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Study of antimicrobial effect of methylene blue incorporated in ZSM-5 zeolite

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Abstract

A new heterogeneous photocatalyst was prepared by incorporation of methylene blue into ZSM-5 zeolite. This photocatalyst effectively absorbs light in visible range of the spectrum and when irradiated with visible light, it generates reactive oxygen species (ROS) in aqueous environment. Efficiency of the photocatalyst was tested on dechlorination of a model compound 4-chlorophenol and on growth inhibition of Gram-negative bacteria *Escherichia coli*.

Keywords: photocatalysis, zeolite, hydroxyl radical, methylene blue, *Escherichia coli*

Introduction

Pollution of water and wastewater with organic pollutants and harmful bacteria is a serious problem worldwide. Photocatalytic oxidation is an alternative technology for detoxification and disinfection of water and wastewater. When photocatalytic semiconductor powders, such as titanium dioxide (TiO_2) are suspended in water and irradiated with near UV ($\lambda < 385$ nm), $^{\bullet}OH$ radicals are generated. The $^{\bullet}OH$ radical is highly toxic towards microorganisms and very reactive in the oxidation of organic substances (Blake et al., 1999; Blough and Zepp, 1995 and Pera-Titus et al., 2004).

Since the photochemical sterilization of *Escherichia coli* using Pt-TiO_2 was for first time reported (Matsunaga et al., 1984), many photocatalytic disinfection studies using TiO_2 have been carried out, not only to determine the major reaction parameters (TiO_2

concentration, light intensity and pH, etc.), but also to improve understanding of the disinfection mechanism responsible for the inactivation of the microorganism (Wei et al., 1994; Watts et al., 1995; Bahnemann et al., 1997; Kikuchi et al., 1997 and Lee et al., 1997). The biocidal action of the TiO_2 photocatalyst has been frequently ascribed to reactive oxygen species (ROS) (Saito et al., 1992; Ireland et al., 1993; Sjogren and Sierka, 1994; Watts et al., 1995; Bekbolet, 1997; Lee et al., 1997; Maness et al., 1999; Huang et al., 2000 and Melian et al., 2000).

Many chemicals, including natural cell constituents, can absorb light and cause damage to organisms. Some of these compounds are used by organisms to attack or defend against other organisms. This process, also called the "photodynamic action", requires oxygen and damages biological target molecules by photosensitized oxidation. Biochemical effects include enzyme deactivation, nucleic acid oxidation, and membrane damage (Heitz, 1987). All of the botanical photosensitizers discovered to date are effective antimicrobial agents capable of killing prokaryotic and eukaryotic organisms (Downum, 1986).

There is a plenty of synthetic dyes, which have the same function as natural photosensitizers – they can produce ROS, when they are irradiated and molecular oxygen is available. One of them is methylene blue (MB). Methylene blue is a photoactive organic sensitizer with light absorption maximum at λ_{max} = 661 nm (http://www.usca.edu/chemistry/spectra/) producing singlet oxygen $^1O_2$.

As MB freely dissolved in aqueous environment may undergo oxidative degradation by ROS, it is necessary to avoid this effect by anchoring it into zeolite channels and surface. Other reason for incorporation of MB to zeolite is the simplicity of separating and reuse of the catalyst.

The aim of this work is, therefore, to prepare a heterogeneous photocatalyst by incorporating the methylene blue into Na-ZSM-5 zeolite structures. Further aims of this work are to test the photoactivity of the catalyst on dechlorination of a model compound 4-chlorophenol and to test its toxicity on a Gram-negative bacteria *Escherichia coli*.

Materials and Methods

Chemicals

Na-ZSM-5 (ZSM-5, M ≈ 43.6) zeolite was obtained from VURUP a.s. Bratislava, 4-chlorophenol (4-CP, 99+ %) and methylene blue (MB, p.a. purity) were purchased from

Sigma Aldrich. Buffer solution with pH = 8.95 consisted of H_3BO_3 (p.a., Lachema Brno), H_3PO_4 (85 %, Lachema Brno), CH_3COOH (p.a., Lachema Brno), NaOH (98 %, Lachema Brno), and demineralised water. The medium used for the bacteria cultivation was meat-peptone broth (MPB, Nutrient Agare Nr. 2, Biomark Laboratory, India).

