Applied Catalysis B: Environmental 183 (2016) 86–95

Contents lists available at ScienceDirect

Applied Catalysis B: Environmental

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

Disinfection capability of Ag/g-C_3N_4 composite photocatalysts under UV and visible light illumination

Mario J. Muñoz-Batista*, Olga Fontelles-Carceller, Manuel Ferrer, Marcos Fernández-García, Anna Kubacka*

Instituto de Catálisis y Petroleoquímica, CSIC, C/Marie Curie 2, 28049 Madrid, Spain

ARTICLE INFO

Article history:
Received 29 July 2015
Received in revised form 6 October 2015
Accepted 12 October 2015
Available online 19 October 2015

Keywords:
Photo-catalysis
Carbon nitride
Silver
Sunlight
Biocide
Germicide

ABSTRACT

The biocidal capability of Ag/g-C_3N_4 composite photocatalysts against *Escherichia coli* was evaluated as a function of the Ag content of the material upon UV and visible light excitation. The Ag/g-C_3N_4 composite system shows significant biocidal activity, presenting a behavior with a strong dependence on the silver content as well as on the excitation wavelength. The physico-chemical characterization of the samples together with a Langmuir–Hinshelwood-type kinetic modelling of biocidal experiments and the (photoluminescence and electron paramagnetic resonance) analysis of charge handling properties of the solids were used to interpret the photocatalytic response of the composite materials. The overall analysis shows that the wavelength dependence observed for all Ag/g-C_3N_4 composite photocatalysts is strongly correlated with the semiconductor—metal heterojunction effect on charge separation, handling and recombination, indicating the key role of the cooperative action between the two components of the system. Such cooperative effect is studied along the sample series and discussed to be related to the efficient use of both hole and electron related species in the disinfection action.

1. Introduction

The biocidal action of semiconductors within the so-called advanced oxidation processes using light as the energy source of the process has been the subject of intensive research in the last decades. Light excitation of the semiconductor can generate energy-rich electron-hole pairs able to degrade chemicals and/or cell components of microorganisms, and rendering innocuous products, mainly water and carbon dioxide [1,2]. Anatase was the first semiconductor tested in the seminal works of Matsunga et al. [3,4], being subsequently the subject of a large number of studies summarized in recent reviews (see, for example, in [5–8]). A weak point of anatase is the need of UV light to generate charge pair carriers and thus its limited utility when visible light or, more importantly, sunlight are used in disinfection processes.

Among the possible alternatives which can profit from a wider (UV and visible) electromagnetic spectrum than anatase, the potential of carbon-based materials has been highlighted [2]. In particular, in very recent times the graphitic carbon nitride (g-C_3N_4) material has been analyzed in connection with biocidal processes [9,10]. This polymeric semiconductor has a band gap energy of ca. 2.7 eV, making it as a viable photocatalyst for fruitful use of sunlight as a renewable energy source of biocidal processes. Such key property is combined with its low cost preparation as well as high thermal and chemical stability to render a very attractive functional material [11,12]. However, the biocidal action of g-C_3N_4 requires improvement mainly as a result of a relatively limited charge handling capability to generate chemistry at the surface [2,12,13]. This goal is commonly achieved through several methods including the doping of the semiconductor and/or the production of hetero-composite materials [2,9,13].

As the biocidal action of g-C_3N_4 has been suggested to be directly related to the nature and quantity of hole-related species located at the material [9,10], the use of electron withdrawing elements within a composite structure would strongly promote the effect of carbon nitride materials on microorganism viability. Among electron seeking components the use of metals and particularly silver have been pursued. Nanoparticulate silver by itself can have bactericidal activity, mostly based in a combination of the release of silver ions, the production of oxygen reactive species and, in specific cases, phagocytosis [14,15]. Moreover, the combination of silver with g-C_3N_4 provides an ideal situation with potential improvement of both components, as silver would be electron enriched while the semiconductor would be hole enriched, opening a way to enhancing simultaneously the biocidal capabilities of

* Corresponding authors. Fax.: +34 915854760.
E-mail addresses: mario.munoz@csic.es (M.J. Muñoz-Batista), ak@icp.csic.es (A. Kubacka).

http://dx.doi.org/10.1016/j.apcatb.2015.10.024
0926-3373/

both components. To the best of our knowledge, the silver-graphitic carbon nitride composite has been tested profusely for the photoelimination of pollutants [16–25] but to date its biocidal potential has not been investigated and reported.

Here we will test the mentioned Ag/g-C_3N_4 composite system biocidal action upon both UV and visible light excitation in order to check the potential of the system in the most interesting UV and visible electromagnetic regions. To this end, we use the pathogenic *Escherichia coli* strain 1337-H as a target microorganism and consider the kinetic analysis of the photokilling tests as a suitable tool for interpreting the properties of the composite system in terms of the action of each component as well as of the system as a whole. The kinetic modelling of the results was carried out following (and modifying) the work of Marugan et al. [26,27]. Modeling of the inactivation profiles is grounded in a simplified (Langmuir–Hinshelwood-like multistep-type) reaction mechanism and considers that microorganism death occurs via a sequential attack of photo-radicals by which "undamaged" cells become "damaged" and eventually progress to an "inactivated" state. The utilization of an "adsorption Langmuir–Hinshelwood" type mechanism allows a reasonable and relatively flexible description of the inactivation. Moreover, the advantage of using this approach appears two-fold: first, (i) its usefulness in analyzing complete sets of inactivation profiles showing (or lacking) initial smooth/fast decays and final tailing section; and, more importantly, (ii) the model renders kinetic parameters allowing physical interpretation of the underlying process, in contraposition with many other simple kinetic laws used previously [26–29]. Such study is completed here with the characterization analysis of the fresh and used materials with the help of XRD, UV–vis and TEM, as well as with a photoluminescence/electron paramagnetic resonance study of the solids charge handling properties after excitation with all wavelengths used in the disinfection tests. With the help of this combined approach we can show that the behaviour of the system strongly depends on the excitation wavelength and pointed out the main physical causes to justify the biocidal properties of the materials.

