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# Reaction kinetics of bacteria disinfection employing hydrogen peroxide

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**Abstract**

The inactivation reaction of *Escherichia coli* bacteria employing hydrogen peroxide at 20 °C and pH = 7 was studied in a well-mixed batch reactor. The proposed objective, as far as the extent of inactivation is concerned, was obtained for $H_2O_2$ concentrations above 100 ppm (1 ppm = $2.94 \times 10^{-5}$ mmol cm$^{-3}$) but, compared with other disinfection technologies, for too long reaction times. Below 40 ppm of the oxidant concentration inactivation was practically ineffective. Results were analyzed employing Modified forms of the Series-Event and Multitarget mechanistic models. At concentrations above 100 ppm the induction time in the semi-logarithmic plot of bacteria concentration versus time was reduced. With both modified models it was found that the reaction order with respect to the hydrogen peroxide concentration was different than one. Both mathematical descriptions provide a good representation of the experimental results in an ample range of the disinfectant concentrations and confirm a methodology that renders the starting point of a reaction kinetic expression useful for further studies regarding the optimization of the operating conditions (pH and temperature, for example), including also combination with other advanced oxidation technologies. An interpretation of the data in terms of a Weibull-like model [1] is also included.


*Keywords:* Water disinfection; Kinetic parameters; Hydrogen peroxide; Modelling; Waste-water treatment; *Escherichia coli*

## 1. Introduction

The antimicrobial and/or antiseptic properties of hydrogen peroxide have been known for many years because of its efficacy, versatility and reasonable manipulation safety. The bactericidal effect of hydrogen peroxide on biological systems has been reported, showing growth inhibition and/or inactivation of pathogenic microorganisms in vegetative bacteria, fungi, viruses, mycobacteria and bacterial spores when using the appropriate disinfectant concentration and operating conditions. In fact it has been used as an antimicrobial agent since the early 1800s and it is well known for its use as a topical skin application in 3% concentrations [2]. In foods, $H_2O_2$ was used as a disinfectant in milk as early as 1904 [3]. During the latest decades of the twentieth century extensive research efforts have been dedicated to study the hydrogen peroxide effects on different varieties of bacteria [4–7].

However, quantitative kinetics results to render conclusive data to decide on its economical feasibility are very scarce. The question is then posed in terms of efficiency rather than efficacy.

It is generally considered that the inhibition of microbial growth by hydrogen peroxide is not the direct result of its oxidative properties in its molecular state, but the consequence of the activity of other strongly oxidant chemical species derived from it. In fact, hydrogen peroxide is an excellent source of singlet oxygen, superoxide radicals ($O_2^{\bullet -}$) and hydroxyl radicals ($^{\bullet}$OH) that are highly reactive and very toxic for microorganisms [8,9]. Although the exact mechanism by which $H_2O_2$ produces lethal products for many microorganisms has not been clearly and completely elucidated, it is well known that, due to its ability to produce the above mentioned derivatives with strong oxidative properties, it can produce damage to nucleic acids, enzymes and membrane constituents [10,11]. As such, it has been considered as one potential advanced oxidation technology (AOT). However, it has also been reported that aqueous solutions of $H_2O_2$ alone will not cause protein, lipid, or nucleic acid modifications without the presence of catalysts for radical formation [12].

Not only the biological effect was investigated in these pathogenic microorganisms [13,14] but also research was conducted trying to understand the kinetics of disinfection processes [15–17]. Among these studies, with different approaches, some work has been specifically done concerning hydrogen peroxide effects on *Escherichia coli* [18–21].

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**Nomenclature**

| | |
|---|---|
| $b$ | inactivation kinetic parameter of Weibull-like model ($s^{-n}$) |
| $C_{B,i}$ | bacteria concentration with state of damage $i$ (CFU $cm^{-3}$) |
| $C_d$ | chemical disinfectant concentration (mmol $cm^{-3}$ or ppm) |
| $C_P$ | hydrogen peroxide concentration (mmol $cm^{-3}$ or ppm) |
| CFU | colony forming units |
| $k_M$ | inactivation kinetic constant of Modified Multitarget model ($s^{-1}$ $(cm^3/mmol)^{\gamma}$) |
| $k_S$ | inactivation kinetic constant of Modified Series-Event model ($s^{-1}$ $(cm^3/mmol)^{\beta}$) |
| $n$ | parameter of Weibull-like model |
| $n_c$ | number of discrete critical targets |
| $n_S$ | events number |
| $R_{B,i}$ | reaction rate corresponding to the bacteria with a state of damage $i$ (CFU $cm^{-3}$ $s^{-1}$) |
| S | survival ratio |
| $t$ | time (s) |
| $T$ | temperature (K) |
| $\underline{x}$ | position vector (cm) |

*Greek letters*

| | |
|---|---|
| $\beta$ | reaction order with respect to $H_2O_2$ in the Modified Series-Event Model. |
| $\gamma$ | reaction order with respect to $H_2O_2$ in the Modified Multitarget Model. |

In this work we studied the disinfection kinetics of *E. coli* using $H_2O_2$ and searched for a representation of the inactivation phenomenon that could be apt for an exact quantification of its effectiveness and eventually for reactor design purposes. If the inactivation rate is sufficiently high, the obtained kinetic parameters with the employed approach should be useful for scaling-up objectives because these results, i.e., the model and the kinetic parameters, are independent of the reactor shape, size and most of the operating conditions (some limitations are unavoidable due to the practically inevitable limits in the extent of the range of explored variables). No effect of interfering substances on the disinfection process has been considered for the moment, as it has been the case of the distinct work on kinetic modelling of these phenomena carried out by Lambert and Johnston [22,23].

