WATER RESEARCH 40 (2006) 630–638

Available at www.sciencedirect.com

SCIENCE DIRECT®

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

Lincomycin solar photodegradation, algal toxicity and removal from wastewaters by means of ozonation

Roberto Andreozzi[a], *Marisa Canterino*[a], *Roberto Lo Giudice*[b], *Raffaele Marotta*[a,*], *Gabriele Pinto*[b], *Antonino Pollio*[b]

[a]*Dipartimento di Ingegneria Chimica, Facoltà di Ingegneria, Università di Napoli "Federico II", p.le V.Tecchio 80, 80125–Napoli, Italia*
[b]*Dipartimento delle Scienze Biologiche, Sezione Biologia Vegetale, Università di Napoli "Federico II", Via Foria, 80125 Napoli, Italia*

ARTICLE INFO

Article history:
Received 14 July 2005
Received in revised form
16 November 2005
Accepted 17 November 2005
Available online 6 January 2006

Keywords:
Lincomycin
Antibiotics
Photodegradation
Algal toxicity
Ozonation
Semicontinuous apparatus

ABSTRACT

Antibiotic molecules have been reported among the xenobiotics present at trace levels in sewage treatment plant (STP) effluents and aquatic environment. Lincomycin, one of the most used in clinical practices whose presence in the STP effluents has been often documented, is submitted to an extensive investigation to assess its persistence in the environment and toxicity towards different algal strains. The possibility to remove the lincomycin from water by means of ozonation is demonstrated and a reduction of toxicity of ozonated solutions on *S. leopoliensis*, with respect to untreated solutions containing this compound, is obtained even just for 1 h of treatment. Kinetic constants for the attack to lincomycin of ozone (from $1.53 \times 10^5\,M^{-1}\,s^{-1}$ at pH = 3.0 and $4.93 \times 10^5\,M^{-1}\,s^{-1}$ at pH = 6.7) and OH radicals ($4.37 \times 10^9\,M^{-1}\,s^{-1}$ at pH = 5.5 and $4.59 \times 10^9\,M^{-1}\,s^{-1}$ at pH = 7.5) are also evaluated.

1. Introduction

Recently pharmaceuticals have been identified as a new class of environmental pollutants. Hundred of tons of drugs are annually sold only in Europe for human and veterinary medicine thus causing the release of many of these species (or their metabolites) to the environment (Heberer, 2002; Halling-Sorensen et al., 1998). Among these, many antibiotic molecules have been found in sewage treatment plant (STP) effluents, in surface waters and in soils (Stackelberg et al., 2004, Andreozzi et al., 2004; Hirsch et al., 1999; Zilles et al., 2005). This is not surprising if one considers that the use of antibiotics is very large for human beings and livestock. Between 30% and 90% of administered dose of most antibiotics is generally excreted with the urine and, often, they are not destroyed by conventional wastewater treatments (Rang et al., 1999). Once these xenobiotics are released to the environment, they may undergo transformation processes in surface waters under the absorption of solar light (direct photolysis) or through the intervention of photosensitizers (indirect photolysis) such as nitrate and humic acids (Zepp et al., 1985). Natural degradation rates of these compounds, depending on their chemical-physical properties, may be very fast or completely ineffective, thus influencing in a relevant way the environmental persistence of the molecules. Although for many pharmaceuticals no adverse effects on living organisms and environment have been shown (Cleuvers, 2003), recent studies demonstrated the capability of some antibacterial agents (flumequine, oxytetracycline) and antidepressants and their metabolites (fluoxetine, sertraline and norfluoxetine) to bioaccumulate in living organisms such as fish and mussels (Delepee et al., 2004; Brooks et al., 2003) whereas anti-inflammatory diclofenac has been reported to have harmful effects on rainbow trout fish

*Corresponding author. Tel.: +39 081 7682968; fax: +39 081 5936936.
E-mail address: rmarotta@unina.it (R. Marotta).
0043-1354/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.watres.2005.11.023

(Laville et al., 2004; Schwaiger et al., 2004). Moreover it has been put forward that the presence of antibiotics in the environment could lead to the selection of resistant bacterial strains (Tendencia and de la Pena, 2001; Boon and Cattanach, 1999). Some evidence has been also collected that this resistance can pass to humans (Rhodes et al., 2000); this fact could render completely useless the related molecules in clinical practice, although this topic is still under debate (Ayscough et al., 2000). Due to their importance for the clinical practice, it is clear that, even when data on the adverse effects on living organisms are collected, the use of these drugs can not be banned and measures devoted to limit their discharge to the environment have to be found. Treatment of sewage waters by means of new removal processes could be a suitable solution. Among these advanced oxidation processes can be proposed as tertiary treatment for municipal wastewaters (Rosenfeldt and Linden, 2004; Vogna et al., 2002). Recently some studies appeared in the literature on the possibility to remove antibiotics from wastewaters by photocatalysis (Addamo et al., 2005), combined chemical and biological oxidation (Arslan Alaton et al., 2004) and by using reverse osmosis and ultrafiltration (Shi-zhong et al., 2004).

