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Photocatalytic inactivation of highly resistant microorganisms in water: A kinetic approach

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ABSTRACT

The mechanism for the photocatalytic inactivation of highly resistant microorganisms (i.e., *Bacillus subtilis* spores) in water was studied using a kinetic approach. This required characterizing the basic processes that occur within the photoreactor. The radiative intensity that entered the photocatalytic system was estimated using the ferrioxalate actinometrical process, the amount of hydroxyl radical produced under a specific photo-assisted Fenton reaction was measured, and a kinetic model to predict the hydroxyl radical generation was proposed to fit the experimental values. These results were then used to suggest new assessment related to the spore inactivation mechanism under controlled photo Fenton reaction conditions. The kinetic model was found to fit the experimental data fairly well ($r^2 > 0.99$) and hydroxyl radical generation was determined to significantly affect the inactivation process. It was determined that a specific amount of hydroxyl radical is required to overwhelm the self-repairing mechanisms of the cell and cause cell death. The amount of hydroxyl radicals generated was found to be a function of radiative intensity and reagent concentration, as previously reported. The proposed relationship between the amount of hydroxyl radical and the inactivation process was supported by adding chloride ions to acting as radical scavengers. It was observed that even the lowest chloride ion concentration was capable of producing a significant delay in the inactivation process by scavenging hydroxyl radicals and generating low reactive species at the pH conditions tested.

1. Introduction

The spread of waterborne diseases resulting from a lack of access to safe drinking water is a critical global concern. It is estimated that over 1.2 billion people around the world do not have access to drinking water supply services, usually because of poverty [1]. The effects of waterborne diseases on sustainable economic development in developing countries and some areas of developed countries are expected to increase because climate-change-related temperature anomalies, which can alter the concentration, persistence, growth rate, and survival of many pathogens in water [2]. Reduced precipitation, changes in rain patterns and the projected rise in seasonal and annual air temperatures are to affect the survival of existing and emerging pathogens and increase the incidence of microbial diseases [3,4]. It has been estimated that there will be increasing impacts to public health because of increased incidences of existing waterborne diseases and the emergence of unknown diseases [5].

Advanced oxidation processes (AOPs) have proven to be a promising alternative to inactivate pathogens in water. AOPs have been tested for their ability to inactivate several different pathogens [6,7]. In particular, Fenton and Fenton-like processes have been identified as effective alternatives for inactivating highly resistant microorganisms [8–10]. *Bacillus subtilis* spores are highly resistant to a wide variety of stressing conditions—such as toxic chemicals, high pressure and temperature, UV, and ionizing radiation—which make them useful test microorganisms for photocatalytic disinfection processes [11] and a conservative water-disinfection model [9,12]. Although current literature has reported successful inactivation of *B. subtilis* spores using Fenton-like AOPs, little is known about the inactivation process. Understanding the mechanisms of the inactivation process will provide the required knowledge to analyze, design, and improve full-scale applications that can be used to implement adaptation measures to prevent the projected effects of climate change on microbiological water quality and the associated effects on public

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health. The goal of this work is to determine the mechanisms for inactivating *B. subtilis* spores in water using Fenton-like AOPs.

2. Experimental procedures

2.1. Reagents

The reagents used in this work were sodium chloride (NaCl, Merck), sulfuric acid (H_2SO_4, Sigma), sodium hydroxide (NaOH, Sigma), *N*,*N*-dimethyl-*p*-nitrosoaniline (pNDA, Sigma), potassium dichromate ($K_2Cr_2O_7$, Aldrich), diphenylamine, 1,10-phenanthroline (Sigma), ferrous sulfate ($FeSO_4$, Sigma), and hydrogen peroxide (H_2O_2, 50% stabilized, Sigma). All reagents were purchased as reagent grade (A.C.S.) and used as received without further purification.

2.2. Experimental setup

Inactivation tests were performed in the experimental setup shown in Fig. 1. Two low-pressure UV lamps (λ_{max} = 365 nm, 15 W, GE F1578/BLB) with an OF-365AUV (black) Filter from Spectroline® (cutting wavelength 365 nm) were used as a radiation source. The photoreactor used included a jacketed reservoir (500 mL) connected to a temperature controller (Polystat®, Cole Parmer) in order to get all the experimental runs at 20 ± 0.1 °C. A magnetic stirrer was used to keep the reaction mixture suspended.