Nevertheless, it must be remarked that this is just the first step in a work intended to analyze the hydrogen peroxide ability to inactivate microorganisms exploring with quantitative, fairly reliable models, its aptitude under, *prima facie*, the most economical conditions, i.e., room temperature and natural pH and compare the results with UVC radiation alone, previously studied employing similar environments [24]. It is clear that depending on the results, very likely several additional variables should be explored in a subsequent work: (i) initial pH variations (ii) temperature effects recognizing that this is a conventional thermal reaction, (iii) effects of bacteria agglomeration, (iv) the role of the water matrix and (v) an eventual combination with other AOTs, particularly UVC radiation.

## 2. Kinetic models

### 2.1. Potential mechanisms for $H_2O_2$ disinfection

The damaging effects of the bacteria cellular components seems to be produced by a particular phenomenon called oxidative stress, resulting from reactive oxygen species (ROS); more specifically $^{\bullet}OH$ radicals. These are oxygen derivable radicals having high capability to produce cellular damage.

In fact, the oxidative stress may be a consequence of the cellular own aerobic metabolism [9,25], or the action of its internal immune system acting on potential competitors or reacting to the attack by undesired pathogenic agents or the result of an aggression by external chemical substances such as hydrogen peroxide.

There are several ways that hydrogen peroxide can be transformed to bring forth hydroxyl radicals; among them it can be included: (i) interaction with transition metal ions existing in the medium, e.g., copper, iron, etc. [26], (ii) participation with the existing intra or extra cellular $Fe^{2+}$ to produce a typical Fenton reaction [27], (iii) acting in combination with UV irradiation [28,29] and (iv) decomposing by a dismutation reaction with a maximum rate at the pH of its $pK_a$ (*ca.* 11.7), [30]. Reactive oxygen species can also be the result of partial reduction of a reactive molecule such as oxygen. ROS can affect the cell in different levels and it is considered, as indicated before, that hydroxyl radicals constitute one of these chemical species with the largest potential to produce cellular damage. At this point it is an indisputable fact that the working context may have a strong influence in the inactivation results. For example, an analysis with atomic absorption spectrometry of the employed culture in the growing broth employed in this work revealed the existence of small concentrations of iron and copper ions. They could be the required catalyst to promote the hydrogen peroxide activity.

Moreover, hydrogen peroxide is not a large molecule and is able to diffuse through the cell membrane and, once inside, to produce hydroxyl radicals ($^{\bullet}OH$) by means of some of the mechanisms previously mentioned [9].

Hydroxyl radicals may impact on different components of the cell producing the oxidative stress that leads to irremediable consequences. Oxidation of different amino acids such as tyrosine, phenylalanine, tryptophan, histidine, methionine and cysteine leads to a loss of the ability of the corresponding protein molecule to properly accomplish its specific function [31]. They may also act on the lipids to yield a peroxidation reaction that severely affects the cellular membrane integrity [32]. One of the consequences of this reaction is the increase in the membrane rigidity resulting in a loss of its permeability or other changes that produce a deterioration of the membrane's internal organization [33,34]. Acting on the cell's DNA, ROS and, more specifically ($^{\bullet}OH$), can produce a break in the double chain or chemical modifications in the nitrogen bases [31]. Noteworthy, the lethal damage can be produced by hydrogen peroxide exist-

ing in the medium (exogenous effect) or the one produced by the cell (endogenous effect). However, bacteria have their own enzymatic mechanisms or catalases that, within limits, exert a self protecting action [35].

### 2.2. Kinetic model proposals

Research on the kinetics of bacteria inactivation with hydrogen peroxide is rather scarce. Imlay and Linn [36] working with *Escherichia coli* K-12 proposed two kinetically distinguishable inactivation modes: (i) mode one, that is exhibited at low hydrogen peroxide concentrations (for instance, $1 \times 10^{-3}$–$2 \times 10^{-3}$ mmol cm$^{-3}$), requires the existence of an active metabolism during the killing action, (ii) mode two, that displays its characteristics at higher hydrogen peroxide concentrations [0.1 mmol cm$^{-3}$], does not require the existence of an active metabolism during its action and needs some form of multihit association of both the hydrogen peroxide and the contact time of the oxidant with the microorganism.

Lambert et al. [16] reported the disinfection kinetics of *Staphylococcus aureus* and *Pseudomonas aeruginosa* employing hydrogen peroxide and peracetic acid and interpreted the results in terms of the well-known and practical Chick–Watson expression and its modifications [37]. Working at a constant temperature, with the same equation, including an arbitrary constant exponent "*n*" to the disinfectant concentration; a similar study had been previously conducted by Hugo and Denyer [38] and was applied by Lambert et al. in their above mentioned work. With the introduction of the empirical model developed by Hom [39] in previous studies, a different approach resulted from the confirmation of the existence of non-linear "log reduction of viable microorganisms versus time" plots rendering, within limits, an improvement to the established basic "law". A tailing effect after several minutes of processing time has also been observed when the surrounding environment had some peculiar, but very frequent characteristics [40–43]. Both, initial shoulders and final tailings cannot be interpreted in terms of the Chick and Watson model.

