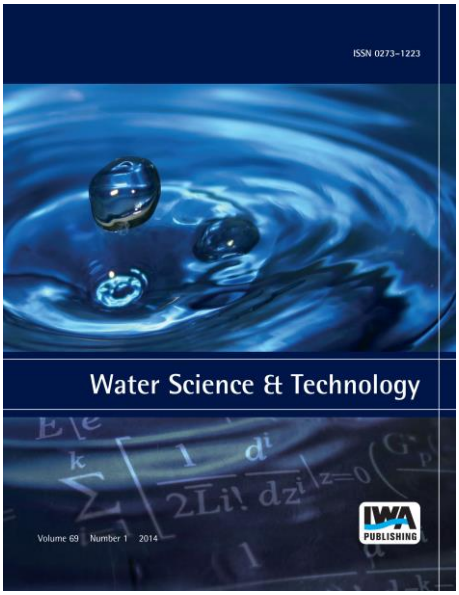

# Kinetic model of water disinfection using peracetic acid including synergistic effects

Marina J. Flores, Rodolfo J. Brandi, Alberto E. Cassano and Marisol D. Labas

## ABSTRACT

The disinfection efficiencies of a commercial mixture of peracetic acid against *Escherichia coli* were studied in laboratory scale experiments. The joint and separate action of two disinfectant agents, hydrogen peroxide and peracetic acid, were evaluated in order to observe synergistic effects. A kinetic model for each component of the mixture and for the commercial mixture was proposed. Through simple mathematical equations, the model describes different stages of attack by disinfectants during the inactivation process. Based on the experiments and the kinetic parameters obtained, it could be established that the efficiency of hydrogen peroxide was much lower than that of peracetic acid alone. However, the contribution of hydrogen peroxide was very important in the commercial mixture. It should be noted that this improvement occurred only after peracetic acid had initiated the attack on the cell. This synergistic effect was successfully explained by the proposed scheme and was verified by experimental results. Besides providing a clearer mechanistic understanding of water disinfection, such models may improve our ability to design reactors.

**Key words** | *Escherichia coli*, kinetic model, peracetic acid, synergism, water disinfection

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## INTRODUCTION

The primary aim of public policies for protecting public health is to ensure access to microbiologically safe drinking water. The efficient removal of pathogenic bacteria, viruses and protozoan parasites from water and wastewaters is critical, since sewage discharges may increase the risks of waterborne infections. An ideal disinfection system should efficiently and reliably destroy infectious agents under normal operating conditions, without producing toxic disinfection by-products (DBPs).

Commercial peracetic acid (PAA) has been increasingly employed in recent years because of its relatively low cost and the confirmation that it is harmless to the environment (Block 2001; Kitis 2004; Flores *et al.* 2014). The main components of commercial PAA, an equilibrium quaternary solution, are PAA and hydrogen peroxide (HP) in varying concentrations. The outstanding attributes of commercial PAA are its ease of implementation, the absence of persistent toxic or mutagenic residual DBPs, short reaction contact time and effectiveness for the treatment of primary and secondary effluents (Kitis 2004).

The fact that the disinfection efficiencies of both disinfectant agents produce a synergistic effect to achieve more effective pathogen inactivation (Flores *et al.* 2014) motivated us to study the contribution that each agent could achieve by acting alone and the global effects when acting together.

Few studies on the interaction of HP and PAA in the mixture (Alasri *et al.* 1992; Wagner *et al.* 2002) have been published in the literature and they focus only on the description of experimental results. To the best of our knowledge, the combined effect of PAA and HP has been reported in one previous work only, which proposes a simple explanation about the synergistic effect of PAA and HP (Flores *et al.* 2014).

This work aims to propose a kinetic model for the chemical disinfection based on the inactivation of bacteria, which is often used to indicate biological contamination in water (*Escherichia coli*). The kinetics includes the joint action of two disinfectant agents, PAA and HP, in order to assess their impact.

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## MATERIALS AND METHODS

A well-stirred, cylindrical batch reactor with a total reaction volume of 2,000 $cm^3$ was employed in all experimental runs. A cooling jacket connected to a thermostatic bath (Haake) kept the reacting system at a constant temperature of 20 °C. The reactor had provisions for sampling, and pH and temperature measurements. Good mixing, essential for the proposed model, was achieved with a specially designed, custom-made stirring device, with an external orbital shaking mechanism.