2. Experimental

2.1. Catalysts preparation

The graphitic carbon nitride was obtained by calcination of melamine (Aldrich), in the semi-closed system (to prevent sublimation) at 580 °C with a heating ramp of 5 °C min^{-1} for 4 h [30]. The composite materials were prepared using a single-pot microemulsion preparation method using *n*-heptane (Scharlau) as the organic medium, Triton X-100 (Aldrich) as a surfactant and hexanol (Aldrich) as a cosurfactant. The g-C_3N_4 was introduced into the organic phase of the microemulsion. After 30 min of stirring a certain quantity of $AgNO_3$ (0.5 M) aqueous solution was added into the organic phase. After 1 h, the adequate amount of a $NaBH_4$ aqueous solution (0.1 M) was quickly added into the solution under continuous vigorous stirring. Water/Ag and water/surfactant molar ratios were, respectively, 110 and 18 for all samples. The resulting mixture was stirred for 24 h and then centrifuged. The separated solid precursors were rinsed with ethanol, distillated water and acetone, and dried at 60 °C for 12 h. The sample names were *x*Ag/g-C_3N_4 for the composite samples, where *x* is the wt.% (1, 2, 5 and, 10) of Ag with respect to g-C_3N_4.

2.2. Catalysts characterization

The BET surface areas and average pore sizes were measured by nitrogen physisorption (Micromeritics ASAP 2010). XRD profiles were obtained with a Seifert D-500 diffractometer using Ni-filtered Cu Kα radiation with a 0.02° step and fitted using the Von Dreele approach to the Le Bail method [31]; particle sizes were measured with XRD and evaluated using the Willianson–Hall formalism [32]. UV–vis diffuse reflectance spectroscopy experiments were performed with a Shimadzu UV2100 apparatus. Ag lixiviation at the liquid phase was measured in centrifuged, rinsed samples using Inductive Coupled Plasma-Mass Spectrometry (ICP-MS Nextion 300XX PerkinElmer).

The electron paramagnetic resonance (EPR) measurements were done with a Bruker ER200D spectrometer operating in the X-band and calibrated with a DPPH standard. For the 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) spin trapping EPR experiments, the samples were suspended in water or methanol (at a concentration of 0.6 g L^{-1}) and were sonicated for 4 min. A solution (0.01 M) of DMPO spin trap (supplied by Sigma) was prepared and kept on ice during the whole set of experiments. Bidistilled water (Elix-10) or methanol (Sigma) were employed for these preparations. 100 μl of the solid suspension and 100 μl of the DMPO solution were mixed into an EPR flat quartz cell under atmospheric air and irradiated at different times, through a spectroscopic Pyrex glass filter with a cut-off at ca. 220 nm, with light excitation source identical to that employed for the photokilling tests and allowing "monochromatic" radiation (±20 nm half width), being then immediately transferred to the spectrometer cavity for EPR analysis. A small radical concentration decay (of ca. 5% on average) was observed in the dark during the course of spectrum recording. The latter were obtained at 298 K at ca. 9.75 GHz microwave frequency, 19.5 mW microwave power, 100 kHz modulation frequency, 1 G modulation amplitude and 2×10^5 spectrometer gain. No significant signal saturation was observed in those conditions. Blank experiments were also performed over mixtures of 100 μl of the DMPO solution and 100 μL of water or methanol to check the absence of radical formation in the absence of solid under the employed conditions.

2.3. Photocatalytic tests

The *x*Ag/g-C_3N_4 samples together with blank tests (using either light without catalyst or the materials at dark conditions) were measured using the same bacterium inoculums (8.9 10^9 colony forming units (CFU) mL^{-1}) for all measurements reported. To prepare bacterial inoculum, cells of *E. coli* 1337-H were grown overnight at 37 °C with shaking at 200 rpm in 10 ml of Luria-Bertani broth (LB) overnight; then, 100 ml of LB medium were inoculated with 2 ml of the overnight culture, and the cells were grown for 4 h (at 37 °C) to 8.9 10^9 CFU ml^{-1}. As demonstrated by blank experiments, care was put of using a sub-lethal, maximum radiation energy fluence of ca. 1 kJ m^{-2} throughout the study. Excitation of 1 ml inoculum was carried with a UV–vis spectrometer (Synergy HT Multi-Mode Reader—BioTek) equipped with filters to obtain "monochromatic" radiation (±20 nm half width). After irradiation and for different time intervals, aliquots of 100 μl were transferred to a 10 ml LB broth test tube. The order of cell dilution at this stage was 10^{-2}. Loss of viability after each exposure time was determined by the viable count procedure on LB agar plates after serial dilution (10^{-2} to 10^{-5}). All plates were incubated at 37 °C for 16 h after which they were scanned using a Bio-Rad Imaging System equipped with Analysis Software 4.6.5 (Bio-Rad) to enable enumeration of bacterial colonies among replicates. Detection limit of the automated method is below 10 colony units. Data reported in this contribution are typically the average of four to six different experiments. A minimum of four experimental runs was performed to determine antimicrobial activity.