chemical compounds [36, 47]. However, it is expected that for both fungal strains the same synergism should be observed. The lack of synergism for *P. chrysogenum* indicates that other factor could be crucial for resultant antifungal activities, e.g., presence of impurities on titania surface. It is proposed that fluorine and sodium may inhibit fungal growth, but this inhibition highly

depends on fungal structure. The worse activity of P25-500 than P25-200 under irradiation for both fungal strains is possibly caused by creating new recombination centers for aggregated titania nanoparticles. Though statistically significant difference between incubation under light and in the dark was not noticed, and thus influence of light could not be clearly confirmed by simple growth of mycelium, it must be pointed that sporulation and mycotoxin generation (observed as formed droplets, which most probably should consist mycotoxins since mycotoxin production is associated with sporulation and has been documented in several mycotoxigenic genera, for example *Aspergillus* [41]) was highly inhibited under irradiation, as shown in the right part of Fig. 7. The reduction of spores amount presented in air is crucial for preventing against impact of mycotoxins on human health. Only slight difference in sporulation and mycotoxin generation was observed between control sample and that supplemented with photocatalyst by simple visual observations. In this regard, further studies on counting of generated spores and mycotoxin production (qualitative and quantitative analyses) are presently under investigation.

Fig. 6 Mean daily radius growth of *Penicillium chrysogenum* (ZUT1) and *Aspergillus melleus* (ZUT21) in dark (D) and light (L) conditions on media supplemented with $20 \text{ g} \times \text{dm}^{-3}$ of photocatalyst; A – anatase washed with NaOH (A_{Na}), R – rutile (R), P25-200 – homogenized P25 thermally post-treated at 200°C , P25-500 – homogenized P25 thermally post-treated at 500°C , C - control (without photocatalyst); black bars mean standard deviations between replications.

Fig. 7. Images of 3-day growth of *A. melleus* (left) in the dark and (right) under irradiation without (top) and in the presence of homo-P25 (bottom).

4. Conclusions

It was found that titania photocatalyst could be efficiently used as antifungal agent for various strains of fungi. Its activity was confirmed under irradiation and also in the dark. It was shown that properties of titania were crucial for antifungal action. Though polymorphic forms of titania (anatase and rutile), isolated from commercial P25 photocatalyst, had similar surface properties (crystalline size), their activity highly differed, possibly due to slight difference in band-gap energy and thus ability of photoabsorption of more photons by rutile phase. In addition, it was shown that purification of isolated phases could result in slight change of their surface properties, i.e., particle aggregation, removal and/or adsorption of chemical species, which resulted in significant change in their antifungal properties. It must be pointed that also differences in fungal structure influenced resultant antifungal properties, and thus for example growth of strains of *P. boydii* (ZUT25) and *A. versicolor* (ZUT13) was highly inhibited by titania and light, but growth of ZUTST was stimulated by titania presence. It is important to remember that different fungi had different demand for water and nutrient, and thus adsorbed water and some impurities (e.g., F^- , Na^+ , HOO^-) on titania surface could either inhibit or stimulate their growth. Another important finding is that even when mycelium growth was similar, the sporulation and mycotoxin generation was highly inhibited

by light and titania presence. The quantitative and qualitative analyses of generated mycotoxin and rate of sporulation are currently under study.

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References

- [1] P. Schneider, G. Lorinci, I.L. Gebefugi, J. Heinrich, A. Kettrup, H.E. Wichmann, J. Expo. Anal. Env. Epid. 9 (1999) 282-292.
- [2] J.G. Hong, J. Maguhn, D. Freitag, A. Kettrup, Fresen. J. Anal. Chem. 371 (2001) 961-965.
- [3] J. Schnelle-Kreis, H. Scherb, I. Gebefugi, A. Kettrup, E. Weigelt, Sci. Total Environ. 256 (2000) 125-132.
- [4] C.S. Mitchell, J.F.J. Zhang, T. Sigsgaard, M. Jantunen, P.J. Lioy, R. Samson, M.H. Karol, Environ. Health Persp. 115 (2007) 958-964.
- [5] S. Dubey, S. Lanjewar, M. Sahu, K. Pandey, U. Kutti, J. Phytol. 3 (2011) 13-14.
- [6] H.M. Ammann, Mycotoxin Res. 21 (2005) 157-163.
- [7] R.K. Bush, J.M. Portnoy, A. Saxon, A.I. Terr, R.A. Wood, J. Allergy Clin. Immun. 117 (2006) 326-333.
- [8] K. Nakata, A. Fujishima, J. Photoch. Photobio. C 13 (2012) 169-189.
- [9] K. Hashimoto, H. Irie, A. Fujishima, Jpn. J. Appl. Phys. 44 (2005) 8269-8285.
- [10] B. Ohtani, Phys. Chem. Chem. Phys. 16 (2014) 1788-1797.
- [11] S.O. Hay, T. Obee, Z. Luo, T. Jiang, Y.T. Meng, J.K. He, S.C. Murphy, S. Suib, Molecules 20 (2015) 1319-1356.
- [12] P. Pichat, J. Adv. Oxid. Technol. 13 (2010) 238-246.
- [13] A. Pottier, C. Chaneac, E. Tronc, L. Mazerolles, J.P. Jolivet, J. Mater. Chem. 11 (2001) 1116-1121.
- [14] A. Fujishima, X.T. Zhang, D.A. Tryk, Surf. Sci. Rep. 63 (2008) 515-582.
- [15] T. Zubkov, D. Stahl, T.L. Thompson, D. Panayotov, O. Diwald, J.T. Yates, Jr., J. Phys. Chem. B 109 (2005) 15454-15462.
- [16] M. Takeuchi, K. Sakamoto, G. Martra, S. Coluccia, M. Anpo, J. Phys. Chem. B 109 (2005) 15422-15428.
- [17] N.S. Allen, M. Edge, G. Sandoval, J. Verran, J. Stratton, J. Maltby, Photochem. Photobiol. 81 (2005) 279-290.
- [18] A. Folli, C. Pade, T.B. Hansen, T. De Marco, D.E. Macphee, Cement Concrete Res. 42 (2012) 539-548.
- [19] T. Matsunaga, R. Tomoda, T. Nakajima, H. Wake, FEMS Microbiol. Lett. 29 (1985) 211-214.
- [20] C. Guillard, T.H. Bui, C. Felix, V. Moules, B. Lina, P. Lejeune, Cr. Chim. 11 (2008) 107-113.
- [21] J. Zhao, X.D. Yang, Build. Environ. 38 (2003) 645-654.