The following chemicals were used: commercial PAA: PAA 15% by vol., HP 20% by vol., acetic acid 25% by vol., and water 40% by vol. (Quimica Agroindustrial Neo); bovine liver catalase from Sigma Aldrich C 1345 [2,000–5,000 U $mg^{-1}$ $s^{-1}$]; potassium permanganate solution 0.1 N (pro analysis), sulphuric acid 95–98% and sodium thiosulfate 0.1 N (pro analysis) from Cicarelli; physiological saline solution (Roux-Ocefa); nutrient broth 20 g/L, EMB agar 37.5 g/L (eosin methylene blue agar) and peptone water 15 g/L from Biokar Diagnostics.

The most widely used method for analyzing solutions containing PAA and HP was proposed by Greenspan & Mackellar (1948). In this method, HP is first titrated with potassium permanganate in acid media and the residual PAA is then determined by adding potassium iodide to the solution and titrating the released diodine with sodium thiosulfate.

*Escherichia coli* strain ATCC 8739 was used throughout this work. The culture was grown in a complex medium (nutrient broth), with beef extract as the main component. The solution used for the experiments was prepared from a culture that had reached the stationary growth phase, bringing it to a 1/1,000 dilution with a physiological saline solution. This dilution ensured that there was no bacteria growth during the disinfection run because the growing culture concentration had been sufficiently diluted (Labas *et al.* 2008; Flores *et al.* 2012, 2014). The prepared culture was mixed with the desired, calculated concentration of PAA in the reactor.

Samples were withdrawn at different intervals, starting from initial concentrations of bacteria ($t = 0$) always around $10^5$ CFU (colony forming units) $mL^{-3}$. Experiments were duplicated and samples subjected to triplicate determinations. The initial pH was 6 and remained practically constant during all experiments. Samples were diluted with sterile peptone water solution to obtain the optimum concentrations for the CFU counting method.

To quench the PAA and HP action during the time interval between sampling and spread plating, a known fraction of the sample was mixed with the required amount of sodium thiosulfate (200 μL) and catalase solution (500 μL), respectively. After spreading them with 100 μL of sample, the plates were incubated for 24 hours at 37 °C in EMB agar.

During the disinfection experiments, two consecutive sets of samples were taken. We studied the inactivation rate and, on the other hand, we analyzed variations in the concentration of the oxidizing agent by analytical methods.

Qualitative studies were performed to evaluate the residual power of the commercial solution of PAA. For this reason, samples taken at the end of each experimental run were spread and incubated at 37 °C in EMB agar at different time intervals: 24, 48 and 72 hours. No regrowth was observed, indicating that *E. coli* was unable to recover from the damage caused by the oxidant. Disinfection always reached more than 99.99% effectiveness.

Therefore, the commercial mixture was also investigated free from HP, which was removed using bovine catalase, thus allowing the study of the efficiency of PAA alone. According to the desired work concentration of HP, liver bovine catalase was added. In the experimental runs when peroxide was removed from the commercial solution using catalase, peroxide formation was observed neither in 300 seconds nor at 60, 120 or 180 minutes.

## KINETIC MODEL DEVELOPMENT

To explain the inactivation process of *E. coli* with PAA, a kinetic scheme was proposed. Table 1 shows the full kinetic scheme of the inactivation process. As indicated before, the individual contribution of disinfecting agents was analyzed separately. In Table 1 $B_{ACT}$ is active bacteria, $B_{SEN}$ is sensitized bacteria, $B_{CIN}$ is catalase-inhibited bacteria, $B_{DAM}$ is damaged bacteria and $B_{ID}$ is irreversibly damaged bacteria; $r$ represents the reaction rate of each stage, $K$ is the kinetic parameter and $C$ represents the concentration.

The model was developed based on the following experimental results: (i) runs with PAA when HP was fully removed; (ii) runs with HP taken from a previous work (Labas *et al.* 2008; Flores *et al.* 2014); and (iii) runs with commercial PAA solution including both oxidants.

Each stage of the inactivation process was modeled as a first-order kinetic expression with respect to both microorganism concentration and disinfectant concentration.