Yamagiwa et al. [17] studied the disinfection kinetics of *Legionella pneumophila* employing $H_2O_2$. They found that the inactivation reaction can be very often characterized by two different steps: (i) in the first, there is a time lag while the concentration of viable cells remains constant and (ii) in the second, the cell concentration typically followed a first order kinetics with respect to the microbe concentration. It was also found that the effect of temperature was well described by an Arrehenius type of equation reinforcing the argument that the disinfectant affects the microorganisms by means of as yet not too clearly elucidated set of damaging chemical reactions.

A sizable proportion of the published literature on inactivation of different microorganisms reports the existence of the above mentioned initial shoulder and/or plateau in a semilogarithmic plot of survivor microorganisms versus time. This behavior has been attributed to an initial resistance to the disinfectant by all or a critical part of the involved cellular chemical species, as well as other more specific features of the steps corresponding to the chemical attack. Two mechanistic types of kinetic models have been proposed to represent this behavior: the Series-Event Model and the Multitarget Model [15].

Working on UV disinfection, we have already introduced some conceptual refinements with several changes in one of these models (the Series-Event) that led to an improvement in the representation of the collected experimental data [24,44]. In chemical disinfection we are proposing that in Severin's models there is no reason to assume that the inactivation rate must be first order with respect to the hydrogen peroxide concentration. There are too many complex reactions involved to expect that they will be well represented by such a simple interpretation. On the other hand, after the initial time lag, according to the available experimental data [16,17], and *in the absence of tailing*, it seems quite acceptable to assume some form of first order dependence with respect to the colony forming units (CFU) concentration.

### 2.3. The Modified Series-Event model

The representation is based on the idea that an event is assumed to be a unit of microorganism damage. Events occur in a stepwise fashion and each step is considered a separate event. The model is thought of as a series of consecutive "damaging reactions" or events. Damage is considered to occur in integer steps and we are also assuming that each event (damaging reaction) can be described, as a reasonable approximation, with the same kinetic constant. The idea can be expressed as a series of chemical reactions where we have already modified the reaction order with respect to the disinfectant concentration:

$$M_{B,0} \xrightarrow{k_1 C_P^{\beta}} M_{B,1} \xrightarrow{k_2 C_P^{\beta}} \cdots M_{B,i} \xrightarrow{k_{i+1} C_P^{\beta}} \cdots M_{B,n_S-1} \xrightarrow{k_{n_S} C_P^{\beta}} M_{B,n_S}$$
$$\text{with } i = 0, 1, 2, \ldots, n_s \text{ and } k_1 = k_2 \cdots = k_{n_S} = k_S \qquad (1)$$

The corresponding kinetics expressions are:

$$\begin{aligned}
&\text{for } i = 0 && \rightarrow R_{B,i}(\underline{x}, t) = -k_S C_P^{\beta}(\underline{x}, t) C_{B,i}(\underline{x}, t) \\
&\text{for } i = 1, 2, \ldots, n_S - 1 && \rightarrow R_{B,i}(\underline{x}, t) = k_S C_P^{\beta}(\underline{x}, t) C_{B,i-1}(\underline{x}, t) - k_S C_P^{\beta}(\underline{x}, t) C_{B,i}(\underline{x}, t) \\
&\text{for } i = n_S && \rightarrow R_{B,i}(\underline{x}, t) = k_S C_P^{\beta}(\underline{x}, t) C_{B,i-1}(\underline{x}, t)
\end{aligned} \qquad (2)$$

In these equations two concentrations are included: hydrogen peroxide concentration ($C_P$) and the *Escherichia coli* concentration ($C_{B,i}$) where the subscript "*i*" indicates the particular "event" under consideration. Although the existence of a unique kinetic constant is an assumption that resulted valid in a previous work employing UV radiation [24,44], it should be tested with experiments in different processes, especially if working with very dissimilar microorganisms or a mix of them.

The special "mass" balance for CFU inside the well-mixed, isothermal, batch reactor is:

$$\frac{dC_{B,i}(t)}{dt} = R_{B,i}(t); \quad i = 0, 1, 2, \ldots n_S - 1 \tag{3}$$

With the following initial conditions:

$$t = 0 \quad \begin{cases} \text{for } i = 0 & \rightarrow C_{B,i} = C_B^o \\ \text{for } i = 1, 2, \ldots, n_S - 1 & \rightarrow C_{B,i} = 0 \end{cases}$$

Combining the kinetic expression with the CFU balance, after integration one gets:

$$\frac{C_B}{C_B^o} = [\exp(-k_S C_P^\beta t)] \sum_{i=0}^{n_S-1} \frac{(k_S C_P^\beta t)^i}{i!} \tag{4}$$

The inactivated bacteria concentrations are given by:

$$C_{B,D} = C_B^o - C_B \tag{5}$$

In Eq. (5) the subscript D refers to "damaged" or inactivated bacteria. Whereas the surviving microorganisms can be obtained from:

$$C_B = \sum_{i=0}^{i=n_S-1} C_{B,i} \tag{6}$$

### 2.4. The Modified Multitarget model

A second approach which has been used to describe the initial resistance of microorganisms is the Multitarget model. In the development of this model, it is assumed that a microorganism contains a finite number, $n_c$, of discrete critical targets, each of which must be hit prior to reach the desired full inactivation of the living "particle". A particle may represent an organism with $n_c$ critical targets or a clump of organisms possessing a total of $n_c$ targets. The model cannot distinguish between a clump of organisms or an individual organism with many targets. If uniform clumping is presumed, rather than the internal resistance of a single organisms, a different rate equation must be devised which includes the decrease in the probability of attaining lethal hits on viable organisms as the number of viable organisms is depleted.

