Toxicity removal by *Daphinia similis* assay in BTEX contaminated groundwater using nanometric TiO_2/ZrO_2 film and black light

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Toxicity removal by *Daphnia similis* assay in BTEX contaminated groundwater using nanometric TiO_2/ZrO_2 film and black light

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Abstract. In this work, a photocatalytic tubular reactor, with a nanometric TiO_2/ ZrO_2 film, immobilized on glass substrate, was developed in order to investigate the degradation of volatile organic compounds, such as, benzene, toluene, ethylbenzene and xylene (BTEX) in groundwater sample, under black light irradiation (365nm). The BTEX compound were determined by solid phase microextraction (SPME) technique and analyzed by Gas Chromatography - Ionization Flame Detection (CG -FID). The efficiency of photocatalytic process was evaluated by determination of BTEX removal and toxicity, using *Daphnia similis* as organism-test. The influence of the initial concentration of BTEX in the degradation process was investigated. The degradation process of sample groundwater contaminated with BTEX (145µg/L) from gasoline, resulted in 90% of efficiency after 120 minutes of treatment. The toxicity (EC50-48 h) of the groundwater sample to *Daphnia similis* was 14.1% (10.5-19.0) for untreated samples. After 30 min of treatment, the EC50-48 h was of 84.3 (71-100%). The benzene efficiency removal was reduced in function of the increase of its initial concentrations that observation was not found for the other compounds studied.

1.Introduction

BTEX (benzene, ethylbenzene toluene and xylenes) are Volatile Organic Compounds (VOCs) of naturally-occurring chemicals found mainly in petroleum [1]. These compounds are found in the environment due to accidental gasoline released from underground storage tanks and pipelines [2, 3]. In Brazil, BTEX environment contamination is extremely worrying due to the solubility of these compounds in water that is increased in the presence of methanol (27 %) in gasoline [4, 5]. Due to their toxicity, BTEX is included by The Agency for Toxic Substances and Disease Registry's list of priority pollutants [2, 6].

Biodegradation, by conventional process, is usually applied for BTEX removal from aquifers and soil; however, these processes may take long time for this removal [7-9]. The advanced oxidation process (AOP) is defined as an oxidation process, in which hydroxyl radical are generated [10-13]. Heterogeneous photocatalysis, using titanium dioxide (TiO_2) as

catalyst, is quite known. [14-17]. One of the problems with this type of photoreaction, is the low efficiency of the light penetration, when the catalyst is used in suspension, but this problem can be resolved by using the TiO_2 films applied on different types of substrates, such as glass [18,19]. TiO_2 is considered the most important material used in the heterogeneous photocatalysis to remove organic compounds from aquatic environment [20]. Studies on binaries metallic oxides as TiO_2/ZrO_2 showed an increase in its photocatalytic effect [21-23,24, 25]. The sol-gel process is appropriated to prepare thin oxide coating, due to several advantages, including excellent photocatalytic properties, homogeneity and large coating area [26].

Heterogeneous photocatalysis (TiO_2) and photo Fenton (Fe^{+2} /H_2O_2), under solar irradiations have been applied in the BTEX treatment [27]. Dezhi et al. [28] applied heterogeneous photocatalysis using TiO_2 doped with Fe in the BTEX degradation [28]. Crittenden et al. (1996) studied the degradation of BTEX in a tubular reactor with Ni doped with Pt immobilized in silica gel substrate [29-30].

In this work, a photocatalytic tubular reactor with internal glass cylinders tubes was developed in order to investigate the degradation of volatile organic compounds, such as, benzene, toluene, ethylbenzene and xylene (BTEX) from gasoline in groundwater sample. The nanometric TiO_2/ZrO_2 film was prepared by sol-gel procedure, characterized and immobilized on glass cylinders tubes. The degradation of BTEX compounds and toxicity (using *Daphnia similis*) were evaluated.