**Table 1** | Kinetic scheme proposal

| | Inactivation stage | Kinetic expression | |
|---|---|---|---|
| PAA acting alone | $\mathrm{PAA} + \mathrm{B_{ACT}} \rightarrow \mathrm{B_{SEN}}$ | $r_{PAA,1} = (K_{PAA,1}C_{PAA})C_{B_{ACT}}$ | (1) |
| | $\mathrm{PAA} + \mathrm{B_{SEN}} \underset{\text{catalase action}}{\rightarrow} \mathrm{B_{CIN}}$ | $r_{PAA,2} = (K_{PAA,2}C_{PAA})C_{B_{SEN}}$ | (2) |
| | $\mathrm{PAA} + \mathrm{B_{CIN}} \rightarrow \mathrm{B_{ID}} \rightarrow \mathrm{Lysis}$ | $r_{PAA,3} = (K_{PAA,3}C_{PAA})C_{B_{CIN}}$ | (3) |
| HP acting alone | $\mathrm{HP} + \mathrm{B_{ACT}} \rightarrow \mathrm{B_{SEN}}$ | $r_{HP,1} = (K_{HP,1}C_{HP})C_{B_{ACT}}$ | (4) |
| | $\mathrm{HP} + \mathrm{B_{SEN}} \underset{\text{catalase attack}}{\rightarrow} \mathrm{B_{DAM}}$ | $r_{HP,2} = (K_{HP,2}C_{HP})C_{B_{SEN}}$ | (5) |
| | $\mathrm{HP} + \mathrm{B_{DAM}} \rightarrow \mathrm{B_{ID}} \rightarrow \mathrm{Lysis}$ | $r_{HP,3} = (K_{HP,3}C_{HP})C_{B_{DAM}}$ | (6) |
| Synergistic mixture system | $\mathrm{PAA} + \mathrm{B_{SEN}} \rightarrow \mathrm{B_{DAM}}$ | $r_{PAA,4} = (K_{PAA,4}C_{PAA})C_{B_{SEN}}$ | (7) |
| | $\mathrm{PAA} + \mathrm{B_{DAM}} \rightarrow \mathrm{B_{ID}} \rightarrow \mathrm{Lysis}$ | $r_{PAA,5} = (K_{PAA,5}C_{PAA})C_{B_{DAM}}$ | (8) |
| | $\mathrm{HP} + \mathrm{B_{CIN}} \rightarrow \mathrm{B_{ID}} \rightarrow \mathrm{Lysis}$ | $r_{HP,4} = (K_{HP,4}C_{HP})C_{B_{CIN}}$ | (9) |

Taken in order, these events represented the injury of increasing severity spanning from the active bacteria to the bacterial lysis.

Firstly, this kinetic proposal studies the separate contribution of both oxidizing agents, PAA and HP and, secondly, their joint effect on the commercial solution for the disinfection of water.

For modeling the reacting system, the disinfectant concentration was considered as follows: (i) uniform throughout the reactor volume and perfectly mixed; and (ii) in excess and constant throughout the reaction. The working experimental conditions were the following: temperature at 20 °C, clear water, pH near circumneutral and perfect mix. This behavior was experimentally verified during the experimental trial.

Under the above assumptions and in the case of water disinfection with PAA acting alone (HP removed), for each state of bacteria involved, a set of equations based on mass balance in the batch reactor was as follows:

$$\frac{dC_{B_{ACT}}}{dt} = -r_{PAA,1} \tag{10}$$

$$\frac{dC_{B_{SEN}}}{dt} = -r_{PAA,2} + r_{PAA,1} \tag{11}$$

$$\frac{dC_{B_{CIN}}}{dt} = -r_{PAA,3} + r_{PAA,2} \tag{12}$$

$$\frac{dC_{B_{ID}}}{dt} = +r_{PAA,3} \tag{13}$$

with the following initial conditions:

$$C_{B_{ACT}} = C^0_{B_{ACT}}\,;\; C_{B_{SEN}} = C_{B_{CIN}} = C_{B_{ID}} = 0 \tag{14}$$

At initial time, the active bacteria concentration was the same as the one that was put into the experimental reactor; no sensitized, catalase-inhibited or irreversibly damaged bacteria were present.