The corresponding kinetic expressions are:

$$\begin{aligned} &\text{for } i = 0 && \rightarrow R_{B,i}(\underline{x}, t) = -n_c\, k_M C_P^\gamma(\underline{x}, t) C_{B,i}(\underline{x}, t) \\ &\text{for } i = 1, 2, ..., n_c - 1 && \rightarrow R_{B,i}(\underline{x}, t) = (n_c - i + 1) k_M C_P^\gamma(\underline{x}, t) C_{B,i-1}(\underline{x}, t) - (n_c - i) k_M C_P^\gamma(\underline{x}, t) C_{B,i}(\underline{x}, t) \\ &i = n_c && \rightarrow R_{B,i}(\underline{x}, t) = (n_c - i + 1) k_M C_P^\gamma(\underline{x}, t) C_{B,i-1}(\underline{x}, t) \end{aligned} \tag{7}$$

The special "mass" balance for a well-mixed reactor, in terms of the CFU, is again:

$$\frac{dC_{B,i}(t)}{dt} = R_{B,i}(t); \quad i = 0, 1, 2, \ldots, n_c - 1 \tag{8}$$

The initial conditions are:

$$t = 0 \quad \begin{cases} \text{for } i = 0 & \rightarrow C_{B,i} = C_B^o \\ \text{for } i = 1, 2, \ldots, n_c - 1 & \rightarrow C_{B,i} = 0 \end{cases}$$

Inserting the kinetic model in the CFU balance, after integration:

$$\frac{C_B}{C_B^o} = 1 - [1 - \exp(-k_M C_P^\gamma t)]^{n_c} \tag{9}$$

The concentration of inactivated bacteria is given by Eq. (5). And the surviving bacteria concentration is:

$$C_B = \sum_{i=0}^{i=n_c-1} C_{B,i} \tag{10}$$

It should be noted that from previously published qualitative information quoted in the introduction, in both models the value of $n_S$ (or $n_c$) could depend on the hydrogen peroxide concentration.

## 3. The reacting system

The employed reactor is a well-stirred batch reactor with a useful volume equal to 1000 $cm^3$. The tank is surrounded by a jacket that is connected to a water thermostatic bath (Haake) to keep the system temperature constant at 20 °C. The tank has a mechanical stirrer, a thermometer, a sampling port and a high flow rate recirculating system (Masterflex Model 7553–76) to improve the mixing operating conditions. Connections between the different components of the recycle were achieved with silicone tubing.

## 4. Experimental procedure

*Escherichia coli* strain ATCC 8739 was used throughout this work. The purity of the strain was verified by conventional methods [45,46]. The culture was grown in a complex medium (nutritive broth) having beef extract as the main component. 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 permitted to ensure that there is no bacteria growth during the disinfection run because the growing culture concentration was sufficiently diluted [24,44]. Atomic spectroscopy analysis 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, weighted amount of hydrogen peroxide (Merck, *pro analysis* 30%) and ultra pure water. $H_2O_2$ concentrations were varied between 15 and 300 ppm and measured with colorimetric techniques at 350 nm (Perkin-Elmer-330 Spectrophotometer) according to Allen et al. [47]. The initial concentrations at $t = 0$ were measured (in all cases the initial values were very close to $10^5$ CFU $cm^{-3}$) and afterwards, samples were withdrawn at different time intervals for several determinations. Runs were duplicated

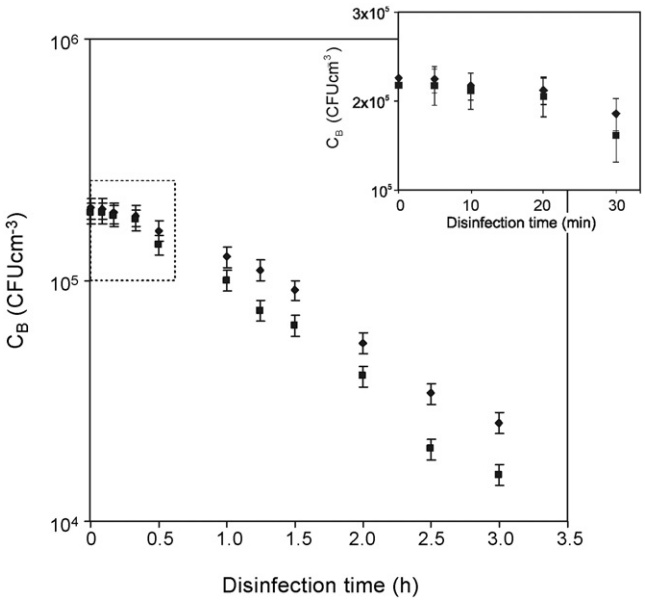


Fig. 1. Bacteria inactivation at low hydrogen peroxide concentrations. (♦) $C_p = 15$ ppm; (■) $C_p = 25$ ppm. Change in *E. coli.* concentration: 1 log (1 ppm = $2.94 \times 10^{-5}$ mmol cm$^{-3}$).

