Journal of Molecular Catalysis A: Chemical 333 (2010) 128–135

Contents lists available at ScienceDirect

Journal of Molecular Catalysis A: Chemical

journal homepage: www.elsevier.com/locate/molcata

Gas-phase photocatalytic degradation and detoxification of *o*-toluidine: Degradation mechanism and Salmonella mutagenicity assessment of mixed gaseous intermediates

Taicheng An[a,*], Lei Sun[a,b], Guiying Li[a,*], Shungang Wan[a,b]

[a] State Key Laboratory of Organic Geochemistry and Guangdong Key Laboratory of Environmental Resources Utilization and Protection, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou 510640, China
[b] Graduate School of Chinese Academy of Sciences, Beijing 100049, China

ARTICLE INFO

Article history:
Received 23 March 2010
Received in revised form 4 October 2010
Accepted 7 October 2010
Available online 15 October 2010

Keywords:
o-Toluidine
Photocatalytic oxidation
Degradation mechanism
Mutagenicity
Ames assay

ABSTRACT

The photocatalytic degradation of toluidine over titanium oxide (TiO_2) thin films under UV irradiation was investigated. The degradation efficiency of 98.7% was obtained for a toluidine concentration of about 4500 $\mu g\,L^{-1}$ and illumination of 240 min. The degradation intermediates produced during photocatalytic oxidation were identified using Fourier transform-infrared spectrometry (FTIR) and gas chromatography–mass spectrometry (GC–MS). Only a small amount of intermediates, including phenol and toluene, were found in the gas phase. Many other trace amount intermediates, such as 2-hydroxybenzaldehyde, 2-nitrobenzaldehyde, 2-hydroxybenzenemethanol, 2-hydroxybenzoic acid, phenol etc., were detected on the TiO_2 surface. An Ames assay of the *Salmonella typhimurium* strains TA98 and TA100 was employed to evaluate the mutagenicity of toluidine and its gaseous photocatalytic degradation intermediates. With or without rat liver microsomal fraction (S9 mix) activation, neither toluidine nor its gaseous intermediates presented mutagenic activity against strains TA98 (±S9) and TA100 (−S9) at all tested doses. Toluidine, however, can induce a weak positive response to the TA100 strain with an S9 mix at doses as high as 4000 $\mu g\,plate^{-1}$. An increase of revertants per plate was obtained after 30 min photocatalysis in the TA100 strain with S9 mix. As reaction time further increased, photocatalytic technology exhibited the ability to completely and efficiently detoxify toluidine. Both our chemical analysis and toxic evaluation indicate that all mutagenic intermediates in the gas can be completely eliminated within 240 min, which further suggests that photocatalytic technology is an effective approach for degrading aromatic amines.

1. Introduction

o-Toluidine, a monosubstituted aniline, has been commercially available since its first synthesis in 1844. It is often used as an intermediate in the dye industry, as well as in a number of other applications in the fields of rubber processing, pharmaceutical production, etc. [1]. *o*-Toluidine also can be released during the manufacturing and processing of coal oil gasoline, etc. According to the International Agency for Research on Cancer (IARC, 1982), the maximum permitted exposure level to *o*-toluidine in the workplace ranges from 3 to 22 $mg\,m^{-2}$ or 5 $mg\,L^{-1}$ in those countries which have set limits [2], especially since *o*-toluidine has been demonstrated to be a carcinogen in mice and rats. In fact, aromatic amines had been recognized to be carcinogenic as far back as 1895, when Ludwig Rehn described the high incidence of bladder cancer in dye industry workers as "aniline cancer" [3]. *o*-Toluidine can also be metabolized in vivo into a number of compounds, some of which are active genotoxins [2]. Due to the various environmental concerns it raised and its adverse effects on human health, *o*-toluidine has received increasing attention in recent decades [4].

