Chemical Engineering Journal 198–199 (2012) 388–396

Contents lists available at SciVerse ScienceDirect

Chemical Engineering Journal

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

Chemical disinfection with H_2O_2 – The proposal of a reaction kinetic model

Marina J. Flores [a], Rodolfo J. Brandi [a,b], Alberto E. Cassano [a,b], Marisol D. Labas [a,b,*]

[a] INTEC, Universidad Nacional del Litoral and CONICET, 3000 Santa Fe, Argentina

[b] Facultad de Ingeniería y Ciencias Hídricas. Universidad Nacional del Litoral, 3000 Santa Fe, Argentina

HIGHLIGHTS

- *Escherichia coli* inactivation using H_2O_2 is modeled as a chemical-like kinetics.
- Bacteria death is assumed to be the result of cellular wall break down by $OH^{\cdot}$ attack.
- The first three steps are modeled as pseudo-homogeneous superficial reactions.
- The next steps are homogeneous oxidations of the lysate chemical components.
- Simulations using an intrinsic kinetic model are fully validated with experiments.

ARTICLE INFO

Article history:
Received 8 February 2012
Received in revised form 29 April 2012
Accepted 30 May 2012
Available online 9 June 2012

Keywords:
Disinfection
Hydrogen peroxide
Kinetic model
Escherichia coli

ABSTRACT

The inactivation (death) of *Escherichia coli* bacteria in water employing hydrogen peroxide has been studied and a five log decrease in CFU cm^{-3} was achieved. The reaction kinetics was modeled as a series of biochemical steps represented by pseudo-homogeneous reactions between hydroxyl radicals and the components of the cellular walls. Afterwards, the lysate was supposed to undergo a group of parallel reactions leading to the oxidation of the chemical components of the cell. It was assumed that the initiation step of hydrogen peroxide dissociation is promoted by the presence of iron or iron-superoxide compounds. In addition the model takes into account that the reaction forming the lysate as well as the ones that follow the destruction of the bacterium wall, compete for the available oxidizing radicals with the steps that involve the attack on active and injured bacteria. A four parameter representation shows good agreement for the whole range of employed hydrogen peroxide concentrations. The results are valid for any form and size of the employed reactor as long as the described operating conditions (pH and concentrations) are maintained. This development constitutes a very general model that is capable to describe inactivation processes whose graphical representation also shows the presence of shoulders at the beginning and tailings in the end of the operation.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

The antimicrobial and/or antiseptic properties of hydrogen peroxide have been known for many years due to its efficacy and reasonable manipulation safety [1,2]. This compound is effective against a wide spectrum of bacteria, yeasts, molds, viruses and spore forming organism [1,3].

Disinfectant agents affect the microorganisḿs viability and survival by attacking their different structures. Depending on the disinfectant agent capacity to break through the cell wall, the disinfectant can cause sequential damage in the different organelles of the cell, DNA molecules, etc. [4]. The toxic effects of H_2O_2 on *Escherichia coli* have been extensively studied [5–8]. The cytotoxic effect exerted by hydrogen peroxide on microorganisms depends on the cell characteristics, its physiological state, extension of time of exposure, environmental conditions and employed H_2O_2 concentration [9,10].

The chemical mechanism that promotes the hydrogen peroxide decomposition to produce the highly oxidative $OH^{\cdot}$ and $O_2^{\cdot-}$ radicals is well known. However, its effect on different microorganism has been usually inferred but not firmly established.

The damaging strokes to the bacteria cellular components have been attributed to a particular phenomenon called oxidative stress resulting from the action of those reactive oxygen species known as ROS [1,11]. Sies [12] defined the oxidative stress, as a disruption of the prooxidant–antioxidant balance in favor of the former, leading to potential injury. Hydroxyl radicals may impact on different components of the cell producing the above mentioned oxidative stress; i.e., ROS can affect the cell in different levels and there is no doubt that, particularly the $OH^{\cdot}$ radical, can produce a severe

* Corresponding author at: INTEC, Universidad Nacional del Litoral and CONICET, 3000 Santa Fe, Argentina.

E-mail address: mlabas@santafe-conicet.gov.ar (M.D. Labas).

1385-8947/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.cej.2012.05.107

Nomenclature

B	bacteria
k	kinetic constant, units depend on the specific reaction step of the model
CFU	colony forming units
E	generic representation of all the elements that constitute the mathematical representation of the process
I	generic representation of all the Ac + In elements that constitute the mathematical representation of the process
R	volumetric reaction rate (CFU cm^{-3} s^{-1})
t	time (s)
HS	hypothetical species of the components of the cell wall
SSA	specific superficial area per unit mass of the cell (cm^2 cm^{-3})
LY	products of bacteria lysis
Y	oxidation yield of reactions involving $OH^{\cdot}$ radicals (CFU mol^{-1})

Greek letters

γ_A	kinetic parameter (cm^3 mol^{-1} s^{-1})
γ_{IN}	kinetic parameter (cm^3 mol^{-1} s^{-1})
γ_0	kinetic parameter (mol CFU^{-1})
γ_1	kinetic parameter (mol CFU^{-1})
γ_2	kinetic parameter (mol CFU^{-1})
α	constant related to dead and lysed bacteria

Subscripts

AC	relative to active bacteria
IN	relative to injured bacteria
DE	relative to death bacteria
CW	relative to cell wall
P_N	relative products of bacteria lysis

Superscripts

0	indicate initial condition

Special symbols

( )	concentration of chemical of species (mol cm^{-3}), or concentration of colonial forming units in the bulk (CFU cm^{-3})

cellular damage that leads to an irremediable outcome; i.e., its death.

Moreover, the extreme reactivity of these radicals provides them the means to become major participants in the toxic processes mediated by free radicals [13], a conclusion that has been demonstrated by numerous studies [14–19]. In the spontaneous mutagenesis and oxidative damage to DNA in *Salmonella typhimurium*, Storz et al. [20] found that $OH^{\cdot}$ can produce a break in the double chain and/or some chemical modifications in the nitrogen bases. In a study of the membrane system, Anzai et al. [21] found that hydroxyl radicals increased lipid peroxidation. The toxicity of $OH^{\cdot}$ radicals generated in vivo has also been well documented by Nunoshiba et al. [11]. Rincon et al. [22] studied the photocatalytic process with TiO_2 for disinfection of drinking water and postulated that the interaction of hydroxyl radicals with bacteria led to its inactivation.

One of the most logical hypotheses is to consider that a Fenton-like or Haber-Weis-like reactions [11,13,23,24] at low iron concentrations [25,26] could be the main cause of formation of the hydroxyl radical, and that this radical will be the most important product of these reactions. Even if Fenton and Fenton-like reactions are usually conducted at acidic pH, it has been shown that they can also be effective working at circumneutral pHs [27–31]. Moreover, problems usually encountered en large scale applications will never be an important issue in biological systems. However, with respect to the participation of type of reactions, their inclusion does not mean that one will ignore that microorganisms have defensive systems to inhibit the destruction of its internal oxidation–reduction balancing [32] and some competition will always be present. These considerations are one of the principal grounds for the model presented in this report.

For disinfection purposes, the non-persistent characteristic of hydrogen peroxide becomes a disadvantage to maintain the water quality in the distribution system. However, its use is widely spread because it is relatively inexpensive, easily removed when desired and unlikely to be hazardous to health if used properly [33]. In addition, it does not give rise to disinfection byproducts, as it is the case of other strong oxidants. But even more importantly, the consequence of the hydrogen peroxide action leads to a complete and irreversible destruction of the bacteria components, hindering the possibility of bacteria recovery or resuscitation.

