STUDY OF THE ANTIBACTERIAL ACTIVITY OF COMPOSITES KAOLINITE/TiO₂

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Abstract
Titanium dioxide (TiO₂) occurs in three basic modifications anatase, rutile and brookite. Anatase is widely studied due to its very good photocatalytic properties, which may be used in additives to paints and construction materials, for degradation of organic pollutants in air and water, etc. Nanoparticles of TiO₂ show good photocatalytic activity, but may also pose some environmental risks, due to their higher biological activity including potential penetration into cells. When TiO₂ nanoparticles are tightly anchored to a suitable type of matrix (e.g. – kaolinite), they demonstrate photodegradable properties together with lowered environmental risk due to decreased mobility in environmental media. Biological activity of titanium dioxide nanoparticles is known and therefore testing of antibacterial activity of kaolinite/TiO₂ composite was performed. Kaolinite/TiO₂ composites with 20 and 40 wt% of TiO₂ were prepared, dried at 105°C and calcined at 600°C. The calcination caused transformation of kaolinite to metakaolinite and origination of the metakaolinite/TiO₂ composite. Spectroscopic methods revealed titanium dioxide to be present only in form of anatase in all the samples evaluated (non-calcined and calcined) and also transformation of kaolinite to metakaolinite after the calcination treatment. Standard microdilution test was used to determine the antibacterial activity using four human pathogenic bacterial strains (Staphylococcus aureus, Escherichia coli, Enterococcus faecalis, Pseudomonas aeruginosa). Antibacterial assays found all the KATI samples to have antibacterial potency. The non-calcined composite with 40 wt% of TiO₂ was found to have highest antibacterial activity to all the strains used.

Key words:
kaolinite/TiO₂ composite, antibacterial activity, Raman spectroscopy, FTIR, Staphylococcus aureus, Escherichia coli, Enterococcus faecalis, Pseudomonas aeruginosa

1. INTRODUCTION
Titanium dioxide is known for its chemical stability, photocatalytic characteristics, durability and antimicrobial activity, which could be attributed to its crystal structure [1]. Anatase has stronger antimicrobial and photocatalytic activity than rutile [2, 3, 4]. Titanium dioxide is known for reactive oxygen species (ROS) generation via photoactivation due to UV-light. Titanium dioxide absorbs photons in the UV range with wavelengths less than 388nm and evolves ROS by reacting with water and oxygen adsorbed in the surface of TiO₂ nanoparticles. However, the promising application has some limitations, due to the fact that the UV region occupies only approximately 4% of the full solar spectrum, because 45% of the energy belongs to the visible light [5].
There are also several studies available which observed the ROS production in the absence of photoactivation [6]. Titanium dioxide can promote the decomposition of inorganic and organic compounds which could be used in potential applications in sanitation and sterilization. Materials coated with TiO$_2$ are already using because of their effective antibacterial properties [7].

Enhancement in material research significantly supports application of titanium dioxide in the field of photocatalytic materials for potential applications in civil engineering. Titanium dioxide is widely used to modification building materials because it has been used as a white pigment for many years. The prime applications of titanium dioxide based on photocatalysis in the field of building materials are self-cleaning and self-disinfecting. The advantage of using solar light and rainwater as driving force has opened a new domain for environmentally friendly building materials [8].

The aim of the study was to evaluate antibacterial activity of the prepared kaolinite/TiO$_2$ composite depending on selected laboratory conditions inducing photocatalysis.

2. MATERIALS AND METHODS

2.1. Studied kaolinite/TiO$_2$ nanocomposites

Titanyl sulphate TiOSO$_4$ (Precheza a.s., Czech Republic) in form of colloid suspension containing 102 g of TiO$_2$ per 1 dm$^3$ of TiOSO$_4$ served as TiO$_2$ precursor for the preparation of the composites. Nanoparticles of TiO$_2$ were anchored to kaolin matrix (SAK47 - LB Minerals s.r.o.).