Preparation of the catalyst

To prepare the impregnating solution 0.4 g of methylene blue was suspended in 100 cm^3 of demineralised water. 5 g of zeolite Na-ZSM-5 was added to solution and the suspension was stirred by magnetic mixer at T = 15 °C. After 15 hours of stirring, the product was filtrated through a paper filter and rinsed with water to gain a colorless filtrate. Furthermore, it was washed in 100 cm^3 of demineralised water, filtrated and dried in a desiccator.

Spectral characterization of the catalyst

The diffuse reflectance UV/VIS spectrum was measured with Specord M-40 apparatus (Zeiss, Germany) and measured data was transformed to Kubelka-Munk parameters. For measuring the spectrum, $BaSO_4$ was used as standard.

Spectral characterization of irradiation lamps

Spectrum of all used lamps was measured with an optical fibre high resolution UV/VIS spectrometer Ocean Optics HR4000 from a distance of 5 cm. Three lamps were used for irradiation: 40 W tungsten light bulb (Tesla), 150 W tungsten spotlight bulb (Philips R95) and 150 W metal-halogen lamp (OSRAM HLX 64642, ~ 35 Wm^2).

Dechlorination of 4-chlorophenol

Dechlorination reaction was performed in a magnetically stirred 100 cm^3 round glass flask, which contained 100 cm^3 aqueous buffer (pH = 8.95) solution of 4-chlorophenol (c_0 = 10^{-2} $mol.dm^{-3}$) with 75 mg of the photocatalyst. This system was irradiated with a 150 W metal-halogen lamp from a distance of 6 cm at room temperature.

Chloride ions released from the 4-chlorophenol molecules during the reaction were measured with a ion-selective chloride electrode Sentek Cl-ISE equipped with a ion-activity meter MS20 (Laboratorní přístroje Praha).

Toxicity test on selected bacteria

The toxicity tests were performed by a macro-dilution method (Dudová et al. 2001) on Gram-negative bacteria *Escherichia coli* (CCM 3988). The bacterial strain was obtained from Czech Collection of Microorganisms, Masaryk's University, Brno, Czech Republic. The experiments were performed in glass tubes designated for direct spectrophotometric measurements. A stock solution of 4-CP (10^{-1} mol.dm^{-3}) was prepared by dissolving 4-chlorophenol in double-distilled water. Test assays with volume of 5 ml with desired 4-CP concentration were prepared by adding different volumes of 4-CP stock solution to liquid MPB inoculated overnight with 2 % vegetative bacterial inoculum of *E. coli* (2 .10^6 ml^{-1}). Depending on the experiment, the ZSM-5 or ZSM-5-MB was added (4 mg, or where indicated 8 mg per 5 ml assay). Assays were cultivated during 5 h at 37 °C in a biological thermostat equipped with a lateral shaker Gallencamp VL. Assays were irradiated during the cultivation with 40 W or 150 W tungsten lamps from the distance of 10 cm, the control assay without 4-chlorophenol and without catalyst was cultivated in the dark. The toxic effect was studied by measuring the light absorbance at λ = 550 nm using Biochrom Libra S2 colorimeter during and after the cultivation in periodical time intervals.

Growth of bacteria in assays was compared with the control assay and characterized by IC_{50} values. The IC_{50} values were read from toxicity curves.

Results and Discussion

Preparation of the catalyst

The photocatalyst was prepared successfully with 4.5 g yield of blue colored powder.

Spectral characterization of the catalyst. Differential reflectance UV/VIS spectrum was achieved by measuring the diffuse reflectance spectra of Na-ZSM-5-MB and raw Na-ZSM-5 and collected Kubelka-Munk's parameters were subtracted to gain the differential spectra shown in Figure 1.

It can be seen that the prepared heterogeneous photocatalyst effectively absorbs light with $\lambda_{max} \approx 645$ nm (Figure 1). Therefore we may assume that the catalyst may be effectively excited by illumination with visible light ($\lambda > 400$ nm) and following photophysical processes of energy transfer to the present molecular oxygen will lead to formation of various ROS ($^1O_2$, $O_2^{\cdot-}$, $^{\cdot}OH$, H_2O_2) (Konovalova et al., 2004).

Spectral characterization of irradiation lamps

Light emission spectra of used lamps are presented in Figure 2.