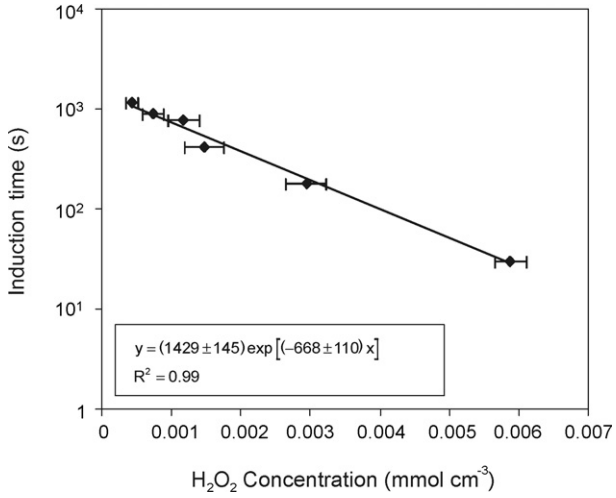


Fig. 2. Variation of induction time as a function of the initial concentration of hydrogen peroxide.

and samples subjected to triplicate determinations. The initial pH was 7 and remained practically constant during all runs. Each sample was examined with the following measurements: absorbance at 350 nm (spectrophotometric analysis for hydrogen peroxide) and CFU counting using specific Pretrifilm$^{TM}$ plates (3M Microbiology Products) for *Escherichia coli* and coliform bacteria. This method has been recognized by the American Public Health Association in Standard Methods for the Examination of Dairy Products [45] and the AOAC (Association of Official Analytical Chemists International) in Official Methods of Analysis [48] as equivalent to the conventional plate method for this type of microorganisms. Dilution of the samples to obtain the optimum concentration for the CFU counting method was made with 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 (Catalase from Aspergillus Niger-BioChemika). Control experiments were conducted to ensure that the employed concentrations of catalase solutions did not affect bacteria concentrations. Likewise, experiments were carried out without hydrogen peroxide to make sure that the starting solution was free from other inactivating agents. The plates were incubated, after spreading with the appropriate volume of sample, for 24 h at 37 °C.

## 5. Results and discussion

The following general results were observed:

1. There is a low limiting hydrogen peroxide concentration below which inactivation is not effective ($C_P < 25$ ppm). Reduction of contamination is poor even for very long reaction times (Fig. 1). The reached inactivation never exceeded 90%.
2. Above a given hydrogen peroxide concentration ($C_P > 100$ ppm) the time lag, during which the viable bacteria concentration is not changed, is reduced (Fig. 2). In addition, inactivation reaches values as high as 99.99%.
3. The time required for this inactivation extent was too large (150 min) to provide good expectations concerning the use of hydrogen peroxide alone, at almost neutral pH and 20 °C, as a recommended disinfection process. Concerning this point, Table 1 shows a comparison with disinfection experiments performed with the same bacteria and growing culture, employing low monochromatic irradiation rates at 253.7 nm.
4. The change in the oxidant concentration during the inactivation run is very low in relation to the initial feed (from 4% at the lowest hydrogen peroxide concentrations to 1% at those above 100 ppm). This behavior was confirmed repeating the same experiment several times and is in agreement with similar observations reported for peracetic acid [49]. It must be stressed that although small, the hydrogen peroxide concentration change along the experiments was clearly observed in all the experiments, indicating that under no circumstances it can be considered constant.
5. In spite of the above mentioned low oxidant consumption the effect of increasing the $H_2O_2$ concentration produces notice-

Table 1
Comparison of reaction times for 99.99% inactivation: UVC radiation vs. hydrogen peroxide

| Process | Time (h) |
|---|---|
| UV lamp. Nominal input power: 15 W. Irradiation rate at the reactor windows: $2.76 \pm 0.07$ mW cm$^{-2}$ at 253.7 nm. Bacteria and growing culture as described in this work. | 0.02 |
| UV lamp. Nominal input power: 15 W. With neutral density filter. Irradiation rate at the reactor windows: $0.45 \pm 0.03$ mW cm$^{-2}$ at 253.7 nm. Bacteria and growing culture as described in this work. | 0.03 |
| Hydrogen peroxide: 100 ppm | 4.00 |

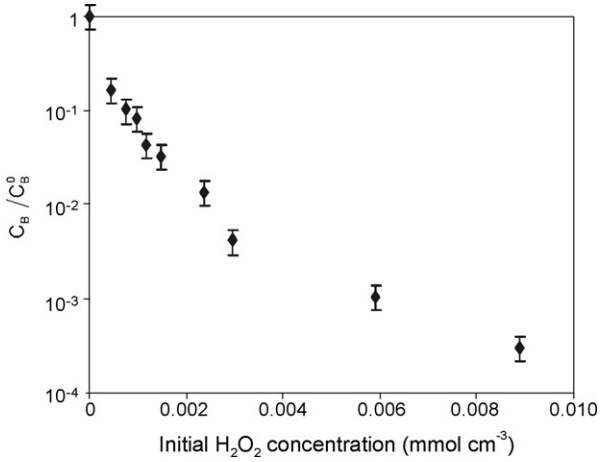


Fig. 3. Change in final concentration of *Escherichia coli* concentration as a function of the initial hydrogen peroxide concentration after 150 min of reaction time.

able changes in the final CFU concentrations for the same reaction time. Fig. 3 shows this effect; in all cases for an arbitrarily chosen 150 min of reaction time. This has to be recognized as a kinetic effect (a specific influence on the rate) and the one described in point 4, figuratively speaking, should be considered as some sort of stoichiometric outcome (relating the hydrogen peroxide consumption with the changes in CFU concentrations).