Several kinetic models to represent the disinfection process have been published. Some of them are empirical equations such as for example those proposed Chick [34], Watson [35], Hom [36], Cerft [37], Buchnann et al. [38], Membre et al. [39] while others, very well represented by the work of Severin et al. [40] with their series and multi target events have a more mechanistic approach. Just recently a much direct interpretation of the involved oxidation process has been published by Marugan et al. [41].

In this work we have made an attempt to develop a general model to describe the chemical disinfection reaction based on a simplified generic interpretation of a very difficult and intricate mechanism. Thus, the membrane disruption, the subsequent lysis of the components of the bacteria and the oxidation of the resulting lysate, were modeled as series of episodes followed by a sequence of parallel events, in which the level of damage will increase until the bacteria not only dies or is severely affected in its vital functions but also, given enough time, the chemical components of the cell will be fully oxidized. Hence, this model should be useful to characterize quite accurately simple as well as complex survival curves of inactivation. In fact, in the present context it appears capable of accommodating initial resistances or retards, as manifested by the shoulder which appears in many survival curves as well as the observed tailings at the end of other methods. This detailed description of the possible pathway through different steps, enhances a virtual visualization of the full process and simplifies further actions concerning reactor design, scale-up or optimization procedures.

2. Experimental

2.1. The reacting system

In all experimental runs we employed a well-stirred, cylindrical batch reactor, having a total reaction volume of 2000 cm^3. Stirring was achieved with an external orbital shaking device. A cooling jacket connected to a thermostatic bath (Haake) surrounds the reactor to keep the reacting system at a constant temperature of

20 °C. The top of the reactor has provisions for sampling, pH and temperature measurements (see Fig. 1).

2.2. Chemicals

The following chemical were used: hydrogen peroxide (Merck, pro-analysis 30%), Catalase (from *Aspergillus niger*, Biochemika), Ammonium molybdate (Cicarelli, pro-analysis), Potassium iodide (Cicarelli, pro-analysis), Potassium acid phthalate (Cicarelli, 95% pro-analysis), Physiological saline solution (Roux-Ocefa), Nutrient broth (Biokar Diagnostics), Sodium hydroxide (Cicarelli, pro-analysis) and Peptone water (Biokar Diagnostic).

2.3. Experimental procedure

Throughout this work *E. coli* strain ATCC 8739 was used. The purity of the strain was verified by conventional methods [42,43]. The culture was grown in a complex medium (Nutrient broth) that had beef extract as the main component. Therefore, the broth composition was: tryptone: 10 g L^{-1}, beef extract 5 g L^{-1} and NaCl: 5 g L^{-1}.

The working solution was prepared from a culture that had reached the stationary phase of growing and, afterwards, was brought to a 1/1000 dilution with physiological saline. This dilution helped to ensure that there was no bacteria growth during the disinfection run due to the fact that the growing culture concentration was sufficiently diluted [44,45]. An atomic spectroscopy analysis (Perkin-Elmer-5000 AAnalyst) detected traces of iron and copper ions in the growing culture (Cu = 7.7 μg g^{-1} and Fe = 43 μg g^{-1}).

The prepared culture was mixed with the desired amount of hydrogen peroxide and distilled water. The hydrogen peroxide concentrations varied between 15 and 300 ppm. They were measured with colorimetric techniques at 350 nm, according to Allen et al. [46] with a Perkin-Elmer-330 spectrophotometer. All initial concentrations (at $t = 0$) were in the order of 10^5 CFU cm^{-3} and afterwards, samples were withdrawn at different time intervals for several determinations. The exact value of the bacteria's initial concentration was measured for each experiment. Runs were duplicated and samples subjected to triplicate determinations. The initial pH was 7 and remained practically constant during all the runs. In addition, experiments were carried out without hydrogen peroxide to make sure that the starting solution was free from other inactivating agents.

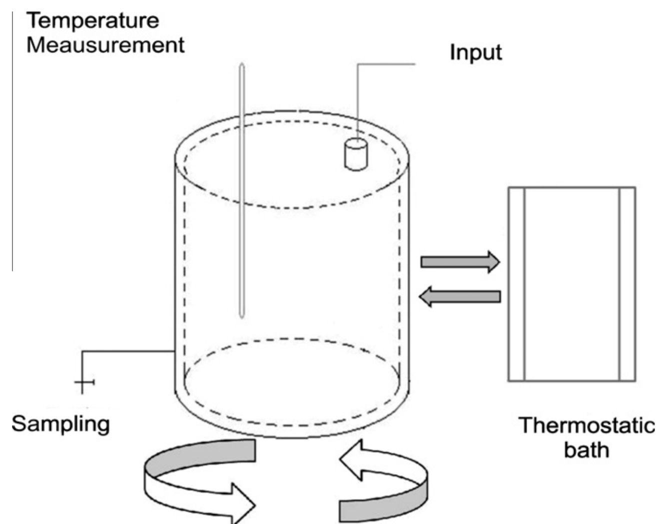


Fig. 1. Schematic representation of the experimental reactor setup.

Each sample was examined with the following measurements: absorbance at 350 nm (spectrophotometric analysis) for hydrogen peroxide and CFU counting using specific Petrifilm™ plates (3 M Microbiology Products) for *E. coli* and coliform bacteria. This method has been recognized by the American Public Health Association in Standard Methods for the examination of Dairy Products [43] and the Association of Official Analytical Chemists International in Official Methods of Analysis (AOAC) [47] as equivalent to the conventional plate method for this type of microorganism. Dilutions of the samples to obtain the optimum concentrations for the CFU counting method were made with a sterile peptone water solution. To quench the hydrogen peroxide action during the time interval between sampling and spread plating, a known fraction of the sample was mixed with the required amount of catalase solution. Control experiments were conducted to ensure that the employed concentrations of catalase solutions did not affect bacteria concentrations. After spreading the plates with the appropriate volume of sample they were incubated for 24 h at 37 °C.

3. Kinetic model proposal

The key point is to search for one acceptable assumption concerning the possible place where the action of ROS can take place. According to Dalrymple et al. [2] in the case of bacteria there are three sites of the cell that could be the targets for ROS invasion: (1) The peptidoglycan layer, (2) the lipopolysaccharide layer (found only in Gram-negative bacteria) and (3) the phospholipid bilayer. In this study *E. coli* was the chosen bacteria to work with and consequently the three layers will be present. Chemical-reactive agents like hydrogen peroxide may display some target specificity like membrane thiol groups and ribosomes but, frequently, hydroxyl radicals have sufficient reactivity to interact with different cellular components obscuring the direct primary damage. It is very likely that, this could be the way to break down the cell membrane. Once this action occurs, ROS could still act on intracellular vulnerable sites such as enzymes, coenzymes and nucleic acids. The enzymatic system appears to be one of the most important and irreplaceable reconstructive agent for bacteria reactivation in UVC disinfection. Consequently, if the disruption of the cellular wall is not enough, this effect of hydrogen peroxide on intracellular components could explain why oxidative disinfection is always an irreversible process.

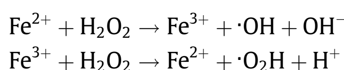
With regard to the most plausible mechanisms for producing damage on components of the cell several detailed studies have been reported, many of them still under discussion. Nevertheless, it is recognized that the process constitute a large sequence of a complex network of interconnected steps before reaching what might be called the "final product" (inactivation). The fundamental question is to know if only one simple reaction path is sufficient or if it is necessary to resort to more than one to complete the pursued objective and, in this case, whether or not it is indispensable to model all the steps involved in the oxidation of the lysis products to adequately represent the disinfection operation leading to cell death.