Other initial conditions can be represented by Equation (15).

$$C_{B_{VIA}} = C_{B_{ACT}} + C_{B_{SEN}} + C_{B_{CIN}} = C^0_{B_{ACT}} - C_{B_{ID}} \tag{15}$$

where $B_{VIA}$ are the viable and culturable bacteria, viable condition involving active, sensitized and catalase-inhibited bacteria.

In the case of water disinfection with HP acting alone, the kinetic scheme proposal (Table 1, Equations (4)–(6)) included various stages in series, which accounted for the overall features of the reaction.

The assumptions of the developed model were the same as those described in the previous case. Hence, a set of equations based on mass balance for each state of bacteria in the reactor was performed as follows:

$$\frac{dC_{B_{ACT}}}{dt} = -r_{HP,1} \tag{16}$$

$$\frac{dC_{B_{SEN}}}{dt} = -r_{HP,2} + r_{HP,1} \tag{17}$$

$$\frac{dC_{B_{DAM}}}{dt} = -r_{HP,3} + r_{HP,2} \tag{18}$$

$$\frac{dC_{B_{ID}}}{dt} = +r_{HP,3} \tag{19}$$

with the same initial conditions as those used in Equations (14) and (15).

The kinetic scheme proposal for the commercial PAA solution can be represented by different stages in series, where the action of both disinfectants in the commercial mixture was considered. The reactions and steps required for cell inactivation are described in a stepwise manner, in a series of elementary stages. An elementary stage means a chemical or catalytic interaction that results in a bacterial lysis. The scheme of the reaction stages is represented by Figure 1.

The assumptions of the model developed were the same as those stated above. A set of equations based on mass balance for each state of bacteria in the reactor was performed as follows, with the same initial conditions as those used in Equations (14) and (15).

$$\frac{dC_{B_{ACT}}}{dt} = -(r_{HP,1} + r_{PAA,1}) \tag{20}$$

$$\frac{dC_{B_{SEN}}}{dt} = -(r_{HP,2} + r_{PAA,2}) + (r_{HP,1} + r_{PAA,1} + r_{PAA,4}) \tag{21}$$

$$\frac{dC_{B_{CIN}}}{dt} = r_{PAA,2} - (r_{PAA,3} + r_{HP,4}) \tag{22}$$

$$\frac{dC_{B_{DAM}}}{dt} = (r_{HP,2} + r_{PAA,4}) - (r_{HP,3} + r_{PAA,5}) \tag{23}$$

$$\frac{dC_{B_{ID}}}{dt} = r_{PAA,3} + r_{PAA,5} + r_{HP,3} + r_{HP,4} \tag{24}$$

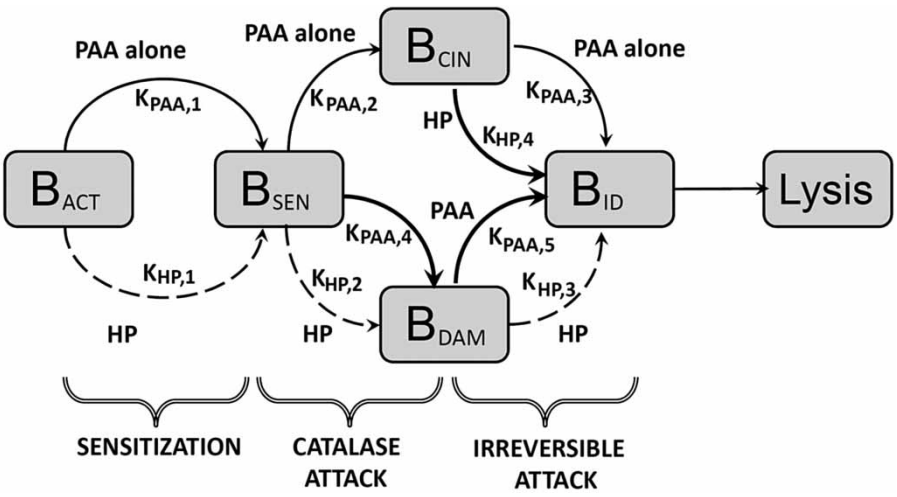


**Figure 1** | Kinetic scheme of commercial PAA leading to disinfection.

To obtain the kinetic parameters corresponding to the model, the experimental data were compared with simulation results obtained from a mathematical representation, employing a computational routine for the resolution of differential equations, the Runge–Kutta method (Press *et al.* 2007), coupled to a non-linear, multiparameter estimator, the Lebenberg–Marquart algorithm (Press *et al.* 2007).