6. For a 99.99% inactivation, the required reaction time is inversely proportional to the hydrogen peroxide concentration and the plot gives an almost perfect straight line (Fig. 4).

The whole set of experimental data was used to investigate the validity of the models proposed in previous sections.

Both expressions (Eqs. (4) and (9)) can be incorporated into a non-linear multiparameter estimator, based on the Levenberg-Marquardt optimizing procedure [50,51]. Then, predicted bacteria concentration as a function of time from the model (with three unknown parameters) can be compared with

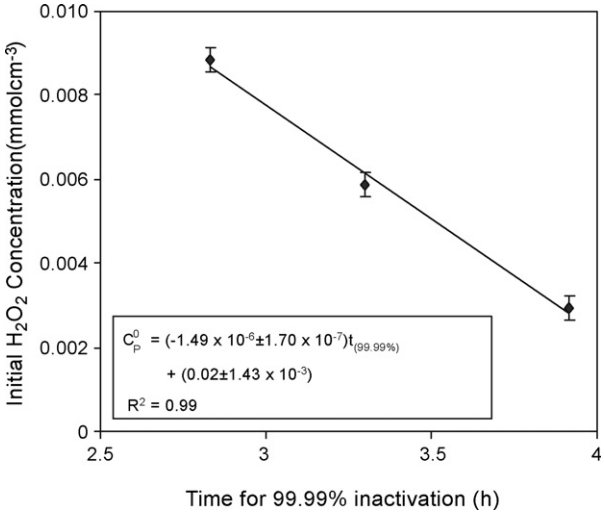


Fig. 4. Effect of the initial hydrogen peroxide concentration on the required reaction time for a 99.99% inactivation.

the corresponding experimental results. The values of $n_S$ and $n_c$ were restricted to integer, positive numbers. The result of the method provides values of $n_S$, $k_S$ and $\beta$ for the Modified Series-Event Model and values of $n_c$, $k_M$ and $\gamma$ for the Modified Multitarget Model. The results within a 95% confidence interval are presented in what follows.

### 5.1. Modified events in Series model

The events number is a function of the $H_2O_2$ concentration:

$n_S = 1$ for $C_P \geq 100$ ppm $(2.94 \times 10^{-3}$ mmol cm$^{-3})$

$n_S = 2$ for $C_P < 100$ ppm$(2.94 \times 10^{-3}$ mmol cm$^{-3})$

The obtained kinetic constant is:

$k_S = (0.37 \pm 0.05) \times 10^{-2}$ s$^{-1}$(cm$^3$/mmol)$^{\beta}$

and the reaction order with respect to $H_2O_2$ is:

$\beta = (0.293 \pm 0.022)$

### 5.2. Modified Multitarget model

The target number is a function of the $H_2O_2$ concentration:

$n_c = 1$ for $C_P \geq 100$ ppm $(2.94 \times 10^{-3}$ mmol cm$^{-3})$

$n_c = 2$ for $C_P < 100$ ppm$(2.94 \times 10^{-3}$ mmol cm$^{-3})$

The obtained kinetic constant is:

$k_M = (0.69 \pm 0.07) \times 10^{-2}$s$^{-1}$(cm$^3$/mmol)$^{\gamma}$

and the reaction order with respect to $H_2O_2$ is:

$\gamma = (0.420 \pm 0.019)$

Fig. 5(a) and (b) shows some of the results in a representation of the model predictions with the experimentally adjusted values of the kinetic parameters (solid lines) compared with the experimental results. For values of $C_P \geq 100$ ppm with $n_S$ (or $n_c$) = 1 and for values of $C_P < 100$ ppm with $n_S$ (or $n_c$) = 2. It becomes clear that in both cases concentrations of hydrogen peroxide lower than approximately 100 ppm prolong the induction time. Additionally, one extra outcome of both models is that there exists some room within the operating conditions for optimization, because the reaction order with respect to the hydrogen peroxide concentration is lower than one. These rather unusual exponents in both models seem to provide undisputable arguments to consider that the attack of hydrogen peroxide may involve a very complex mechanism on different chemical constituent of the cell, leading to this atypical overall reaction order. However, the observed long reaction times for a reasonable degree of inactivation (99.99%) do not provide too much hope to use hydrogen peroxide alone under the explored, preconceived more economical operating conditions.