In view of these difficulties, and searching for some form of plausible, simplified and at the same time, conceptually undistorted representation of all these processes, one could try to adopt a model that takes into account the previously mentioned actions on the biological metabolism, without assigning a detailed composition to each of the representative family of cell components. Once this model is mathematically stated, it will be necessary to find out the minimum set of kinetic parameters that are required to depict, within an acceptable error, the series or series–parallel events that

lead to the dead of the bacteria. In this sense, the membrane disruption, the subsequent lysis of the components of the bacteria and the oxidation of the resulting lysate, can be modeled as series of episodes followed by a sequence of parallel events, in which the level of damage will increase until the bacteria is severely affected in its vital functions [1,41,48] without pursuing further oxidation reactions if they are not necessary.

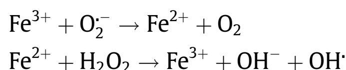
The kinetic model put forward here for *E. coli* disinfection is a conceptual modification of the one described by Marugan et al. [41] where a reaction pathway is proposed for the undamaged, damaged and inactivated populations of bacteria and particularly, for the structural mechanism of the bacteria decease. Instead of considering the attachment of titanium dioxide particles on the bacterium wall that afterwards is followed by the classical oxidative mechanism of the photocatalytic system, in this work we begin by considering the full sequence of hydroxyl radicals formation and then, explicitly takes into account that there is one attack on the cell membrane components according to Dalrymple et al. [2] that could involve one or more steps until the cell anatomy is disrupted causing the bacteria's death; i.e. it is neither active, nor culturable [49]. At this point a lysate is achieved. Afterwards, the most important families of cells components are involved in a net of parallel-series oxidative reactions that can compete with the active and injured cells for the hydroxyl radicals and thus affecting the disinfection rate.

In modeling the intrinsic reaction kinetics of disinfection with hydrogen peroxide one of the major difficulties is to include the iron or iron-superoxide participation in the mechanism to producing hydroxyl radicals [11,13]; i.e. the Fenton-like or Haber-Weis-like reactions:



$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \cdot OH + OH^-$$
$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + \cdot O_2H + H^+$$

or



$$Fe^{3+} + O_2^{\cdot -} \rightarrow Fe^{2+} + O_2$$
$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\cdot}$$

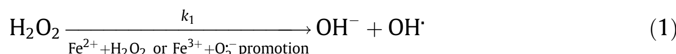
There are two reasons for this complication: (1) The real mechanistic interaction of iron compounds inside the cell to explain the effects produced by the oxidative radicals is not fully understood yet and (2) the concentrations of the ferric and the superoxide ions are not precisely known.

One artificial way to deal with this problem is to assume that the reaction is effectively mediated by iron or the couple ferric-superoxide ions, that they are at constant concentrations and that this process can be included as part of a hypothetical kinetic step. The respective pseudo specific constant of this stage represents the mechanism to generate the hydroxyl radicals.

3.1. A proposal of a tentative reaction representation of the bacteria death

The reactions that lead the hydroxyl radical generation are known to the point that, if desired, all the values of the specific kinetic constants of each step have been well established. Assuming an initiation step promoted by iron (III) or iron (III)-superoxide to split the hydrogen peroxide, the following sequence can be proposed:

3.1.1. Initiation



$$H_2O_2 \xrightarrow[Fe^{2+}+H_2O_2 \text{ or } Fe^{3+}+O_2^{\cdot -} \text{ promotion}]{k_1} OH^- + OH^{\cdot} \quad (1)$$

Note that, when the reaction is written as above, without including specifically the Fe^{3+} or the $Fe^{3+} + O_2^{\cdot -}$ promotion, k_1 is not known.

3.1.2. Propagation

$$H_2O_2 + OH^{\cdot} \xrightarrow{k_2} HO_2^{\cdot} + H_2O \quad (2)$$

$$H_2O_2 + HO_2^{\cdot} \xrightarrow{k_3} OH^{\cdot} + H_2O + O_2 \quad (3)$$

3.1.3. Termination

$$2OH^{\cdot} \xrightarrow{k_4} H_2O_2 \quad (4)$$

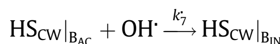
$$2HO_2^{\cdot} \xrightarrow{k_5} H_2O_2 + O_2 \quad (5)$$

$$OH^{\cdot} + HO_2^{\cdot} \xrightarrow{k_6} H_2O + O_2 \quad (6)$$

All these steps have been studied in details for several years now and are fully accepted in the scientific literature Buxton et al. [50], Laat and Gallard [51], Zalazar et al. [52] just to mention a few examples.

3.1.4. Membrane disruption

The process that ends up with the membrane breakdown may be treated by resorting to a pseudo-homogeneous interpretation of the intricate network of superficial reactions that leads to the rupture of the protective envelope. Let $HS_{CW}|_{B_{AC}}$ represent a hypothetical species of the components of the cell wall of the active bacteria whose concentration can be expressed in units of $mol\ cm^{-2}$ and that reacts with the $OH^{\cdot}$ radical. This composition is the same for every bacterium of a given family of bacteria. Then,



$$HS_{CW}|_{B_{AC}} + OH^{\cdot} \xrightarrow{k_7} HS_{CW}|_{B_{IN}}$$

At this point, it is possible to think of $SSA|_{B_{AC}}$ as the specific superficial area per unit volume of one cell (in units of $cm^2\ cm^{-3}$), $V|_{B_{AC}}$ as the volume of one cell per CFU (in units of $cm^3\ CFU^{-1}$) and $[B_{AC}]$ as the whole instantaneous concentration of the active bacteria (in units of $CFU\ cm^{-3}$ of reacting medium). The concentration of hypothetical species per unit volume of the fluid may be calculated from the above mentioned superficial concentration of this species as:

$$\underbrace{\underbrace{[HS_{CW}|_{B_{AC}}]}_{\text{Superficial concentration of of hypotetical species of one bacterium}} \times \underbrace{SSA|_{B_{AC}}}_{\text{Superficial area per unit volume of bacterium}} \times \underbrace{V|_{B_{AC}}}_{\text{volume of one CFU}} \times \underbrace{[B_{AC}](t)}_{\text{Instantaneous concentration of active bateria per unit volume}}}_{\text{Instantaneous representation of the total volumetric concentration of hypotetical species of the active bacteria}}$$

Taking into account averaging values for specific family of bacteria, $\left[HS_{CW}|_{B_{AC}}\right]$ in the cell wall, $SSA|_{B_{AC}}$ and $V|_{B_{AC}}$ may be assumed almost constant. Then,

$$[HS_{CW}|_{B_{AC}}] \times SSA|_{B_{AC}} \times V|_{B_{AC}} = \text{constant} \qquad \underbrace{k_7}_{\text{Pseudo-homogeneous volumetric kinetic constant}} = \underbrace{k_7^*}_{\text{Superficial kinetic constant}} \times [HS_{CW}|_{B_{AC}}] \times SSA|_{B_{AC}} \times V|_{B_{AC}}$$

However, it is very unlikely that just one hydroxyl radical will produce a serious damage to the cell wall. Neither the number of moles of OH**.** which are necessary to consider that the bacterium has been injured, nor the number of bacterium that form a CFU are known. It is always possible to conceive an oxidation yield as the ratio of injured CFU with respect to the spent hydroxyl radicals to produce this event:

$$k_7^{\#} = \frac{k_7}{Y_7}; \quad Y_7 = \frac{\text{Injured CFU}}{\text{OH}^{\cdot}\text{spent this event}} [=] \frac{\text{CFU}}{\text{mol}}; \quad k_7^{\#} [=] \frac{\text{cm}^3}{\text{CFU}_s}$$