2. Methods

2.1. TiO_2/ZrO_2 photoreactor

The schematic diagram of the experimental setup is shown in Fig. 1. Two identical reactors and a feed reservoir (1.175 L) are connected. Each photoreactor had 37 cm in length and 9 cm diameter and an useful volume of 1.325 L. The black lamp, wavelength of 365 nm and power of 15 W (Sylvania F15W/BL) were inserted in the center of the photoreactor. The substrate for the photocatalytic reactor loading consisted in an apparatus with 30 quartz tubes of 4 mm external diameter and 2 mm of internal diameter, supported in two Teflon discs fixed to the lamps extremity and to the reactor. The catalyst film of TiO_2/ZrO_2 was immobilized in the quartz tubes surfaces (Fig. 2).

The oxygen flow (10 mg/L) from the inner part of the reservoir was maintained during all the process. The oxygen flow was constantly controlled by a flux-meter (model AALBORG) and the oxygen dissolved in the water was monitored continuously with an oxygen-meter.

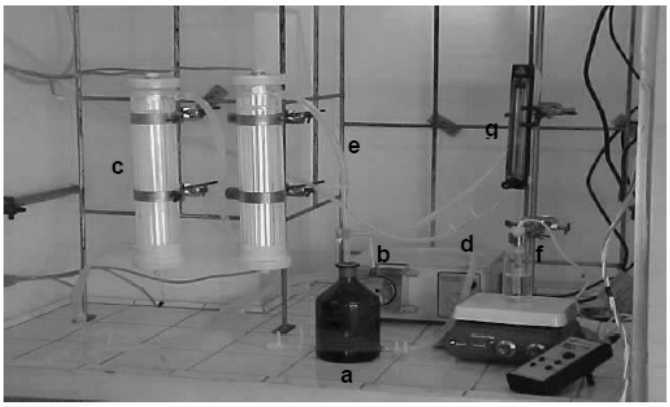

Figure 1. Detailed schematic diagram of the experimental setup: a) feed reservoir, b) glass tap, c) photochemical reactor, d) peristaltic pump, e) silicone tube, f) dissolved oxygen-meter, g) air flux-meter.

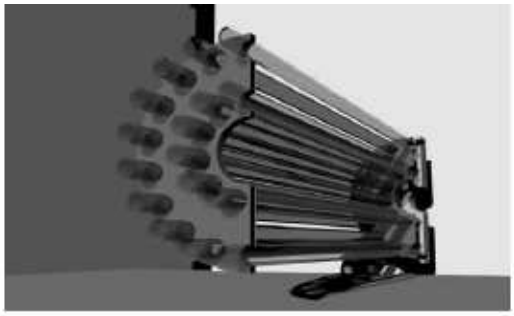

Figure 2. Photocatalytic reactor in transversal and with internal views.

2.2. Nanometric thin film preparation

Sol-gels of titanium and zirconium were prepared according Zorn et al. [21]. The glass tubes were capping with the mixture of sols of TiO_2 and ZrO_2 in a 7:3 ratio (88% and 12% (m/m)), respectively, and dried at 100ºC. After the deposition of 15 film layers on the substrate, the quartz tubes were calcined at 450ºC for 1 h. The TiO_2 film was developed in agreement of and US Patent and other researchers [21, 25, 31, 32].The films were characterized by Scanning Electronic Microscopy (SEM) (Jeol JSM-6360LV microscopy) and Transmission Electronic Microscopy (TEM) (Zeiss CEM-902),

2.3.Photocatalytic activity measurements

In order to evaluate the nanometric TiO_2/ZrO_2 photocatalytic reactor efficiency in the degradation of BTEX, samples were prepared *in situ*, using groundwater (from artesian well) and gasoline. The gasoline and water were added in a beaker of 4 L, under magnetic stirring for 90 min, and submitted to the photodegradation process. The sample were characterized by determination of pH (from Weilheim, model WTW82362, with glass combined electrodes), by total organic carbon (TOC 5000A, Shimadzu) and BTEX concentration.

The feed flow rate was adjusted to 280 mL/min. Samples were collected after 30, 60, 90 and 120 min of the treatment and the concentration of BTEX were determined. The concentration of oxygen dissolved was maintained at 10 mg/L during the experiment.