From previous results (shown in Fig. 5(a) and (b)) and the small errors in the estimated parameters it can be concluded that, although the model was not independently validated, expressions (4) and (9) represent very well the experimentally observed

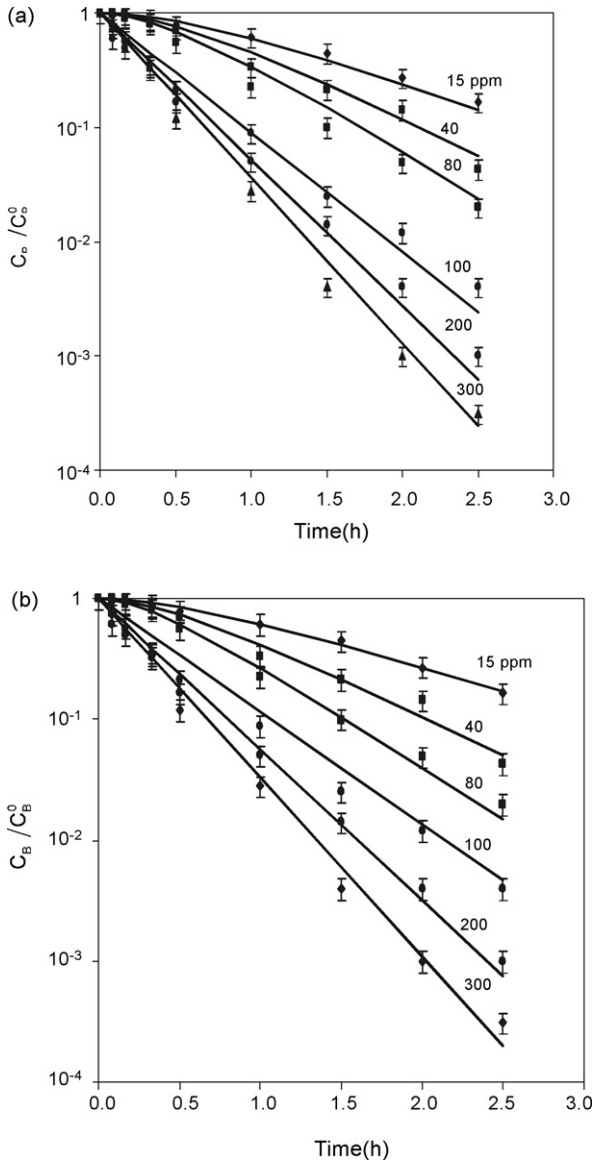


Fig. 5. Bacteria inactivation as a function of time. Comparison of model predictions (solid lines) with experimental data. Hydrogen peroxide concentration is the parameter. (a) Modified Series-Event Model. (b) Modified Multitarget Model.

data. Both models describe with almost the same accuracy the experimental results. However, it is fair to recognize that the assumptions involved in the formulation of the Modified Multitarget model seem to be, to some extent, more realistic.

It is very interesting to note that for concentrations of hydrogen peroxide above 100 ppm, both models give $n_S = n_c = 1$ and Eqs. (2) and (7) reduce to the modified Chick and Watson model with a variable exponent as reported by Hugo and Denyer [38]. Thus, with a $H_2O_2$ concentration above 100 ppm the mathematical model is greatly simplified.

## 6. A comparison with a Weibull-like model

It may be interesting to compare the obtained results with the two models employing three parameters, with a well-known model widely employed in bacteria inactivation and sterilization in the food industry. Significant contributions have been made in this area by Peleg and Cole [1], Campanella and Peleg [52], Peleg et al. [53] and Corradini and Peleg [54]. The proposed model for thermal inactivation has the following general expression (the decimal logarithm is used in the original work):

$$\ln S(t) = -b(T)t^{n(T)} \quad \text{where } S = \left(\frac{C_B}{C_B^0}\right) \tag{11}$$

In this equation $b$ and $n$ are function of the temperature. For chemical disinfection, Peleg [55] has proposed the following expression:

$$\ln S(t) = -b(C_d)t^{n(C_d)} \tag{12}$$

In Eq. (12) the subscript d stands for the chemical disinfectant. In this work, d will be substituted by $H_2O_2$. The problem is to describe the functionality of "$b$" and "$n$" with the chemical disinfectant concentration. Different trials were made with the experimental data, assuming initially that $n = 1$ for $C_P \geq 100$ ppm and $n = 1.2$ for $C_P < 100$ ppm. In Fig. 6 the change in the coefficient $b$ with the hydrogen peroxide concentration ($C_P$) is shown. It gives a typical sigmoidal curve.

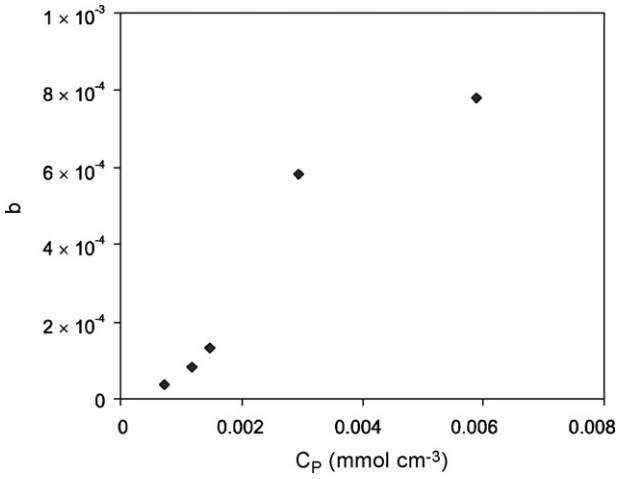


Fig. 6. A representation of the constant $b$ of the Weibull-like model as a function of the hydrogen peroxide concentration.