The obtained composites were denoted as KATI12, KATI14, KATI62 and KATI64 respectively, where the first number means that the composite was dried at 105°C or calcined at 600°C and the second number denotes the amount of TiO$_2$ in the final composite (20 or 40wt%).

The initial kaolinite was mixed with titanyl sulphate water solution and stirred for 30 minutes. Distilled water was then added to the mixture in 1:1 volume ratio to titanyl sulphate solution. Afterwards, the temperature of the dispersion was raised to 100°C and kept for 60 minutes. The dispersion was left to cool down to the laboratory temperature and filtrated using the Büchner funnel. The solid content separated from the dispersion was rinsed with distilled water and filtrated until the conductivity of the filtrate was below 3 μS. The solid content was dried at 105°C and calcined at 600°C. Thus prepared samples were used for further characterization and assessment.

2.2. Microscopic and Phase Analysis

Scanning electron microscope Quanta FEG 450 (FEI) with EDS analysis APOLLO X (EDAX) was used as a microscopic method for characterization of morphology and elemental composition of the studied samples. Raman and FTIR spectroscopic techniques were used for qualitative characterization and phase analysis of all samples. Raman spectra were recorded on Smart Raman Microscopy System XploRA™ (Horiba Jobin Yvon, France). Laser 532 nm and grating 1200 gr/mm were used. ATR technique with diamond crystal on Spectrometer Nicolet 6700 FT-IR (Thermo Nicolet, USA) was used for obtaining the mid-FTIR spectra.

2.3. Antibacterial Assessment

Four different bacterial strains were used for the testing of antibacterial activity. Glucose broth (HiMedia) was used as a growth media for the purpose of antibacterial assay of the KATI composites. Turbidity of the inoculums was measured using Densi-La-Meter (LACHEMA). Incubation of bacteria was conducted in Biological thermostat BT 120M at 37°C. The lamp (Hagen) with wide spectral bulb which simulates daylight (Hagen) was used to induce photoactivation of the sample.

The antibacterial activity of the nanocomposite KATI was tested using standard micro dilution method which enables to determine the minimum inhibitory concentration (MIC) of tested substances. Disposable micro
titration plates were used for the testing. Commercial solid blood agar plates for cultivation bacteria without any additional modifications were used. Liquid growth media were prepared by instruction of producer and after this sterilized in an autoclave. Suspension of KATI in growth media was diluted to achieve concentrations 10, 3.3, 1.1, 0.37, 0.12, 0.041, 0.014 mg/L of KATI in growth media. Staphylococcus aureus 3953, Escherichia coli 3954, Enterococcus faecalis 4224 and Pseudomonas aeruginosa 1960 were achieved from the Czech Collection of Microorganisms (Czech Republic). Bacterial inoculums used had a cell concentration of 1.9x10^9 (S.aureus), 1.3x10^9 (E.faecalis, E.coli) and 9.3x10^8 (P.aeruginosa). Each cell at the micro titration plate was inoculated. This plate is called the reaction plate. The lamp with wide spectral bulb was placed 10 cm above the reaction plate to induce a photoactivation and 12 hours irradiation of the plate was applied. After the exposure only living bacterial cells were transferred from the reaction plate to pure growth media using the inoculation hedgehog. These re-inoculated plates were incubated at 37°C for 24h and then the MIC values were determined according to visible growth inhibition [9].

3. RESULTS AND DISCUSSION

Scanning electron microscopy confirmed supposed structure of the KATI nanocomposite and also content of TiO_2 in the prepared composites. The SEM images of the studied samples (Fig. 1 and 2) proved matrix consisting of micro-sized kaolinite particles having layered structure with sub-micron particles of TiO_2 attached onto the surface of the clay matrix.

