Available online at www.sciencedirect.com
ScienceDirect
Solar Energy 84 (2010) 703–709
SOLAR ENERGY
www.elsevier.com/locate/solener

Inactivation of *Clostridium perfringens* spores and vegetative cells by photolysis and TiO_2 photocatalysis with H_2O_2

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Received 28 October 2009; received in revised form 22 January 2010; accepted 27 January 2010
Available online 23 February 2010

Communicated by: Associate Editor Gion Calzaferri

Abstract

Due to public health concerns related to the generation of dangerous by-products from conventional systems of water disinfection, innovative technologies based on the generation of oxidant radicals are being developed. The aim of this work is to evaluate the bactericidal activity of different treatments with light (λ: 320–800 nm), TiO_2 (1 g L^{-1}) and H_2O_2 (0.04 mM) on the viability of vegetative cells and spores of *Clostridium perfringens*. After spiking a natural water sample (from the Ebro River, Zaragoza (Spain)), the population of vegetative cells was of 10^8 CFU·100 mL^{-1} and of spores about 10^3 CFU·100 mL^{-1}. Treatments without radiation source (TiO_2, H_2O_2, TiO_2/H_2O_2) show a poor level of inactivation (<0.5 log) on both bacterial forms. The light treatment achieves a vegetative cell inactivation of 1.2 log after 5 min of treatment and <0.5 log on spores after 30 min. The combined light/TiO_2 system increases the level of disinfection with a vegetative cell removal in the order of 6 log after 5 min and 0.6 log of spores after 5 min. Light/H_2O_2 and light/TiO_2/H_2O_2 treatments also significantly increase the disinfection of vegetative cells of *C. perfringens* (>6 log). Regarding spores, light/H_2O_2 and light/TiO_2/H_2O_2 treatments achieve constant inactivation of 1 log after 5 min of treatment. The application of a light/TiO_2/H_2O_2 treatment does not increase the level of inactivation with regard to the level reached by the light/TiO_2 and light/H_2O_2 systems. This fact shows there is no a significant interaction between TiO_2 and H_2O_2 under the conditions studied.

Keywords: *Clostridium perfringens*; Water disinfection; Spores; Sunlight; Photocatalysis

1. Introduction

One of the great achievements of the 20th century for the well-being of mankind has been, from a public health and disease prevention viewpoint, the provision of drinking water. This improvement is evident in developed countries, although nowadays frequent outbreaks by waterborne transmission are still common (OECD, 2000). However, this improvement is not a reality in many other countries in Latin America and Africa (Rodrigues et al., 2007).

Conventional disinfection treatments of natural waters are commonly based on chlorine. Due to its low cost and easy handling, chlorine has been the most commonly used agent for achieving an acceptable removal of pathogenic microorganisms in natural waters. However, dangerous by-products detected in chlorinated water are generating a substitution of chlorine as primary disinfectant little by little (Boorman et al., 1999).

Of these by-products, the most important are trihalomethanes (THMs), potentially dangerous substances for

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0038-092X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.solener.2010.01.023

human health (Environmental Protection Agency, 1999). The quantity of THMs generated depends on the NOM concentration existing in the water undergoing treatment. Research has therefore been directed towards new techniques which avoid the generation of these harmful substances.

Nowadays, photolysis is an important alternative in the field of drinking water and wastewater disinfection strategies. The disinfecting properties of photolysis have been studied previously (McGuigan et al., 1998; Robertson et al., 2005). However, factors as water turbidity or regrowth of bacteria after solar treatments can affect in the effectiveness of process (Rincón and Pulgarin, 2004).

To obtain better results, photolysis can be combined with a catalyst (photocatalysis) which increases the reaction rate after being activated with the energy from light. The use of titanium dioxide as a photocatalyst for water treatment has frequently been reported (Robertson et al., 2005; Maness et al., 1999; Rincón and Pulgarin, 2004). TiO_2 photocatalysis is an advanced oxidation technology that could be an alternative to conventional disinfection processes (Rincón and Pulgarin, 2003). This technique does not require the addition of consumable reagents and harmful by-products are not generated.