In order to have the proper application of this yield as well as achieving unit's homogeneity it must be considered that:

$$R_{7,B_{AC}} = -k_7 [B_{AC}][OH^{\cdot}]$$
$$R_{7,OH} = -k_7^{\#} [B_{AC}][OH^{\cdot}]$$

With this pseudo homogeneous, biological reaction approach, step 7 can be finally expressed as:

$$B_{AC} + OH^{\cdot} \xrightarrow{k_7} B_{IN} \quad (7)$$

In a similar way, the same procedure can be applied to the injured bacteria in the reaction with the OH$^{\cdot}$ radicals according to:

$$\underbrace{k_8}_{\text{Pseudo-homogeneous volumetric kinetic constant}} = \underbrace{k_8^*}_{\text{Superficial kinetic constant}} \times [HS_{CW}|_{B_{IN}}] \times SSA|_{B_{IN}} \times V|_{B_{IN}}$$
$$k_8^{\#} = \frac{k_8}{Y_8}; \; Y_8 = \frac{\text{Dead CFU}}{\text{OH}^{\cdot}\text{spentin this event}} [=] \frac{\text{CFU}}{\text{mol}}; \; k_8^{\#} [=] \frac{\text{cm}^3}{\text{CFUs}}$$
$$R_{8,B_{IN}} = -k_8 [B_{IN}][OH^{\cdot}]$$
$$R_{8,OH} = -k_8^{\#} [B_{IN}][OH^{\cdot}]$$

$$B_{IN} + OH^{\cdot} \xrightarrow{k_8} B_{DE} \quad (8)$$

This step leads to the rupture of the cellular wall and the death of the bacterium.

3.1.5. Lysis of dead bacteria

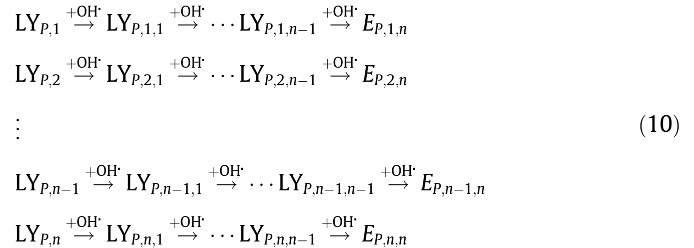
The death of the bacterium implies that the cellular wall has been broken permitting the access of the oxidant to the intracellular components, rendering the lysate:

$$B_{DE} + OH^{\cdot} \xrightarrow{k_9} \text{LYSATE} \quad (9)$$

where:

$$\underbrace{k_9}_{\text{Pseudo-homogeneous volumetric kinetic constant}} = \underbrace{k_9^*}_{\text{Superficial kinetic constant}} \times [HS_{CW}|_{B_{DE}}] \times SSA|_{B_{DE}} \times V|_{B_{DE}}$$
$$k_9^{\#} = \frac{k_9}{Y_9}; Y_9 = \frac{\text{Lysed CFU}}{\text{OH}^{\cdot}\text{spentin this event}} [=] \frac{\text{CFU}}{\text{mol}}; \quad k_9^{\#} [=] \frac{\text{cm}^3}{\text{CFU}_s}$$
$$R_{9,B_{LY}} = -k_9 [B_{DE}][OH^{\cdot}]$$
$$R_{9,OH} = -k_9^{\#} [B_{DE}][OH^{\cdot}]$$

Once the anatomic morphology of the cell has been altered producing the lysate, the OH$^{\cdot}$ can interact with its internal components in typical homogeneous reactions. From the chemical point of view the activity of the OH$^{\cdot}$ radicals can be interpreted in terms of several parallel-series reactions with an undefined number of hypothetical components of the resulting lysis. In the model they are represented by $LY_{P,1}, LY_{P,2}, \ldots LY_{P,n-1}, LY_{P,n}$, that correspond to important groups of compounds which, after ulterior oxidations will produce the end products of the disinfection process:



$$\begin{aligned} &LY_{P,1} \xrightarrow{+OH^{\cdot}} LY_{P,1,1} \xrightarrow{+OH^{\cdot}} \cdots LY_{P,1,n-1} \xrightarrow{+OH^{\cdot}} E_{P,1,n} \\ &LY_{P,2} \xrightarrow{+OH^{\cdot}} LY_{P,2,1} \xrightarrow{+OH^{\cdot}} \cdots LY_{P,2,n-1} \xrightarrow{+OH^{\cdot}} E_{P,2,n} \\ &\vdots \\ &LY_{P,n-1} \xrightarrow{+OH^{\cdot}} LY_{P,n-1,1} \xrightarrow{+OH^{\cdot}} \cdots LY_{P,n-1,n-1} \xrightarrow{+OH^{\cdot}} E_{P,n-1,n} \\ &LY_{P,n} \xrightarrow{+OH^{\cdot}} LY_{P,n,1} \xrightarrow{+OH^{\cdot}} \cdots LY_{P,n,n-1} \xrightarrow{+OH^{\cdot}} E_{P,n,n} \end{aligned} \quad (10)$$

3.2. Mathematical representation of the model

Operational parameters of the process might be obtained from the solution of a kinetic model, derived from the proposed sequence of processes that leads to the cell destruction. The calculation of the reaction rates for each step is carried out in every position of the reactor. However one should note that for a well mixed reactor in the absence of external effects (for example, irradiation or ultrasonic enhancements) compositions are uniform and correspond to the local values. The reaction's kinetics was formulated in terms of the mass action law for all the proposed steps to generate bacterial destruction by hydrogen peroxide. We can assume that:

(i). The system is isothermal with perfectly mixed compositions.
(ii). The micro steady state approximation is valid.
(iii). The first set of lysate products oxidation is enough to represent the disinfection process (to be confirmed with the results).

If our third assumption is valid, considering only the first oxidation reaction of lysate byproducts should be sufficient to represent the disinfection reaction even if it does not reach the total oxidation of the cell's chemical components.

From the reaction scheme we can derive the following expressions:

$$\begin{aligned} R_{OH^{\cdot}} = &\, k_1[H_2O_2] - k_2[H_2O_2][OH^{\cdot}] + k_3[H_2O_2][HO_2^{\cdot}] - k_4[OH^{\cdot}]^2 \\ &- k_6[OH^{\cdot}][HO_2^{\cdot}] - k_7^{\#}[B_{AC}][OH^{\cdot}] - k_8^{\#}[B_{IN}][OH^{\cdot}] \\ &- k_9^{\#}[B_{DE}][OH^{\cdot}] - \sum_{i=1}^{n} k_{10,i}[LY_{P,i}][OH^{\cdot}] \end{aligned} \quad (11)$$

$$\begin{aligned} R_{HO_2^{\cdot}} = &\, k_2[H_2O_2][OH^{\cdot}] - k_3[H_2O_2][HO_2^{\cdot}] - k_5[HO_2^{\cdot}]^2 - k_6[OH^{\cdot}] \\ &\times [HO_2^{\cdot}] \end{aligned} \quad (12)$$

The reaction rate expression for the Active and Injured bacteria are:

$$R_{B_{AC}} = -k_7[B_{AC}][OH^{\cdot}] \tag{13}$$

$$R_{B_{IN}} = k_7[B_{AC}][OH^{\cdot}] - k_8[B_{IN}][OH^{\cdot}] \tag{14}$$

Applying the Micro Steady State Assumption (MSSA), from steps (1) to (6) the hydroxyl radical concentration ($OH^{\cdot}$) can be readily calculated.