- [22] A. Markowska-Szczupak, K. Ulfig, W.A. Morawski, *Catal. Today* 161 (2011) 249-257.
- [23] M. Bekbolet, C.V. Araz, *Chemosphere* 32 (1996) 959-965.
- [24] J.A. Byrne, P.S.M. Dunlop, J.W.J. Hamilton, P. Fernandez-Ibanez, I. Polo-Lopez, P.K. Sharma, A.S.M. Vennard, *Molecules* 20 (2015) 5574-5615.
- [25] D. Mitoraj, A. Janczyk, M. Strus, H. Kisch, G. Stochel, P.B. Heczko, W. Macyk, *Photochem. Photobiol. Sci.* 6 (2007) 642-648.
- [26] H.A. Foster, I.B. Ditta, S. Varghese, A. Steele, *Appl. Microbiol. Biot.* 90 (2011) 1847-1868.
- [27] A.G. Rincon, C. Pulgarin, *Appl. catal. B-Environ.* 44 (2003) 263-284.
- [28] C. Maneerat, Y. Hayata, *Int. J. Food Microbiol.* 107 (2006) 99-103.
- [29] F.N. Chen, X.D. Yang, Q. Wu, *Build. Environ.* 44 (2009) 1088-1093.
- [30] S. Thabet, M. Weiss-Gayet, F. Dappozze, P. Cotton, C. Guillard, *Appl. catal. B-Environ.* 140 (2013) 169-178.
- [31] S. Thabet, F. Simonet, M. Lemaire, C. Guillard, P. Cotton, *Appl. Environ. Microb.* 80 (2014) 7527-7535.
- [32] A. Markowska-Szczupak, K. Janda, K. Wang, W.A. Morawski, E. Kowalska, *Cent. Eur. J. Public Health* (2015) in press.
- [33] P. Dantigny, A. Guilmar, M. Bensoussan, *Int. J. Food Microbiol.* 100 (2005) 187-196.
- [34] B. Ohtani, O.O. Prieto-Mahaney, D. Li, R. Abe, *J. Photoch. Photobiol.* 216 (2010) 179-182.
- [35] B. Ohtani, Y. Azuma, D. Li, T. Ihara, R. Abe, *Trans. Mater. Res. Soc. Jpn.* 32 (2007) 401-404.
- [36] T. Ohno, K. Sarukawa, M. Matsumura, *J. Phys. Chem. B* 105 (2001) 2417-2420.
- [37] O.O. Prieto-Mahaney, N. Murakami, R. Abe, B. Ohtani, *Chem. Lett.* 38 (2009) 238-239.
- [38] K. Wang, Study on photocatalytic activity of anatase-rutile titania particles by isolation of each componen, MSc thesis, (2014) Hokaido University.
- [39] A. Zielińska-Jurek, E. Kowalska, J.W. Sobczak, W. Lisowski, B. Ohtani, A. Zaleska, *Appl. catal. B-Environ.* 101 (2011) 504-514.
- [40] J. Reszczynska, T. Grzyb, J.W. Sobczak, W. Lisowski, M. Gazda, B. Ohtani, A. Zaleska, *Appl. Surf. Sci.* 307 (2014) 333-345.
- [41] A.M. Calvo, R.A. Wilson, J.W. Bok, N.P. Keller, *Microbiol. Mol. Biol. Rev.* 66 (2002) 447-459.
- [42] Y. Paz, A. Heller, *J. Mater. Res.* 12 (1997) 2759-2766.
- [43] M.E. Kurtoglu, T. Longenbach, Y. Gogotsi, *Int. J. Appl. Glass Sci.* 2 (2011) 108-116.
- [44] A. Fujishima, T.N. Rao, *Proc. Indian Acad. Sci. (Chem. Sci.)* 109 (1997) 471-486.
- [45] B. Boumaaza, M. Benkhelifa, M. Belkhouja, *International Journal of Microbiology* 2015 (2015) Article ID 57626.
- [46] G.D. Yang, Z. Jiang, H.H. Shi, M.O. Jones, T.C. Xiao, P.P. Edwards, Z.F. Yan, *Appl. catal. B-Environ.* 96 (2010) 458-465.
- [47] D.C. Hurum, K.A. Gray, T. Rajh, M.C. Thurnauer, *J. Phys. Chem. B* 109 (2005) 977-980.

Graphical abstract