When a semi-conductor such as TiO_2 is irradiated with near ultraviolet light ($\lambda < 400$ nm), electron (e^-)/hole (h^+) pairs are generated in the conduction band and valence band respectively. These holes and electrons are involved in the development of redox reactions. The holes on the catalyst surface react with ^-OH ions and with H_2O molecules generating hydroxyl radicals. At the same time, the excited electrons react with oxygen molecules present in the water, generating more oxidant intermediate species and precursors of $^{\bullet}OH$ radicals, such as superoxide anions ($O_2^{\bullet -}$), peroxide radicals ($HO_2^{\bullet}$) and H_2O_2 molecules. These reactive oxygen species (ROS) contribute to renew the radical attack mechanisms (Maness et al., 1999).

Some authors maintain that the addition of hydrogen peroxide to TiO_2 photocatalysis enhances the photodegradation of the existing pollutants because H_2O_2 has the capacity to react directly with the electrons in the conduction band giving rise to hydroxyl radicals (Hartmann and Eisenstark, 1978). Moreover, the peroxide also directly adsorbs UV wavelengths and its peroxidic bond breaks, forming more oxidant radicals by itself (Mamane et al., 2007) and thus further improving the disinfection efficiency.

Many authors have investigated the mechanisms of photocatalysis bactericidal action since the first research carried out by Matsunaga and co-workers (1985). These studies have been recently reviewed (McCullagh et al., 2007). Most of these researches are been performed in distilled water or buffer solution. However, the matrix of the natural water has influence on the effectiveness of treatments on pathogens. Microorganisms can hide away from the radiation behind particles without being affected.

On the other hand, despite the intense existing literature, the system of photocatalytic disinfection is still not well understood. The fact of that polyunsaturated phospholipids are an integral component of the bacterial cellular membrane and they are susceptible to attack by ROS is well documented (Maness et al., 1999). The loss of the membrane structure and consequently its derived functions (semi permeability, respiration, oxidative phosphorylation reactions) are the main cause of cellular death. Cho et al. (2003) shows that there are different photocatalytic inactivation behaviours depending on the kind of microorganisms, based on the differences in the sizes and cellular surface structure.

Escherichia coli has been the most studied microorganism (Rodrigues et al., 2007; Rincón and Pulgarin, 2004; Rincón and Pulgarin, 2004; Rincón and Pulgarin, 2003; Rincón and Pulgarin, 2007; Gumy et al., 2006). However, only a few works have focused on other bacterial parameters which present greater resistance to disinfection treatments such as *Clostridium perfringens* (Dolin, 1959; Ando and Tsuzuki, 1986; Dunlop et al., 2008). European Directive 98/83/CE has included *C. perfringens* as a new microbiological parameter to check in systematic drinking water analysis because in adverse situations, it forms spores and survives in water for much longer than coliforms. Its presence in disinfected water can indicate that the treatment has been deficient and maybe other resistant pathogens will also have survived (Payment, 1999).

Therefore, in this work, the effectiveness of photolysis and TiO_2 photocatalysis and their combination with hydrogen peroxide has been compared for the inactivation of the anaerobic bacterium *C. perfringens* and its spores in natural water.

2. Methods

2.1. Sample preparation

The natural water studied in this research comes from the River Ebro (Zaragoza, Spain). The sampling was carried out 1 km away from the entrance point to the drinking water plant in the city of Zaragoza (Spain). The samples were conserved at −20 °C. The water quality of the surface water is presented in Table 1.

Because the presence of *C. perfringens* in the natural samples was low (see Table 1), it was decided to increase this population artificially by the preparation of concentrated suspensions of vegetative cells and spores. Before mixing the water with the vegetative cell suspension or the spore suspension, the water sample was sterilized at 121 °C/15 min to remove any endogen microorganism.

2.1.1. C. perfringens vegetative cells

A strain of *C. perfringens* of clinical origin was kindly supplied by the Microbiology Service of the Lozano Blesa Clinical University of Zaragoza (Spain). This strain was confirmed as *C. perfringens* after carrying out the following