![Fig.1 SEM images of KATI12 (A) and KATI62 (B)](image1)

![Fig.2 SEM images of KATI14 (A) and KATI64 (B) samples](image2)

Raman spectra of the non-calcined KATI 12 and KATI 14 samples (Fig. 3A) clearly show one main band of anatase at positions 153 and 157 cm\(^{-1}\) respectively [10]. Other three characteristic bands had very low intensities barely visible due to noise of fluorescence background of clay matrix [11]. All characteristic bands for anatase are clearly visible in the spectra of the calcined KATI 62 and KATI 64 samples (Fig. 4A) at positions 147, 398, 520, and 641 cm\(^{-1}\) [10]. Thus, we can assume that calcination at temperature 600°C did not cause transformation of anatase to rutile.

Contrary to the Raman spectra the FTIR spectra provided qualitative information about the kaolinite structure, but less significant information about TiO_2 due to its very broad and weak bands [12]. The
characteristic bands for kaolinite are detectable in the spectra of KATI 12 and KATI 14 samples (Fig. 3B). The bands with maxima at 3691, 3651, 3619, and 910 cm\(^{-1}\) correspond to the vibration of inner and outer structural hydroxyl groups in Al-OH, bands at 1113, 1029, 1006, 787, and 460 cm\(^{-1}\) belong to Si-O vibrations, and bands at 749, 688, and 526 cm\(^{-1}\) correspond Si-O-Al vibrations. The broad bands at higher wavenumbers (~3000 cm\(^{-1}\)) and at 1635 cm\(^{-1}\) belong to vibrations of O-H bond in adsorbed water [12]. The disappearance of the bands in the region between 3700-3500 cm\(^{-1}\) is clearly evident and this is the evidence of the transformation of kaolinite to metakaolinite [13]. The broad band at 1050 cm\(^{-1}\) corresponds to the amorphous SiO\(_2\) [12].

Fig. 3: Raman (A) and mid-FTIR (B) spectra of the KATI sample

Fig. 5: Minimum inhibitory concentrations (MIC) for the KATI samples and four bacterial strains used
Antibacterial activity expressed as the MIC values of the KATI samples was evaluated using four bacterial strains and the experimental results achieved are presented in Fig. 5. The highest antibacterial activity was obtained for the KATI14 sample. The determined MIC values of 3.3 mg/L for S. aureus and E. coli, and 10 mg/L for E. faecalis and P. aeruginosa confirmed best potential of the KATI14 in term of growth inhibition of all bacterial strains used. The other KATI samples caused growth inhibition (10 mg/L) of S. aureus and E. coli only. The MIC values for E. faecalis and P. aeruginosa was not determined, which can be caused by the MIC value being higher than 10 mg/L, and thus above our highest concentration of the composite applied in the growth media. Thus it can be stated that calculation of the samples does not lead to increased antibacterial activity as was observed for photodegradation properties of the same materials by Mamulová-Kutláková K. et al. [14].

4. CONCLUSIONS

Composite kaolinite/TiO₂ (KATI) was prepared using titanyl sulphate as a precursor of TiO₂. Scanning electron microscopy proved the presence of TiO₂ particles and confirmed that nanoparticles of TiO₂ are bound onto the kaolinite surface. Spectroscopic methods revealed titanium dioxide to be present only in form of anatase in all the samples evaluated (non-calcined and calcined) and also transformation of kaolinite to metakaolinite after the calcination treatment. Antibacterial assays using four bacterial strains found the KATI samples to have antibacterial potency. The non-calcined composite with 40 wt% of TiO₂ was found to have higher antibacterial activity to all strains used. Due to the previously confirmed photocatalytic activity of the KATI samples we can assume that the biological effect of the studied samples is also based on photocatalysis and interaction its products with bacterial cells. Our experimental data are in accordance with Gurr et al. [6] who observed that daylight can induce photocatalysis and usage of UV light is not necessary. This finding is important in term of potential applications of these nanocomposites for antibacterial modification of various surfaces.

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