2.4. BTEX quantification

The analyses were performed by using a gas chromatograph (Autosystem XL,Perkin Elmer), splitless injection mode at inlet temperature of 230ºC and FID detector. The helium (high purity) was used as carrier gas at 1 mL min-1 flow rate. The separation of compounds were performed by HP-5 (30 m x 0.32 mm x 0.25 m) column with temperature programmed as follows: the initial oven temperature was set at 40ºC for 2 min, increased to 110ºC at 7ºC/min then ramped at 20ºC/min to 130ºC (hold 2 min). The total run was of 15 min. The retention times of 2.2, 3.5, 5.4 and 5.6 were attributed to benzene, toluene, ethylbenzene and xylene respectively. For the extraction, 3 mL of the BTEX solution (variable concentrations) were added to the reaction vials of 7 mL with silicon septum and maintained under magnetic agitation at 1200 rpm for 5 min to form the headspace. After that, fiber exposed to headspace and maintained for 5 min in equilibrium, being posterior injected to chromatograph for the measurements.

The analytical curves were constructed in the range 1 – 50 µg/L for benzene, 50 -1000 µg/ L for toluene and 1-10 mg/L for ethylbenzene and xylene. All the samples were analyzed in triplicate and the average value were calculated by the standard deviation (SD) (not shown). The typical chromatogram is showed in Fig. 3.

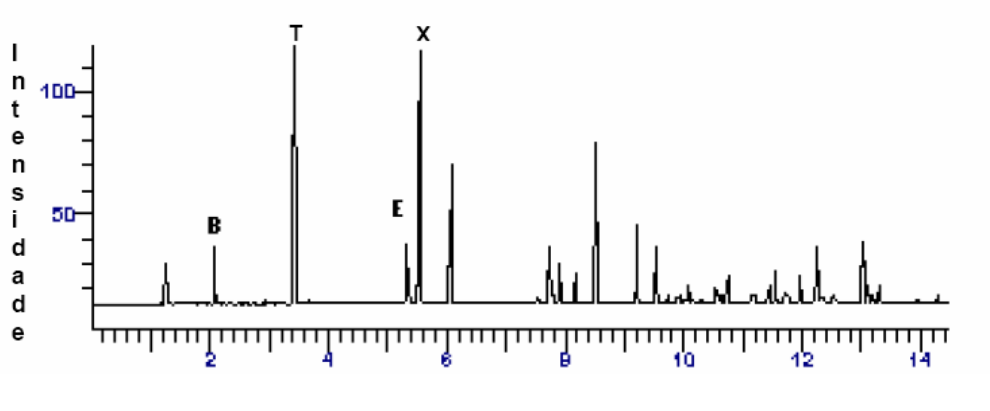

Figure 3. Chromatogram obtained for the extraction through headspace of a sample of water contaminated with gasoline, where B: Benzene, T: Toluene, E: Ethylbenzene and X: Xylenes, by FID-GC.

2.5. *Toxicity assay using Daphnia similis*

This assay was carried out at the Ecotoxicology Laboratory at EMBRAPA, Jaguariúna, Campinas, S.P., Brazil, following a published methodology [33]. The age of test organism was approximately 24 h. The culture and exposure media were prepared according Jonsson and Maia [34].

In the toxicity assay with *Daphnia similis* a preliminary test containing contaminated water without treatment and for the sample irradiated at 30, 90 and 120 min of treatment to calculate the mortality of the organisms was carried out. Doses 15, 30 and 50% of the problem-samples were used for the intervals of times.

The immobility of the organisms towards different concentrations of the chemical were subjected to Probit analysis in order to determine the median effective concentration EC50-48h and its confidence interval 95%. It was performed using the Statgraphics Plus Version 5.1 software [35, 36].

3.RESULTS AND DISCUSSION

3.1.Characterization of TiO_2/ZrO_2 film

TiO_2/ZrO_2 film was prepared by the sol-gel method since this material let to form a thin film with high adherence in a solid matrix surface. This material after many experiments the film was still strongly adhered to the surface, being this an excellent vantage for the use as catalyst for long periods. The thin film led to an excellent UV light penetration through the external wall of the glass tube.