Additionally, on the basis of previous confirmed evidences it is assumed that the rates of termination steps (4) and (6) are negligible as compared with (5) that is the predominant chain termination step and that stage (2) is the predominant propagation step [53–55]; then the hydroxyl radical concentration is:

$$[OH^{\cdot}] = \frac{k_1[H_2O_2]}{k_2[H_2O_2] + k_7^{\#}[B_{AC}] + k_8^{\#}[B_{IN}] + k_9^{\#}[B_{DE}] + \sum_{i=1}^{n} k_{10,i}[LY_{P,i}]} \tag{15}$$

The value of $\sum_{i=1}^{n} k_{10,i}[LY_{P,i}]$ may be considered proportional to the concentration of dead bacteria. This simplification is possible because it involves the same level of arbitrariness that all the terms included in the summation symbol.

$$\sum_{i=1}^{n} k_{10,i}[LY_{P,i}] = \alpha k_{10}[B_{DE}] \tag{16}$$

$$[OH^{\cdot}] = \frac{k_1[H_2O_2]}{k_2[H_2O_2] + k_7^{\#}[B_{AC}] + k_8^{\#}[B_{IN}] + (k_9^{\#} + \alpha k_{10})[B_{DE}]} \tag{17}$$

Replacing Eq. (17) in into Eq. (13) and rearranging the result the final expression for the disappearance rate of Active bacteria is:

$$R_{B_{AC}} = -\frac{\gamma_A[B_{AC}][H_2O_2]}{[H_2O_2] + \gamma_0[B_{AC}] + \gamma_1[B_{IN}] + \gamma_2[B_{DE}]} \tag{18}$$

where

$$\gamma_A = \left(\frac{k_1 k_7}{k_2}\right); \quad \gamma_0 = \frac{k_7^{\#}}{k_2}; \quad \gamma_1 = \frac{k_8^{\#}}{k_2}; \quad \gamma_2 = \frac{(k_9^{\#} + \alpha k_{10})}{k_2}$$

Similarly, replacing Eq. (17) into Eq. (14) and operating on the result, the final expression for the disappearance rate of injured bacteria is:

$$R_{B_{IN}} = \frac{[B_{AC}][H_2O_2]\gamma_A - [B_{IN}][H_2O_2]\gamma_{IN}}{[H_2O_2] + \gamma_0[B_{AC}] + \gamma_1[B_{IN}] + \gamma_2[B_{DE}]} \tag{19}$$

where $\gamma_{IN} = \left(\frac{k_1 k_8}{k_2}\right)$

4. Results and discussion

Fig. 2 displays all the experimental data obtained in this work. Two direct conclusions can be extracted from these results. The first is that concentrations of hydrogen peroxide below approximately 300 ppm are impractical regardless the disinfection time. The second is that in all cases, the required processing extent does not appear to offer an attractive prospect for large-scale applications.

Moreover, from these results, it was not considered necessary a further increase in the concentration of hydrogen peroxide because, having reached five orders of magnitude in the change of the CFU population, the evolution of the figure suggests that a small reduction in the processing time would not have introduced a major improvement in the above mentioned disadvantageous circumstance.

A significant portion of the curves exhibits a linear behavior. However, simple first-order kinetics, as one knows, cannot represent the shoulder that is shown at the beginning of all experiments. Later we will see another advantage that has this model showing some aspects of the process that the first-order approximation would not be able to unveil.

Experiments were carried out in an isothermal, well mixed, batch reactor. Then the analysis was made with a very simple mass balance:

$$R_j = \frac{d[E]}{dt} \quad E = B_{AC}, B_{IN}, B_{DE}, LY_{P_i} \tag{20}$$

$$t = 0 \begin{cases} [B_{AC}] = [B_{AC}]^0 & \text{for } B_{AC} \\ [E] = 0 & \text{for } E = B_{IN}, B_{DE}, LY_{P_i} \end{cases}$$

The simulation results for $I = B_{AC} + B_{IN}$ (surviving bacteria) were compared with the data obtained from the cell culture of the sampling employing a non-linear, multiparameter optimization computer program, in order to obtain the values of the kinetic constants γ_k for $k = A, B, 0, 1$ and 2.

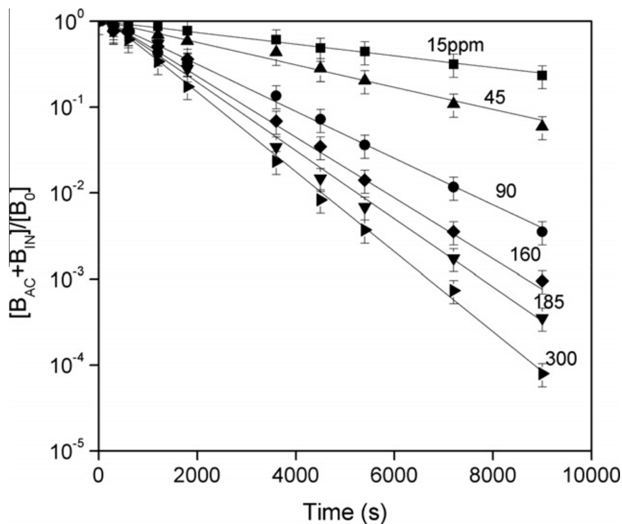


Fig. 2. Simulation results with the five parameters model (solid lines) compared with experimental data. The plot portrays the values of culturable cells.

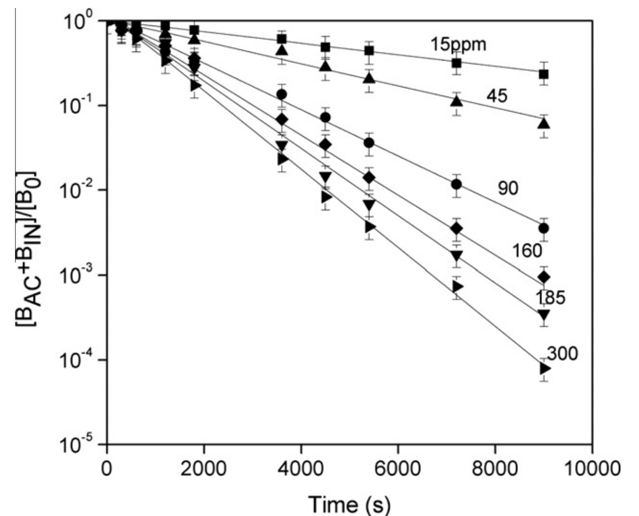


Fig. 3. Simulations results with the four parameters model (solid lines) compared with experimental data. The plot portrays the values of culturable cells.

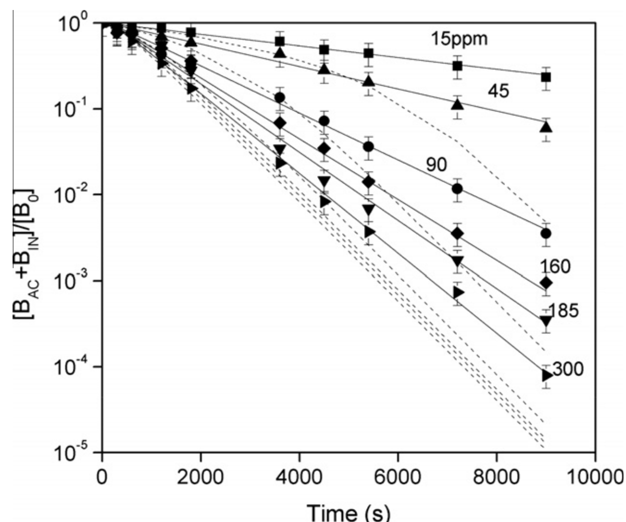


Fig. 4. Results assuming that the step producing the lysate of the dead bacteria is omitted. For $[B_{AC+}B_{IN}]/[B_0]$ vs. time: solid lines: complete model; broken lines: $\gamma_2 = 0$.