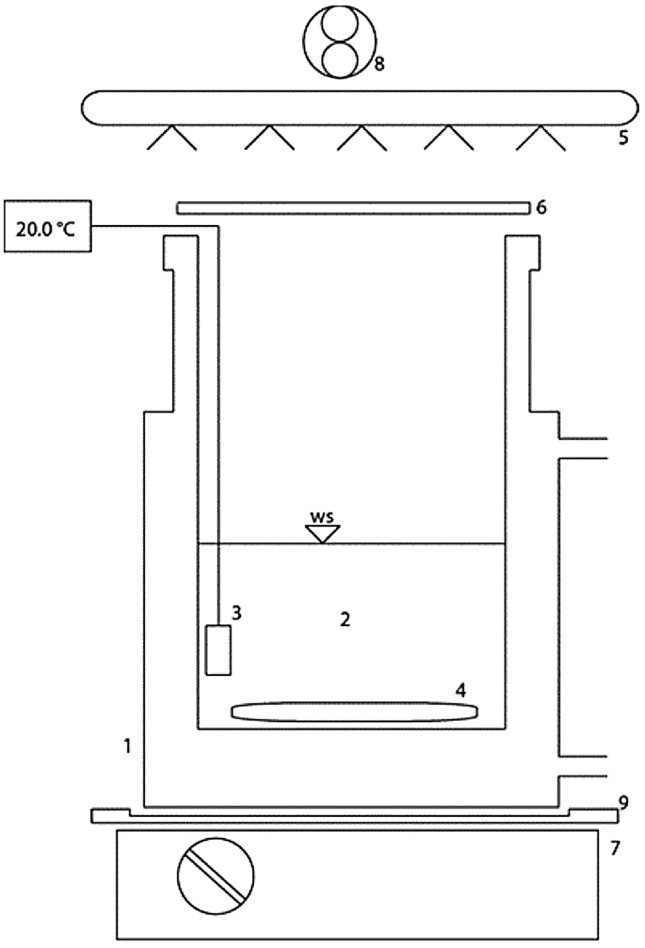


Fig. 1. Experimental setup used in this work. The main arrangement elements are: 1) jacketed photoreactor connected to a controlled temperature water bath, 2) reaction mixture, 3) temperature measurement, 4) magnetic stirrer, 5) BL lamps, 6) OF-365AUV (black) optical filter, 7) magnetic stirring plate, 8) air extraction system, and 9) horizontal positioner.

2.3. Actinometry measurements

The radiative intensity entering the photoreactor during the inactivation process was measured using the widely reported ferrioxalate actinometry procedure [13]. It is well-known that potassium ferrioxalate can absorb UV radiation in agreement with Eq. (1) as follows:

$$2Fe^{3+} + C_2O_4^{2-} \xrightarrow{h\nu} 2Fe^{2+} + 2CO_2 \quad (1)$$

The amount of ferrous ion produced can be quantified by complexation with 1,10-phenanthroline and measuring its absorbance at 510 nm [14].

The amount of radiation arriving into the system by unit of time (I) can be estimated in agreement with Eq. (2) as follows:

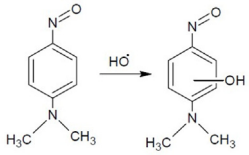
$$I = \frac{d[Fe^{2+}]V}{dt \quad \phi} \quad (2)$$

where I is the photonic flux (Einstein min^{-1}), [Fe^{2+}] is the amount of ferrous ion measured by complexation with 1,10-phenanthroline at 510 nm, V is the irradiated volume (L), and ϕ is the ferrous ion quantum yield (1.21 Fe^{2+} quantum^{-1} at 365 nm) [15].

The radiative intensity was determined at four distances (8, 10, 13, and 15 cm) from the lamps to determine the highest value and was used to perform the inactivation processes and hydroxyl radical production processes with a known radiative intensity.

2.4. Hydroxyl radical production

The amount of hydroxyl radicals generated by the system was estimated using *N*,*N*-dimethyl-*p*-nitrosoaniline (pNDA) as a radical scavenger (Bors et al., 1978; Barashkov et al., 2010). As has been shown in previous reports, pNDA reacts with hydroxyl radicals with a 1:1 stoichiometry and at a high reaction ratio (k = 1.25 × 10^{10} $M^{-1}s^{-1}$), which is shown in Eq. (3) as follows (Farhataziz, 1977; Bors et al., 1978; Barashkov et al., 2010):



The determination of hydroxyl radical production was performed using 300 mL of pNDA (10 μM) and three different concentrations of ferrous ion (0.0, 0.018, and 0.036 mM) and hydrogen peroxide (0.0, 0.55, and 1.1 mM) for the Fenton-like process. Once the pNDA solution was poured in the photoreactor, the pH was adjusted to 3.0 with 0.1 M H_2SO_4, and then the required amount of ferrous sulfate was added and the mixture was stirred for 3 min to allow iron dissolution. After iron dissolution, the initial (t = 0) sample was taken (c.a. 1 mL). Finally, the required amount of hydrogen peroxide to get the desired final concentration was added to the reaction mixture and submitted under the lamps (previously warmed up for 15 min). The photocatalytic process was considered to have started once the hydrogen peroxide was added and the lamps were turned on. Further sampling was performed at 5, 10, 15, 25, 35, 45, and 60 min and the remaining concentration of pNDA was measured by absorption at 440 nm in a UV–vis spectrophotometer (Cary 100, Agilent).