3.2.*Scanning electronic microscopy (SEM)*

The morphology of the obtained materials, covered 15 folds with TiO_2/ZrO_2 sol it was investigated by SEM coupled to microprobe of dispersive energy (EDS) at 7 kV. The Fig. 4 shows the picks of the elements of our interest as titanium, and zirconium and the SEM microscopy (inserted) of the sample.

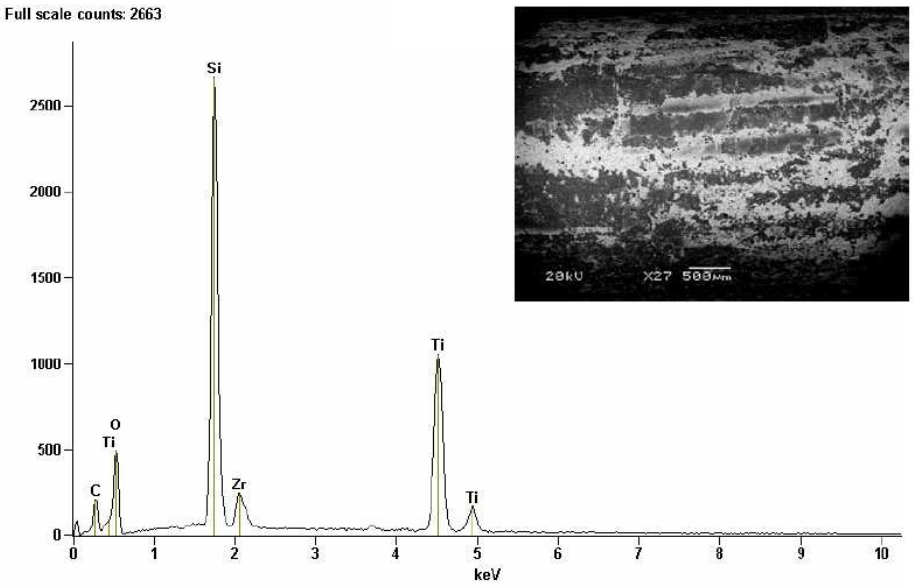

Figure 4. Micrography obtained by SEM/EDS for the sample covered with 15 folds of films of the titanium/zirconium oxides.

Three different regions were analyzed inside the same sample and showed the presence of Ti and Zr. The average concentration (in weight) between them was 24.2% and 3.7%, for Ti and Zr, respectively, in a 6.5:3.5 ratio that corroborate the data previously obtained by Zorn et al. [21].It was observed also carbon from the carbon ribbon used to fix the sample and iron and silica from brass support and quartz tubes, respectively.

The images showed also that were not homogeneous surface, since the procedure was only possible to follow the *dip-coating* manually and not automatic as should be desirable, due to the size of the sample (30 quartz tubes of 33 cm length).

3.3.*Transmission electronic microscopy (TEM)*

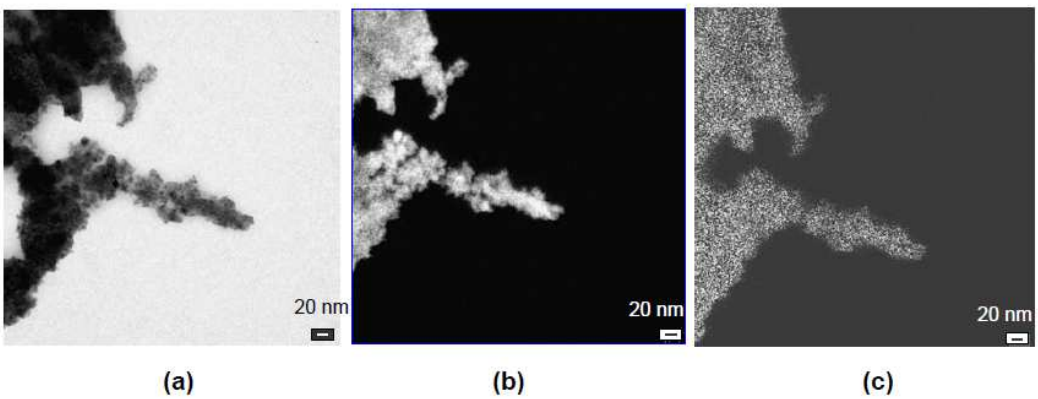

Figure 5. Elementary images obtained in bright-field (a), for Ti and (b) for Zr (c), where the trace indicate 20 nm.