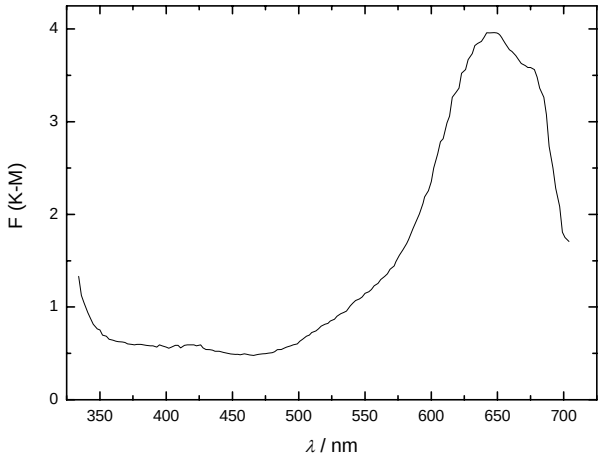

Figure 1. Differential reflectance UV/VIS spectrum of Na-ZSM-5-MB photocatalyst.

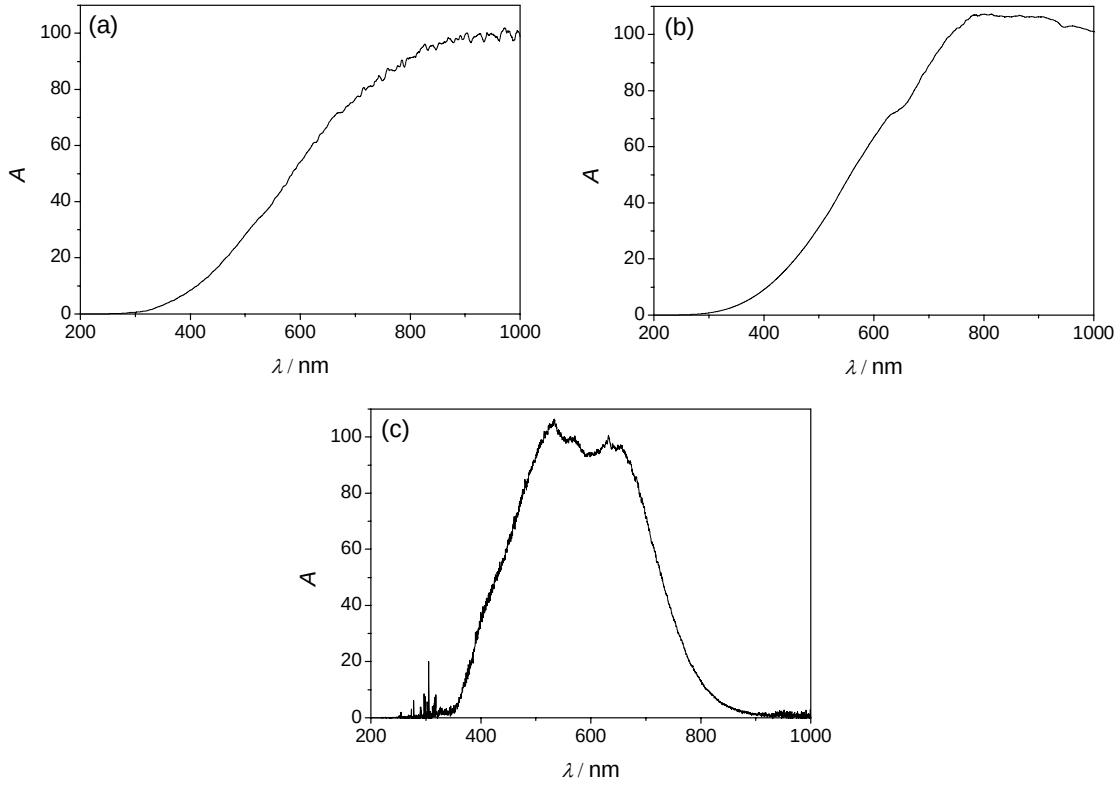

Figure 2. Spectral characteristic of irradiation lamps (A – absorbance, λ - wavelength): (a) 40 W (Tungsten), (b) 150 W (Tungsten), (c) 150 W (Metal-halogen).

All lamps are emitting light in visible region of the spectrum and partially in the UV region. Therefore they were considered as suitable for our experiments.