The present work aims to study the solar photodegradation and the effects of lincomycin (Fig. 1), one of the most used antibiotics whose occurrence in surface waters and STP effluents (Calamari et al., 2003), on simple living organisms such as algae along with the possibility of removing it from aqueous solution by means of ozonation. Lincomycin is one of the antibiotics of the lincosamines class. It is generally used as Lincomycin hydrochloride, a well-established antibiotic drug used in human and veterinary medicine. It is effective primarily against gram-positive pathogens, and not effective against gram-negative bacteria. It is currently employed against susceptible strains of streptococci, pneumococci, and staphylococci which usually can also be treated with penicillin or erythromycin. The antibacterial activity of Lincomycin hydrochloride is similar to the group of macrolide antibiotics to which erythromycin belongs.

First of all, the toxicity of lincomycin towards microalgae has been assessed using a battery of microalgae; the most sensitive ones have been chosen to carry out tests on liquid medium.

The effect of pH and starting concentration on the ozonation process have been investigated and the total organic carbon (TOC) abatement also evaluated. A mathematical model has been developed and the kinetic constant for the ozone attack to the substrate estimated by using the data collected during the experiments in a semicontinuous laboratory apparatus. The efficiency of ozonation in reducing toxicity has been measured by liquid-phase algal bioassays.

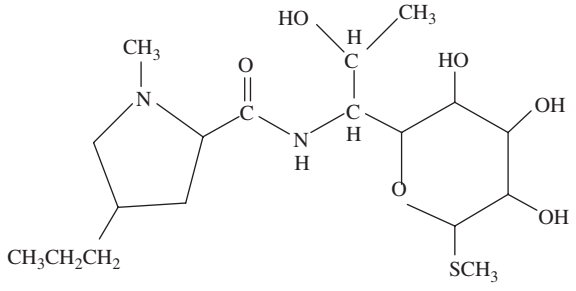

Fig. 1 – Chemical structure of lincomycin.

2. Experimental

2.1. *Solar irradiations set-up*

Sunlight irradiation runs were performed in Naples (40°N–14°E) in glass disk-reactors placed horizontally in thermostated bath at 25 °C. Actinometry was carried out by using a solution of *p*-nitroacetophenone (PNAP, 2.0×10^{-5} M) and different concentrations of pyridine (Dulin and Mill, 1982). The pyridine concentration was chosen to adjust the quantum yield of the PNAP ($\phi_{Act} = 0.0169$ [pyridine]) to modify the rate of loss of PNAP to match the rate of consumption of lincomycin (Organisation for Economic Cooperation and Development (OECD), 2000). The molar extinction coefficients of lincomycin at different wavelength were determined by means of a UV–VIS diode array spectrophotometer (HP 8452A).

2.2. *Toxicological assessments*

2.2.1. *Disk diffusion assay*

To detect antialgal activity of lincomycin, agar diffusion tests with different microalgal strains were performed. Eight blue-green and 10 green algal strains (Table 1) were grown in 100 ml Erlenmayer flasks containing 50 ml of Bold Basal Medium (Nichols, 1973). The cultures were incubated on a shaking apparatus at 24 °C and continuously illuminated with fluorescent lamps (Philips TDL 30w/55) at approximately 85 μE m^{-2} s^{-1}. Growth was daily determined microscopically with a Bürker blood-counting chamber and by monitoring absorbance at 550 nm with a Secoman 250 I spectrophotometer. Plates were prepared with the media described above containing 15.0 g of agar/l.

For preparation of the test plates, 1 ml of each culture in the mid-log phase of growth, were spread with a sterile glass rod on the surface of the plates containing the agarized Bold Basal Medium. The plates were incubated for 24 h, under the same conditions of light and temperature above described. Then, lincomycin (20 μl of solutions corresponding to 4.5 or 9 μg/l) was added to sterile paper disks (Becton Dickinson, diameter 6 mm). Once dry, each paper disks was placed onto the agar surface containing the test alga, and the plates were incubated for a week under the same experimental conditions. The antibiotic activity was recorded as the diameter of clear zones of inhibited algal growth around the paper disk.

2.2.2. *Bioassay on liquid medium*

The stock solutions of lincomycin were prepared by dissolving a known quantity of the compound in water. No added solvent were used for their preparation. The solutions were stirred for 24 h in the dark at ambient temperature. The test solutions were prepared by mixing the appropriate volumes of the stock solutions and of the culture media. The pH of all dilutions prepared in culture media and used for liquid bioassays was 6.8 ± 1.