The obtained values of the kinetics parameters were

$$\gamma_A = (2.7 \pm 0.1) \times 10^{-3} \text{cm}^3 \text{ mol}^{-1} \text{ s}^{-1}$$
$$\gamma_{IN} = (9.6 \pm 3.1) \times 10^{-3} \text{ cm}^3 \text{ mol}^{-1} \text{ s}^{-1}$$
$$\gamma_0 = (1.5 \pm 0.2) \times 10^{-10} \text{ mol CFU}^{-1}$$
$$\gamma_1 = (4.9 \pm 2.2) \times 10^{-8} \text{ mol CFU}^{-1}$$
$$\gamma_2 = (1.7 \pm 0.2) \times 10^{-8} \text{ mol CFU}^{-1}$$

With these parameters, the agreement with the experimental data is shown in Fig. 3. The simulation results fit quite well all the experimental data for the whole range of explored hydrogen peroxide concentrations.

However, it can be seen that the value of γ_0 is rather small. It is possible to reduce the parameters of the model if we assume that:

$$\gamma_0[B_{AC}] \ll [H_2O_2] + \gamma_1[B_{IN}] + \gamma_2[B_{DE}] \tag{21}$$

Rearranging the resulting equations, the final expressions for Active and Injured bacteria result:

$$R_{B_{AC}} = -\frac{\gamma_A[B_{AC}][H_2O_2]}{[H_2O_2] + \gamma_1[B_{IN}] + \gamma_2[B_{DE}]} \tag{22}$$

$$R_{B_{IN}} = \frac{\gamma_A[B_{AC}][H_2O_2] - \gamma_{IN}[B_{IN}][H_2O_2]}{[H_2O_2] + \gamma_1[B_{IN}] + \gamma_2[B_{DE}]} \tag{23}$$

Using this assumption, a new set of four kinetic constants was obtained:

$$\gamma_A = (2.7 \pm 0.0) \times 10^{-3} \text{ cm}^3 \text{ mol}^{-1} \text{ s}^{-1}$$
$$\gamma_{IN} = (10.0 \pm 2.8) \times 10^{-3} \text{ cm}^3 \text{ mol}^{-1} \text{ s}^{-1}$$
$$\gamma_1 = (5.3 \pm 2.0) \times 10^{-8} \text{ mol CFU}^{-1}$$
$$\gamma_2 = (1.7 \pm 0.1) \times 10^{-8} \text{ mol CFU}^{-1}$$

The new plot is shown in Fig. 3.

It can be observed that the four parameters model also renders a good agreement with the experimental data. In this process exists a competion for the OH· radicals between two groups: (1) the dead bacteria and the compounds resulting from the lisate formation and (2) the active and the injured bacteria. The important question is to know to what extent the subsequent reactions after the bacteria's death affect the desinfection rate. These reactions correspond to the kinetic constants k_9 and αk_{10}. Both are grouped in γ_2. The importance of this conflict can be seen by making $\gamma_2 = 0$ (either because $k_9 = 0$ or $\alpha k_{10} = 0$ or both are cero). The results are shown in Fig. 4.

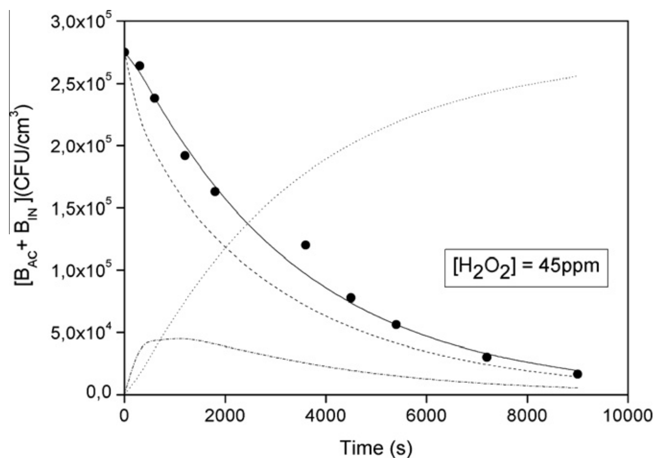


Fig. 5. A graphical description of the concentration evolution of the principal species existing in the reacting medium as seen by the model. The plot follows changes in concentration of the active, injured and dead bacteria at 45 ppm concentration of H_2O_2. Experimental data: solid line: culturable bacteria [BAC + BIN]; broken line: active bacteria; broken and dotted line: injured bacteria; dotted line: dead bacteria.

It becomes clear that with this assumption the model predicts a much faster degradation of the bacteria. The implication is that the existing competition for the OH· radicals should have been wrongly disregarded if γ_0 is made equal to zero, permitting to conclude that at least the step corresponding to the formation of the lysate cannot be ignored. The isolation of the effect corresponding to either k_9 or αk_{10} cannot be obtained from this model, but certainly αk_{10} cannot be different from zero if k_9 is equal to zero before.

The model also provides good information about the way in which the inactivation reaction proceeds. With the obtained parameters it is possible to follow the changes in concentration of the active, injured and dead bacteria. They are shown in Figs. 5 and 6 for two different concentrations of hydrogen peroxide.

It becomes clear that the life of the injured bacteria is very short and its concentration never reaches very high values. This effect can be seen by looking at the values of the obtained parameters.

From these two figures it can also be observed a consistent behavior. When lower concentrations of hydrogen peroxide are

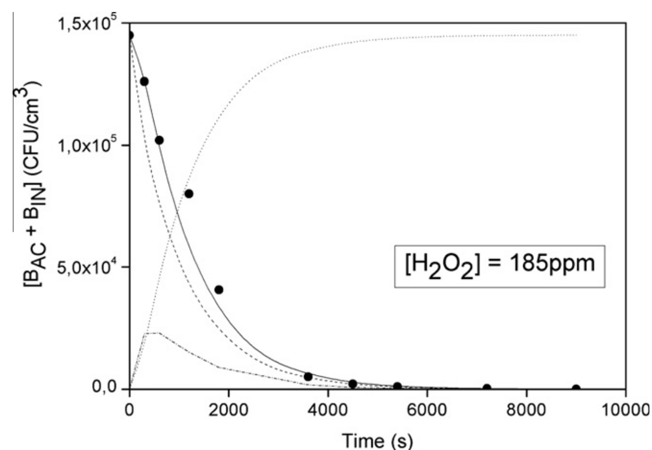


Fig. 6. A graphical description of the concentration evolution of the principal species existing in the reacting medium as seen by the model. The plot follows changes in concentration of the active, injured and dead bacteria at 185 ppm concentration of H_2O_2. Experimental data: solid line: culturable bacteria $[B_{AC} + B_{IN}]$; broken line: active bacteria; broken and dotted line: injured bacteria; dotted line: dead bacteria.

used (45 ppm) the survival of a major number of active and injured bacteria is seen, indicating that the oxidation is not sufficiently intense to overcome the resistance of the cell wall. Conversely, at higher concentrations, (185 ppm) the number of survival and undamaged bacteria gradually decreases and there is a breaking of the bacteria envelope in a shorter time. Then, very rapidly, it changes from the state of injured bacteria to one of irreversible death. For that reason, the concentration of injured bacteria is always low. This is, at the same time, a confirmation that oxidant concentration is the most important factor to affect bacterial target sites [56].