Dechlorination of 4-chlorophenol

During the experiment, 6 cm^3 samples were withdrawn from the reaction flask in certain intervals (every 1 hour) and immediately measured with the ion-selective electrode. Figure 3 depicts the chloride ion concentration increase during the irradiation.

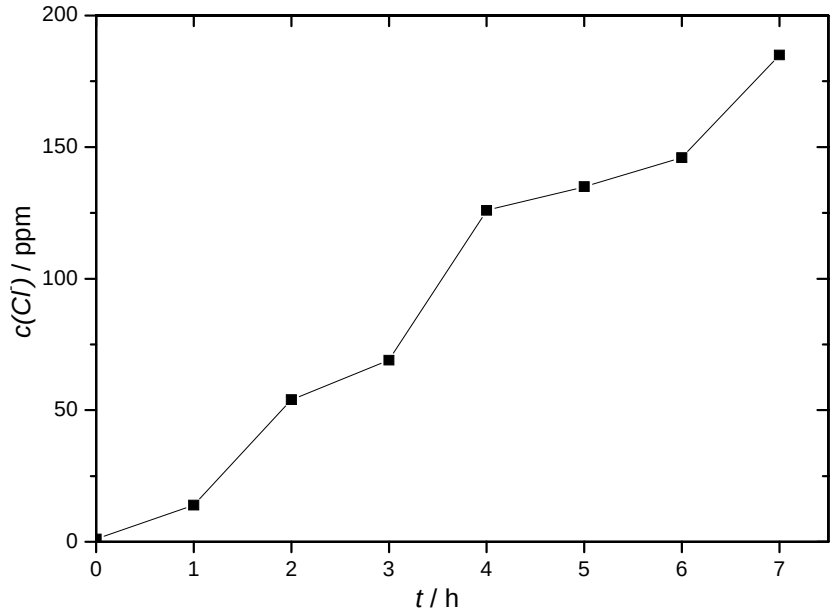

Figure 3. Chloride ion concentration change in time of irradiation.

From the Figure 3 it is clearly visible that chloride concentration increased during the irradiation time. This effect is caused by oxidative dechlorination of 4-chlorophenol by ROS produced by irradiated photocatalyst. These ROS can be easily detected by the EPR spectroscopy (not documented) and can be utilized for inactivation of harmful bacteria.

Toxicity test on selected bacteria

The IC_{50} value of 4-CP, 4-CP with ZSM-5-MB in dark conditions and 4-CP with ZSM-5-MB irradiated with 40 W lamp for *E. coli* were experimentally determined by macro-dilution method (Figure 4). From this Figure we may assume that addition of the catalyst decreases the toxicity of 4-chlorophenol.

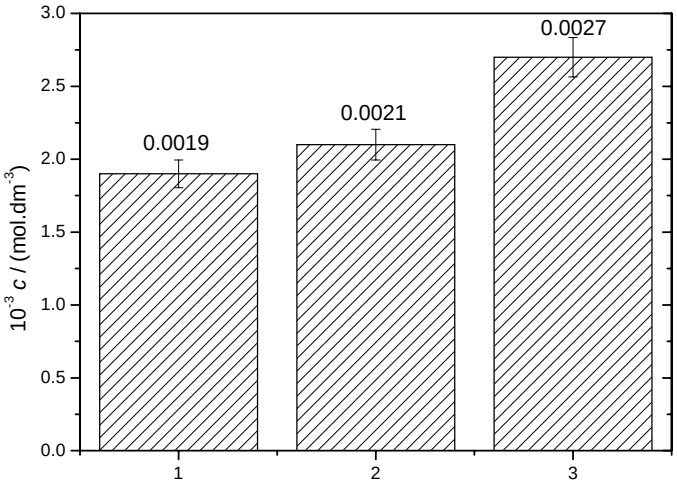

Figure 4. IC_{50} values of selected tested systems: (1) 4-CP, (2) 4-CP with ZSM-5-MB in dark, and (3) 4-CP with ZSM-5-MB irradiated with 40 W light.

Results of experiments with unmodified and modified zeolite photocatalysts on *E. coli* are presented below.