Following similar approaches for thermal and chemical disinfection, it is possible to propose a sigmoidal logistic expression for $b = b\,(C_P)$ and a Boltzmann-type function for $n = n\,(C_P)$:

$$b(C_P) = \frac{k_1}{1 + k_2 \exp(-k_3 C_P)} \tag{13}$$

$$n(C_P) = 1.25 - \frac{0.25}{1 + k_4 \exp(-k_5 C_P)} \tag{14}$$

In Eqs. (13) and (14) $k_1$, $k_2$, $k_3$, $k_4$ and $k_5$ are parameters to be obtained from experiments.

The results within a 95% confidence interval are:

$$k_1 = (7.84 \pm 0.29) \times 10^{-4}\ \text{s}^{-n}$$

$$k_2 = (7.24 \pm 0.09) \times 10$$

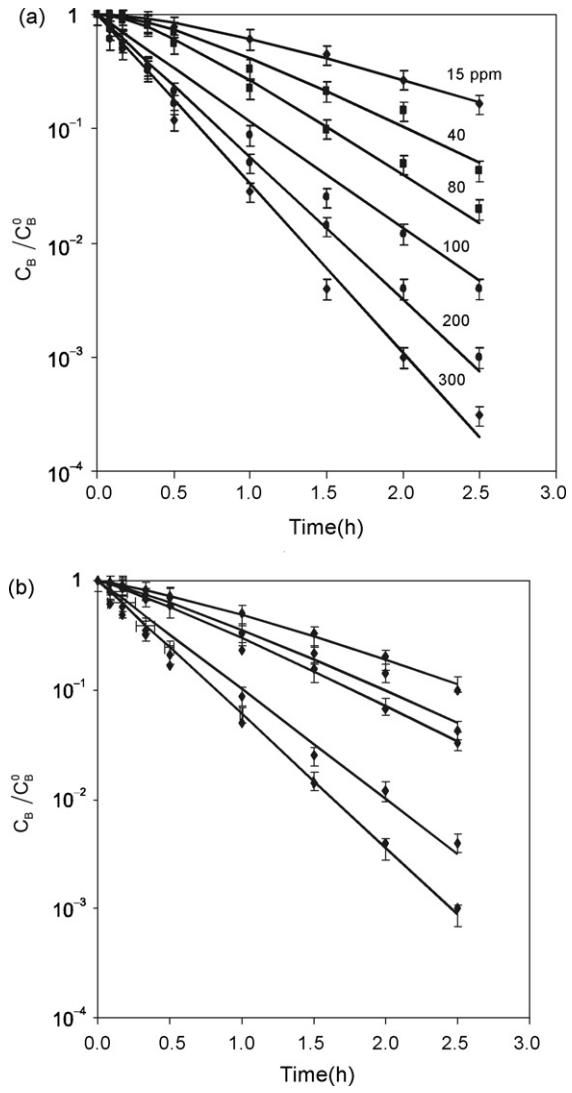


Fig. 7. Comparison of the interpretation of the experimental results with the Weibull-like model (a) and the Modified Multitarget model (b). Solid lines are results from the models.

$$k_3 = (1.82 \pm 0.06) \times 10^3\ \mathrm{cm^3\ mmol^{-1}}$$

$$k_4 = (1.79 \pm 0.08) \times 10$$

$$k_5 = (2.07 \pm 0.10) \times 10^3\ \mathrm{cm^3\ mmol^{-1}}$$

In Fig. 7(a) and (b) the ratio of the ln $C_B/C_B^0$ versus time is represented for the Weibull-like model and the Modified Multitarget model. Solid lines correspond to the models.

Even though, the first one has five empirical parameters, the results are very satisfactory.

## 7. Conclusions

The described results permit to summarize the following conclusions:

- The inactivation rate presents a clear dependence on the $H_2O_2$ concentration.
- The initial time lag for bacteria inactivation is noticeably reduced when the hydrogen peroxide concentration is increased.
- The dependence of the inactivation rate with the $H_2O_2$ concentration is not of the first order.
- The change in hydrogen peroxide concentration along the disinfection process is very small but detectable.
- Both proposed models, employing just three experimentally adjusted parameters, represent reasonably well the experimental data and have shown to be useful for describing the process for an ample range of hydrogen peroxide concentrations, and to show that the initial concentration of the oxidant is very critical to expect an applicable method for the prescribed, acceptable inactivation results.
- The reaction time, even for rather large values of the initial hydrogen peroxide concentrations is too long, turning this method unadvisable under the stated operating conditions, particularly when compared with UVC alone [24].
- However, the developed models pave the way to analyze other operating conditions, i.e., the employed approach will be a valuable instrument in order to search for more favorable results (modification of pH or temperature) and eventually render a useful tool for scaling-up purposes.
- A representation of the results employing a Weibull-like model gave satisfactory results employing five experimentally fitted parameters.

The natural extension of this work is to analyze the effects of pH and temperature, as well as bacteria agglomeration and changes in the water composition, and include these variables in the model, as well as the modeling of the more complex, combined UVC + $H_2O_2$ process. In this last case, the obtained results will be very useful to describe the parallel dark reaction or, eventually, a very good argument to neglect it.

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