Both figures show in the curves represented by the solid lines, the small shoulder mentioned in the beginning of this section.

It is also very interesting to note that taking the ratio between k_7 and k_8 (which is equal to 0.28) one gets the relationship between injured bacteria and fully active bacteria. This is shown more clearly by setting Eq. (14) equal to zero resulting $[B_{IN}]/[B_{AC}] = 0.28$. This is good evidence that the first stage is slower than the second. But this relationship is not much less than one to allow grouping both stages is a single one. Consideration of the mechanistic series process, allows unequivocally demonstrating the existence of the initial resistance observed in the shoulders of the figures.

5. Conclusions

A new kinetic model for chemical disinfection with hydrogen peroxide has been developed. It was validated with experiments using *E. coli* as a surrogate bacterium. Simulation results with a four parameters mathematical description provide very good agreement with the experimental data for a wide range of hydrogen peroxide concentrations.

The proposed mechanism involves a series of three heterogeneous steps (analyzed as pseudo-homogeneous reactions) followed by a group of parallel-series oxidation reactions in the homogeneous phase.

The disinfection process is characterized by the following steps: (1) Production of the hydroxyl radicals which is supposed to be promoted by a Fenton like or Haber-Weis-like reactions; (2) A first attack by the hydroxyl radical to damage the cellular wall; (3) A second attack by the same oxidant, that produces the complete destruction of the bacterium envelope; (4) The formation of a lysate with all the cell components; (5) Subsequent oxidation of the chemical species resulting from the lysis of the bacterium.

The reactions leading to the formation of the lysate and the further oxidation of the cells components compete with the active and injured bacteria for the hydroxyl radical, a phenomenon that with the help of the model can be clearly unveiled.

In accordance with the model, the action that leads to the complete destruction of the bacterium cell wall is faster than the one that produces the initial damage that transforms the active bacterium into injured bacterium.

The reported model is based on an idealized description of the chemical disinfection, but does not introduce a conceptually distorted representation of the processes involved in the bacterium death. Consequently, the obtained kinetic parameters should be independent of the type and size of the employed laboratory reactor, as long as the reported operating conditions are reproduced; i.e. isothermal performance, pH and oxidant concentrations. Then, they should be useful for scale-up or reactor design as well as optimization procedures.

Finally, it should be remarked that this is a very general model that by the simple expedient of adding one set of parallel reactions (that were not needed in this work) it is possible to represent typical tailings of disinfection curves very often shown when a different disinfectant is used.

Acknowledgments

The authors are grateful to Universidad Nacional del Litoral (UNL), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), and Agencia Nacional de Promoción Científica y Técnica (ANPCyT) for its financial support.

References

[1] M.D. Labas, C.S. Zalazar, R.J. Brandi, A.E. Cassano, Reaction kinetics of bacteria disinfection employing hydrogen peroxide, Biochem. Eng. J. 38 (2008) 78–87.
[2] O.K. Dalrymple, E. Stefanalos, M.A. Trotz, D.Y. Goswami, A review of the mechanisms and modeling of photocatalytic disinfection, App. Catal. B 98 (2010) 27–38.
[3] B.R. Cords, S.L. Burnett, J. Hilgren, M. Finley, J. Magnuson, Sanitizers: halogens, surface-active agents, and peroxides, in: P.M. Davidson, J.N. Sofos, A.L. Branen (Eds.), Antimicrobials in Food, 3rd ed., CRC Press Taylor & Francis Group, Florida, 2005.
[4] S. Malato, P. Fernandez-Ibañez, M.I. Maldonado, J. Blanco, W. Gernjak, Decontamination and disinfection of water by solar photocatalysis: recent overview and trends, Catal. Today 147 (2009) 1–59.
[5] S. Rafellini, M. Schenk, S. Guerrero, S.M. Alzamora, Kinetics of *Escherichia coli* inactivation employing hydrogen peroxide at varying temperatures, pH and concentrations, Food Control 22 (2011) 920–932.
[6] J.A. Imlay, S. Linn, Toxic DNA damage by hydrogen peroxide through the Fenton reaction in vivo and in vitro, Science 240 (1988) 640–642.
[7] G. Brandi, F. Cattabeni, A. Albano, O. Cantoni, Role of hydroxyl radicals in *Escherichia coli* killing induced by hydrogen peroxide, Free Radic. Res. Commun. 6 (1989) 47–55.
[8] J.J. Schurman, Antibacterial Activity of Hydrogen Peroxide Against Escherichia coli 2001.
[9] B.J. Juven, M.D. Pierson, Antibacterial effects of hydrogen peroxide and methods for it detection and quantization, J. Food Prot. 59 (1996) 1233–1241.
[10] B. Halliwell, Oxidative stress in cell culture: an under-appreciated problem?, FEBS Lett 540 (2003) 3–6.
[11] T. Nunoshiba, F. Obata, A.C. Boss, S. Oikawa, T. Mori, S. Kawanishi, K. Yamamoto, Role of iron and superoxide for generation of hydroxyl radical, oxidative DNA lesions and mutagenesis in *Escherichia coli*, J. Biol. Chem. 274 (1999) 34832–34837.
[12] H. Sies, Role of the reactive oxygen species in biological process, Klin. Wochenschr. 69 (1991) 965–968.
[13] J.P. Kehrer, The Haber-Weiss reaction and mechanisms of toxicity, Toxicology 149 (2000) 43–50.
[14] E.B. Freese, J. Gerson, H. Taber, H.J. Rhaese, E. Freese, Inactivating DNA alterations induced by peroxides and peroxide-producing agents, Mutat. Res. 4 (1967) 517–531.
[15] S.P. Wolff, A. Garnera, R.T. Dean, Free radicals, lipids and protein degradation, Trends Biochem. Sci. 11 (1986) 27–31.
[16] V. Vallyathan, Generation of oxygen radicals by minerals and its correlation to cytotoxicity, oxygen radicals and lung injury, Environ. Health Perspect. 102 (1994) 111–115.
[17] P.J. Barnes, Reactive oxygen species and airway inflammation, Free Radical. Biol. Med. 9 (1990) 235–243.
[18] A.R. Krapp, V.B. Tognetti, N. Carrillon, A. Acevedo, The role of ferredoxin-NADP super (+) reductase in the concerted cell defense against oxidative damage. Studies using *Escherichia coli* mutants and cloned plant genes, Eur. J. Biochem. 249 (1997) 556–563.
[19] F. Buyuksonmez, T.F. Hess, R.L. Crawford, R.J. Watts, Toxic effects of modified fenton reaction on *Xanthobacter flavus* FB 71, Appl. Environ. Microbiol. 64 (1998) 3759–3764.
[20] G. Storz, M.F. Christman, H. Sies, B.N. Ames, Spontaneous mutagenesis and oxidative damage to DNA in Salmonella typhimurium, Proc. Natl. Acad. 84 (1987) 8917–8921.
[21] K. Anzai, M. Hamasuna, H. Kadono, S. Lee, H. Aoyagi, Y. Kirino, Formation of ion channels in planar lipid bilayer membranes by synthetic basic peptides, Biochem. Biophys. Acta 1064 (1991) 256–266.
[22] A.G. Rincón, C. Pulgarin, N. Adler, P. Peringe, Interaction between *E. coli* inactivation and DBP-precursors-dihydroxybenzene isomers – in the photocatalytic process of drinking-water disinfection with TiO_2, J. Photochem. Photobiolog. A: Chem. 139 (2001) 233–241.
[23] B. Halliwell, J.M. Gutteridge, Biologically relevant metal ion-depend hydroxyl radical generation. An update, FEBS 307 (1992) 108–112.
[24] R.J. Watts, D. Washington, J. Jowsawkeng, F.L. Loge, A.L. Tell, Comparative toxicity of hydrogen peroxide, hydroxyl radical, and superoxide anion to *Escherichia coli*, Adv. Environ., Res. 7 (2003) 961–968.
[25] I. Yamazaki, L.H. Piette, EPR spin-trapping study on the oxidizing species formed in the reaction of the ferrous ion with hydrogen peroxide, J. Am. Chem. Soc. 113 (1991) 7588–7593.
[26] S. Croft, B.C. Gilbert, J.R. Lindsay Smith, A.C. Whitwood, An E.S.R. investigation of the reactive intermediate generated in the reaction between Fe(II) and H_2O_2 in aqueous solution. Direct evidence for the formation of the hydroxyl radical, Free Radicals Res. 17 (1992) 21–39.