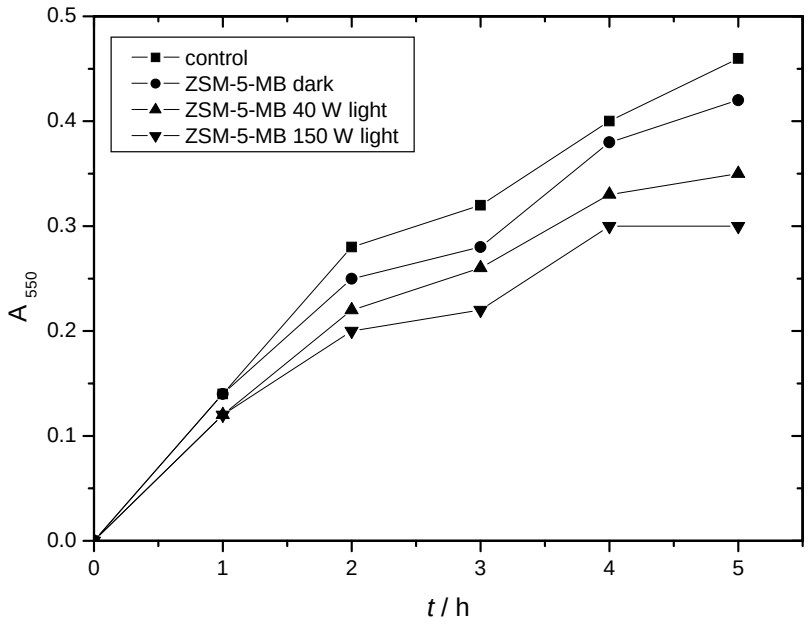

Figure 5. The growth of *E. coli* (A_{550}) in the presence of: (■) control – *E.coli* (100 % growth), (●) ZSM-5-MB in dark conditions (92 % growth), (▲) ZSM-5-MB irradiated with 40 W light (76 % growth) and (▼) ZSM-5-MB irradiated with 150 W light (65 % growth).

Figure 5 presents growth curves of *E. coli* in presence of modified ZSM-5-MB without the presence of 4-chlorophenol. From these growth curves it may be assumed, that production of ROS increases with increasing light intensity and therefore the *E. coli* culture is growing slower. Similar effect can be observed when comparing the effect of modified zeolite ZSM-5-MB and original unmodified ZSM-5 zeolite, both either in dark or irradiated with 40 W light (Figure 6).

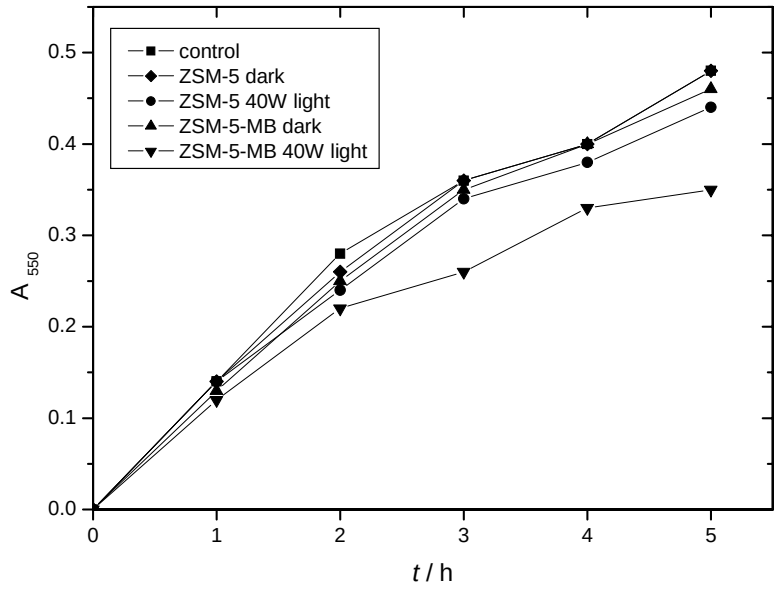

Figure 6. The growth of *E. coli* (A_{550}) in the presence of: (■) control, (♦) ZSM-5 in dark (100 % growth), (●) ZSM-5 irradiated with 40 W light (76 % growth), (▲)ZSM-5-MB in dark (90 % growth), (▼) ZSM-5-MB irradiated with 40 W light (57 % growth).

From Figure 6 we may assume that addition of both zeolites in the dark cause almost no growth inhibition (100 % growth for ZSM-5 and 90 % growth for ZSM-5-MB). Despite the very low photoactivity of ZSM-5 zeolite, irradiation of ZSM-5 with 40 W light causes decrease in absorbance of samples which can be explained as decreased *E. coli* growth (76 % growth) caused by attack of ROS. Obviously, modified catalyst ZSM-5-MB decreases the growth even more (57 %), because of enhanced ROS production.