The images are exhibited in Fig. 5, showing the presence of Ti and Zr and the Ti domain region are superimposed in a defined form to the bright-filed image, however the Zr was more expanded in relation to the domain of the crystals. This is probably due to that Zr was no totally incorporated to the crystal structure. Other groups reported similar structures and have shown that doped TiO_2 supports were better catalyst than the TiO_2 alone [37, 23].

The intensity of the black light in the reactor was measured with a radiometer and showed that an exponential decrease of the radiation intensity in function of the increase of the distance between the lamp and reactor surface, reaching values near to 2 Mw/cm^2 at 30 mm of distance. Taking the first 5 first points, from the most near to the lamp and close to the reactor wall showed an average value of 3.6 Mw/cm^2 [27, 29]. Cho et al. [27] and Crittenden et al. [29] in their experiments with sun light of heterogeneous photocatalysis the intensities measured were of 1.4 and 1.7 Mw/cm^2, respectively.

3.4. *Photocatalytic degradation of BTEX groundwater samples*

The characteristics of groundwater samples were showed in table 1.

Table1. Characteristics of groundwater samples

Characteristics	Groundwater sample 1	Groundwater sample 2
pH	7.0	7.2
[benzene] (µg/L)	22.6	93.3
[toluene] (µg/L)	31.8	1738.3
[ethylbenzene] (µg/L)	51.5	315.5
[xylene] (µg/L)	39.5	1546.4
[BTEX] (µg/L)	145.4	3693.5
TOC (mg/ L)	99.2	103.2

The dissolved oxygen was monitored in each groundwater sample and it was maintained around 6-10 mg/L during all the experiment. For the groundwater sample 1, the efficiency removal after 30 minutes of treatment was 70%, for all compounds studied, and after 120 min, the efficiency removal was around 90%. The initial pH of the water before the photocatalytic system was 7.0, decreasing to 5.8 after 120 min of treatment. The pH decreasing can be attributed to the CO_2 formation in the treated water in function of the compounds mineralization.

For the groundwater 2, the efficiency removal obtained by the photocatalytic treatment for toluene, ethylbenzene and xylene was of 80% after 120 min, while benzene removal was around 50% in the same period of treatment. Results reported by Silva et al. [38] for BTEX biodegradation showed that benzene removal is less efficient in comparison with other BTEX compounds. Comparing the results obtained from both groundwater samples, the benzene efficiency removal, was reduced in function of the increase of its initial concentrations, that observation was not found for the other compounds studied.

3.5. *Toxicity assay using Daphnia similis*

It was observed that in the untreated water 100% died and with 30 min of treatment had a 90% survival and with 90 and 100 min a 100% survival.

In function of these results it was calculated de EC50-48 h with and without photocatalytic treatment with 30 min. The values of the EC50-48 h for untreated groundwater sample 1 (145.4µg/L BTEX) was 14.1 (10.5-19.0) while for the treated sample (30 minutes) the value obtained was 84.3 (71-100%), indicating significative toxicity reduction.

However, the BTEX concentration was low (145.6 µg/ L) in the contaminated water with gasoline, it presented toxicity to *Daphnia similis* till 30 min of treatment. This is probably due

to the benzene concentration was 22.6 μg /L, or 4 folds over the permitted concentration by the legislation (5 μg/ L) and decreased 70% after 30 min treatment.

4. Conclusion

The photocatalytic reactor developed in this study was effective in the BTEX treatment from groundwater sample. The degradation of BTEX achieved 90 % of efficient after 120 minutes of treatment. The benzene removal efficiency was reduced in function of the increase of its initial concentrations, and this fact was not found for the other compounds studied. The toxicity was removed after 30 min of treatment with a EC50-48 h of 84.3 (71-100%).

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