[27] Y. Sun, J.J. Pignatello, Chemical treatment of pesticide wastes, evaluation of iron(III) chelates for catalytic hydrogen peroxide oxidation of 2,4-D at circumneutral pH, J. Agric. Food Chem. 40 (1992) 322–327.
[28] S.S. Lin, M. Gurol, Catalytic decomposition of hydrogen peroxide on iron oxide: kinetics, mechanism, and implications, Environ. Sci. Technol. 32 (1998) 1417–1423.
[29] R.J. Watts, M.K. Foget, S.H. Kong, A.L. Teel, Hydrogen peroxide decomposition in model subsurface systems, J. Hazar. Mater. B69 (1999) 229–243.
[30] W. Kwan, M. Voelker, Decomposition of hydrogen peroxide and organic compounds in the presence of dissolved iron and ferrihydrite, Environ. Sci. Technol. 36 (2002) 1467–1476.
[31] Romero, A. Santos, T. Cordero, Soil remediation by Fenton-like process: phenol removal and soil organic matter modification, Chem. Eng. J. 170 (2011) 36–43.
[32] B. Halliwell, J.M. Gutteridge, Role of free radicals and catalytic metal ions in human disease: an overview, Methods Enzymol. 186 (1990) 1–85.
[33] A. Romero, A. Santos, F. Vicente, S. Rodriguez, A. Lopez Lafuente, In situ oxidation remediation technologies: kinetic of hydrogen peroxide decomposition on soil organic matter, J. Hazard. Mater. 170 (2009) 627–632.
[34] H. Chick, An investigation into the laws of disinfection, J. Hyg. 8 (1908) 92–158.
[35] H.E. Watson, A note on the variation of the rate of disinfection with change in the concentration of the disinfectant, J. Hyg. 8 (1908) 536–542.
[36] L.W. Hom, Kinetics of chlorine disinfection in an ecosystem, J. Environ. Div. ASCE 98 (1972) 183–194.
[37] O. Cerf, Tailing of survival curves of bacterial spores, J. Appl Bacteriol 42 (1977) 1–19.
[38] R.L. Buchanan, M.H. Golden, J.G. Philips, Expanded safety concerns, the proposed method should be used models for non-thermal inactivation of *Listeria monocytogenes*, J. Appl. Microbiol. 82 (1997) 567–577.
[39] J.M. Membre, V. Majchrzak, I. Jolly, Effects of temperature, pH, glucose, and citric acid on the inactivation of *Salmonella typhimurium* in reduced calorie mayonnaise, J. Soc. Chem. London. Food Prot. 60 (1997) 1497–1501.
[40] B. Severin, M. Suidan, R. Engelbrecht, Kinetic modeling of U.V. disinfection of water, Water Res. 17 (1983) 1669–1678.
[41] J. Marugan, R. van Grieken, C. Pablos, M.L. Satuf, A.E. Cassano, O.M. Alfano, Rigorous kinetic modeling with explicit radiation absorption effects of the photocatalytic inactivation of bacteria in water using suspended titanium dioxide, Appl. Catal. B: Environ. 102 (2011) 404–416.
[42] M.L. Speck (Ed.), Compendium of Methods for the Microbiological Examination of Foods, second ed., APHA, American Public Health Association, Washington, DC, 1984.
[43] R. Marshall, Standard Methods for the Examination of Dairy Products, 16th ed., Editorial APHA, American Public Health Association, Washington, DC, 1992.
[44] M.D. Labas, C.A. Martin, A.E. Cassano, Kinetics of bacteria disinfection with UV radiation in an absorbing and nutritious medium, Chem. Eng. J. 114 (2005) 87–97.
[45] M.D. Labas, R.J. Brandi, C.S. Zalazar, A.E. Cassano, Water disinfection with UVC radiation and H_2O_2, a comparative study, Photochem. Photobiol. Sci. 8 (2009) 670–676.
[46] A.O. Allen, C.J. Hochanadel, J.A. Ghormley, T.W. Davis, Decomposition of water and aqueous solution under mixed fast neutron and gamma radiation, J. Phys. Chem. 56 (1952) 575–586.
[47] Official Methods of Analysis, 15th ed., AOAC, Arlington, VA, 1990.
[48] J. Marugan, R. van Grieken, C. Sordo, C. Cruz, Kinetics of the photocatalytic disinfection of *Escherichia coli* suspensions, Appl. Catal. B: Environ. 82 (2008) 27–36.
[49] D.B. Kell, A.S. Kaprelyants, D.H. Weichart, C.R. Harwood, M.R. Barer, Viability and activity in readily culturable bacteria: a review and discussion of the practical issues, Antonier van Leeuwenhoek 73 (1998) 169–187.
[50] G. Buxton, C. Greenstock, W. Helman, A. Ross, A critical review of data constants for reactions of hydrated electrons, hydrogen atoms and hydroxyl radicals in aqueous solutions, J. Phys. Chem. Ref. Data 17 (1988) 513–886.
[51] H. Gallard, J. De Laat, Kinetic modelling of Fe(III)/H_2O_2 oxidation reactions in dilute aqueous solution using atrazine as a model organic compound, Water Res. 34 (2000) 3107–3116.
[52] C.S. Zalazar, M.D. Labas, R.J. Brandi, A.E. Cassano, Dichloroacetic acid degradation employing hydrogen peroxide and UV radiation, Chemosphere 66 (2007) 281–286.
[53] C.H. Liao, M.D. Gurol, Chemical oxidation by photolytic decomposition of hydrogen peroxide, Environ. Sci. Tech. 29 (1995) 3007–3014.
[54] O.M. Alfano, R.J. Brandi, A.E. Cassano, Degradation kinetics of 2-4-D in water employing hydrogen peroxide and UV radiation, Chem. Eng. J. 82 (2001) 209–218.
[55] C.S. Zalazar, M.E. Lovato, M.D. Labas, R.J. Brandi, A.E. Cassano, Intrinsic kinetics model of the oxidative reaction of dichloroacetic acid employing hydrogen peroxide and ultraviolet radiation, Chem. Eng. Sci. 62 (2007) 5840–5853.
[56] A.D. Russell, G. McDonnell, Concentration: a major factor in studying biocidal action, J. Hosp. Infect. 44 (2000) 1–3.