Figure 7 presents more complex experiment where certain amount of 4-chlorophenol was added into every sample (c_0 (4-CP) = 1 $.10^{-2}$ mol.dm^{-3}). The shape and position of growth curves in Figure 7 indicates that the highest growth inhibition was observed in presence of 4-CP (26 % growth) and the growth rate slightly increases with addition of the catalyst (33 % growth). This is most probably caused by adsorption of 4-CP on catalyst surface.

Furthermore, when the samples were irradiated with light, the bacteria grew even faster. Increase of the light source intensity from 40 W to 150 W led to increase in *E. coli* growth (37 % at 40 W and 44 % at 150 W).

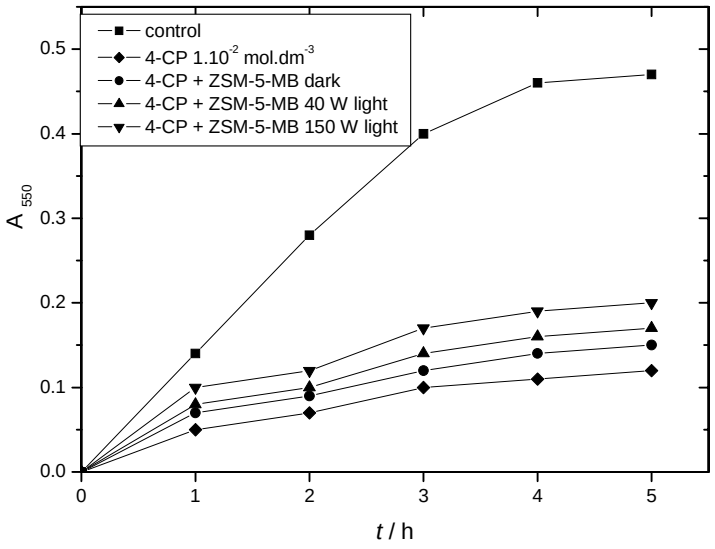

Figure 7. The growth of *E. coli* (A_{550}) in the presence of: (■) control, (♦) pure 4-CP with initial concentration of 10^{-2} mol.dm^{-3} (26 % growth), (●) ZSM-5-MB in dark (33 % growth), (▲) ZSM-5-MB irradiated with 40 W light (37 % growth) and (▼) ZSM-5-MB irradiated with 150 W light (44 % growth).

Several experiments were carried out to show the influence of different initial 4-CP concentrations and the influence of doubled load of the photocatalyst in the system (8 mg per 5 ml of medium). Different light sources and different initial 4-CP concentrations were used: 40 W light and 10^{-2} mol.dm^{-3} of 4-CP in Figure 8, and 150 W light and 5 .10^{-3} mol.dm^{-3} of 4-CP in Figure 9.

Figure 8 indicates that the toxic effect of 4-chlorophenol (28 % growth) is slightly suppressed by addition of the catalyst ZSM-5 and irradiating it with 40 W light (31 % growth). Irradiated ZSM-5-MB catalyst decreases the toxicity of 4-CP even more (37 % growth) than unmodified ZSM-5 and double load of ZSM-5-MB reduced the toxicity to 57 % growth.

Lower initial concentration of 4-CP causes slightly lower growth inhibition of *E. coli* (35 % growth, Figure 9) and in combination with stronger light source (150 W) there is more significant, that growth inhibition of 4-CP is lower due to 4-CP decomposition by ROS produced by ZSM-5-MB (57 % growth). Double load of ZSM-5-MB causes even faster growth (76 %) of *E. coli* bacteria in samples.