Research on organic genotoxicity has become increasingly understood with the application of bacteria [5] and mammalian cells [6] in animals, as well as through epidemiological investigations among large groups of people [7,8]. Among many assay methods, the Salmonella mutagenesis assay (Ames assay) has been conveniently and reliably used [9]. Most of the research available has focused on the genotoxicity of *o*-toluidine and its co-mutagenic action with norharman [10,11], rather than the degradation intermediates produced.

Photocatalytic technology is a highly effective method for the complete degradation of a wide spectrum of organic pollutants into less toxic or harmless compounds without selection [12–16]. Most of the previous works in this field were carried out in aqueous systems [17–19], but only limited investigations focused on

* Corresponding authors. Tel.: +86 20 85291501; fax: +86 20 85290706.
E-mail addresses: antc99@gig.ac.cn (T. An), ligy1999@gig.ac.cn (G. Li).

1381-1169/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcata.2010.10.009

the evaluation the detoxification of gaseous organic pollutants. Mo et al. [20] theoretically evaluated the toxicity of the photocatalytic by-products of gas-phase toluene at indoor levels and concluded that the by-products had no negative effects to human health via the health-related index (HRI). However, HRI values do not represent a standard method. Rather, they are the user-defined sum of all HRI_i parameters (i.e., the ratio of the concentration versus its recommended exposure limit of compounds *i*). To date, few studies have been reported that experimentally assess the mutagenic change trends of volatile organic compounds (VOCs) and their gaseous mixed degradation intermediates during the photocatalytic degradation.

In this work, a typical aromatic amine, *o*-toluidine, is used as a model VOC to determine photocatalytic detoxification feasibility by chemically identifying its degradation intermediates. We aim to experimentally examine the potential mutagenicity of *o*-toluidine before and during photocatalytic degradation and evaluate the safety discharge of photocatalytic technology as compared to conventional methods for the treatment of aromatic amines. A tentative degradation pathway is also proposed to explain why photocatalytic technology is an effective method for detoxifying gaseous *o*-toluidine.

2. Materials and methods

2.1. Reagents

Dimethyl sulfoxide (DMSO) (purity: >99.9%), glucose-6-phosphate (purity: >98%) and dexon (purity: 99%) were purchased from Sigma Chemical Corp. (Saint Louis, MO, USA). 2-Aminofluorene (purity: >97.0%) was purchased from Fluka Chemical Corp. (Ronkonkoma, NY, USA). Nicotinamide adenine dinucleotide phosphate (NADP), D-biotin, L-histidine, agar, nutrient broth, and other chemicals of analytical grade were purchased from Huankai (Guangzhou, China). Rat liver enzymes (S9) and *S. typhimurium* strains, TA98 and TA100, were obtained from the Guangzhou Sanitation Prevention Station, China. *o*-Toluidine (purity: 99.5%) was purchased from Acros Organics (Geel, NV, Belgium). The methanol used in the procedures was of chromatography grade (Germany). N,O-bis (trimethylsilyl)-trifluoroacetamide (BSTFA) (purity: 98%) was obtained from Acros Organics (NJ, USA). All other reagents were used as received without further purification.

