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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 = 2.94×10^{-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

β	reaction order with respect to H_2O_2 in the Modified Series-Event Model.
γ	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-