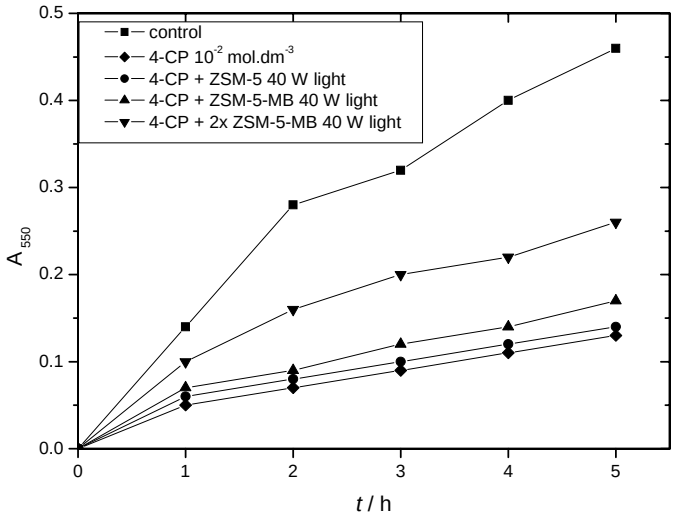

Figure 8. The growth of *E. coli* (A_{550}) in the presence of: (■) control, (♦) 4-CP with initial concentration 10^{-2} mol.dm^{-3} (28 % growth), (●) ZSM-5 with 4-CP irradiated with 40 W light (31 % growth), (▲) modified ZSM-5-MB with 4-CP irradiated with 40 W light (37 % growth) and (▼) double load of ZSM-5-MB with 4-CP irradiated with 40 W light (8 mg per 5 ml of medium, 57 % growth).

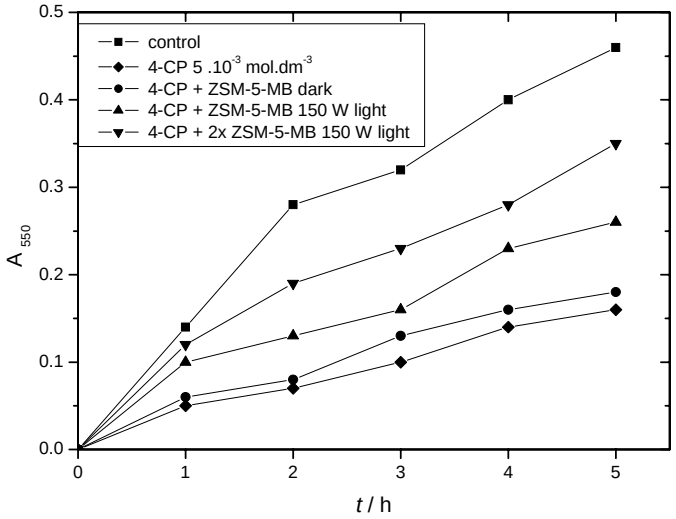

Figure 9. The growth of *E. coli* (A_{550}) in the presence of: (■) control, (♦) pure 4-CP with initial concentration 5 .10^{-3} mol.dm^{-3} (35 % growth), (●) ZSM-5-MB with 4-CP in the dark (39 % growth), (▲) ZSM-5-MB with 4-CP irradiated with 150 W light (57 % growth) and (▼) double load of ZSM-5-MB with 4-CP irradiated with 150 W light (8 mg per 5 ml of medium, 76 % growth).

Addition of ZSM-5-MB in dark influenced the bacteria growth only slightly (39 % growth) probably by adsorption of 4-CP from the solution.

Conclusion

The goal of this work was to prepare a new heterogeneous photocatalyst, which by photoactivation with visible light effectively produces reactive oxygen species (ROS), utilizable in photodegradation of environmental pollutants and inactivation of harmful microorganisms. The synthesized system effectively absorbs light in the visible region of the spectra with $\lambda_{max} \approx 645$ nm.

Chloride ion formation from photodegradation of 4-chlorophenol proved that formation of ROS was effective. Furthermore, the effective formation of ROS was proved on "photokilling" of *E. coli* bacteria.

Based on obtained results it may be concluded that in the dark phase of experiments without 4-chlorophenol addition almost no toxic effect of the ZSM-5 was observed. Addition of ZSM-5-MB catalyst caused very low inhibition. Irradiation of both added catalysts caused significant growth inhibition of *E. coli*.

Addition of toxic 4-chlorophenol suppressed the growth of *E. coli*, but addition of the catalyst decreased the toxicity of 4-CP by adsorbing some of it from the solution. Irradiation of added catalyst caused production of ROS which predominantly attacked 4-CP molecules and thus the toxic effects of 4-CP on *E. coli* were suppressed.

Therefore we may conclude that this catalyst may be suitable for disinfection of water with low organic matter content (e.g., potable water) but in the presence of photodegradable organics, the disinfection effect may be impaired.

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