2.2. Photocatalytic characterization

Nanocrystalline titanium dioxide(TiO_2) thin films were prepared via our reported method [21] with the experimental flow chart shown in Fig. 1. All photocatalytic experiments were performed in a sealed Pyrex gas–solid reactor with a total volume of 5 L [22]. Briefly, a 125-W high-pressure mercury lamp that emits maximum radiation of 365 nm (GGZ125, Shanghai Yaming Lighting Co., Ltd.) was used as the light source. Film photocatalysts were installed facing the light source, which was sleeved in the center of a double-walled quartz cylinder. The reaction temperature was maintained at room temperature by a continuous circulation of cooling water in the quartz glass jacket around the light source (light intensity: 5.3 mW cm^{-2}). A mini-type fan stirrer at the bottom was used as the air blender for the reaction gas during the operation. For a typical experiment procedure, four identically prepared TiO_2/ITO films were installed into the reactor, which was then filled with dry air. Liquid *o*-toluidine and a small fraction of distilled water were injected into the vaporizing tube from the injection port. The vaporizing tube was heated and *o*-toluidine was allowed to vaporize, mix, and reach gas–solid adsorption equilibrium. The photocatalytic degradation of *o*-toluidine was carried out via a circulation reaction process with a gas circulation pump joining with short circulation Teflon tubing. The mixed reaction gas was circulated from bottom to top. The *o*-toluidine equilibrium concentration was about 4500 μg L^{-1} and the relative humidity was ca. 45%. Once the concentration of *o*-toluidine has stabilized, the *o*-toluidine gas was then irradiated with UV light, signaling the start of photocatalysis. Subsequently, at photocatalytic intervals of 30, 60, 120, 180, and 240 min, a portion of the gaseous samples was collected and sealed in a 1-L volumetric Teflon bag for mechanism studies. Other portions of the gaseous samples were trapped with DMSO solution for mutagenicity assessment.

2.3. Analysis

A gas chromatography (GC) (HP 5890, Series II, equipped with a split/splitless injector and a flame ionization detector) was used to analyze the concentration of gaseous *o*-toluidine before and after photocatalytic degradation. The injector temperature and the detector temperature were set at 250 and 280 °C, respectively. Gaseous samples amounting to 500 μL were taken using a 500 μL gas-tight locking syringe (Agilent, Australia) at given intervals and injected into the GC for *o*-toluidine determination in splitless mode. The typically programmed temperature of the column was maintained at 40 °C for 2 min, then increased to 100 °C at a rate of 6 °C min^{-1}, and finally increased to 280 °C at a rate of 10 °C min^{-1}. The concentrations of the target compounds were quantified by external standard calibration. The degraded gaseous intermediates were analyzed by a GC (Agilent 7890A)–mass spectrometer detector (Agilent 5975C with Triple-Axis Detector) (GC–MSD) with a DB-5 capillary column (30 m × 0.32 mm × 0.25 μm, Agilent Technology). The carrier gas was ultra-high purity helium fed at a constant flow rate of 1.3 mL min^{-1}. The analysis method and programmed procedure were the same as for GC quantification. The MSD was operated in full scan mode with *m*/*z* 40–300 amu. The gaseous target species were identified via their mass spectrum using the Wiley database (Wiley Mass Spectral Library).

To identify the intermediates adsorbed onto the surface of TiO_2 thin films, two sets of experiments were performed. First, solid intermediates deposited onto the thin films were directly analyzed by Nicolet 330 Fourier transform-infrared spectroscopy (FTIR). Next, these intermediates were extracted ultrasonically using a small amount of methanol for 30 min. The extract was filtered through a 0.45 μm filter membrane. The filtrate was collected, concentrated with a gentle stream of high-purity nitrogen, averaged into two similar 1.5 mL vials, and then completely dried with a gentle stream of high-purity nitrogen. One portion of the sample was re-dissolved in 1.0 mL ethyl acetate and then injected into the GC–MSD for the direct determination of intermediates. The remaining portion was derived at room temperature using 50 μL BSTFA as the derivatization reagent and 50 μL pyridine as the catalyst. The resulting solution was injected into the GC–MSD to identify polar intermediates. Samples amounting to 1.0 μL were injected in the splitless mode. The analysis method was the same as for the detection of gaseous target species. These intermediates were also identified via their mass spectrum according to the library spectra as described previously.

2.4. Mutagencity assessment

Ames assays were performed according to the standard plate incorporation method with the classic *S. typhimurium* strains, TA98 and TA100, which are capable of detecting base frame shift types and base pair substitution-type mutagenicity, respectively [23]. *o*-Toluidine and its photocatalytic degradation intermediates at 30, 60, 120, 180, and 240 min were trapped and dissolved completely