## RESULTS AND DISCUSSION

This section presents and discusses results of the kinetic model proposal. The whole set of experimental data was used to investigate the validity of the model proposed in the previous section.

### Peracetic acid acting alone

Three different concentrations of PAA acting alone (4, 5 and 6 ppm) were selected to determine the coefficients of the kinetic model. These values were chosen since they can be used in practical applications (when possible, high rate of inactivation at low contact times). Different stages in series were proposed for the kinetic model (Equations (1)–(3)). At each efficient contact with the disinfectant agent, the bacterium changed, giving rise to a different physiological state.

Applying a linear regression, a correlation coefficient ($R^2$) equal to 0.959 indicated that the model adequately represented the measured experimental data.

Figure 2 shows the good fit between the model results and the experimental data obtained for the inactivation of *E. coli* using PAA as the only oxidizing agent.

The kinetic parameters obtained in the study of peracetic alone were: $K_{PAA,1} = (4.86 \pm 0.25) \times 10^{-1}\ \mathrm{mM^{-1}\,s^{-1}}$; $K_{PAA,2} = (1.24 \pm 0.18)\ \mathrm{mM^{-1}\,s^{-1}}$; $K_{PAA,3} = (8.34 \pm 2.02) \times 10^{2}\ \mathrm{mM^{-1}\,s^{-1}}$. The constant $K_{PAA,1}$ seems to correspond to the controlling stage of the reaction. This result is in agreement with our theoretical claims and with the experimental results shown in a previous publication: the presence of shoulders in the disinfection curves (Flores *et al.* 2014). This result is also in agreement with other results reported in the literature in which the cell outer membrane is shown as the first barrier for the disinfectant action (Block 2001; Ortega Morente *et al.* 2013). The first reaction of any antibacterial agent involves interaction with the cell outer membrane, in the case of Gram-negative bacteria, and the subsequent attack of the biocide to the target site. Although

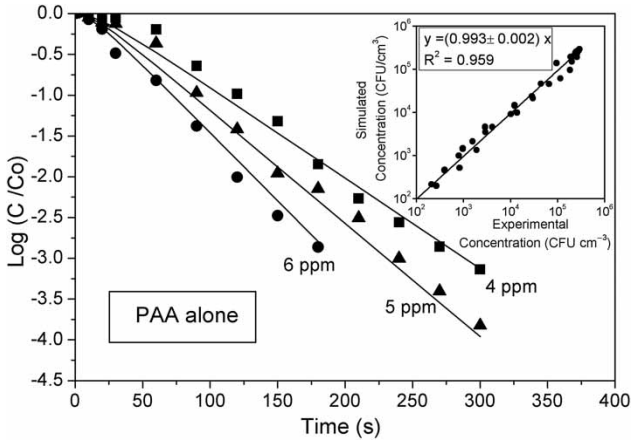


**Figure 2** | Experimental and simulated concentrations of *E. coli* vs. time for different initial concentrations of peracetic acid alone (experimental data: symbols; model simulation results: solid lines). In the box: simulated concentrations of *E. coli* vs. experimental values, for all experiments, using peracetic acid as the only disinfectant agent.

the reaction mechanisms of PAA-bacteria are not fully elucidated, it has been proposed that peroxygen compounds act through radical reactions and oxidation–reduction reactions with transition metals present on the surface of the cell (Marquis *et al.* 1995; Block 2001).

It is suggested that the constant $K_{PAA,2}$ represents a sensitization reaction and a noncompetitive inhibition of an enzymatic reaction. The sensitization stage involves the cytoplasmatic membrane, often considered as the major target site for biocide attack.

The last parameter obtained, $K_{PAA,3}$, is the fastest stage of this mechanism. This result is consistent with that obtained from the literature: the bacterial cell chemical structure has already been altered and its cell defense mechanisms inhibited in previous steps. Once the PAA penetrates into the cytoplasm, it quickly interacts with the main constituents of the cell, such as purine nucleotides and sulfhydryl bonds, causing an irreversible damage (Russell 2003; Park *et al.* 2009; Flores *et al.* 2012).

### Hydrogen peroxide

From previous experimental tests, three different concentrations of HP (15, 33 and 45 ppm) were chosen, using *E. coli* as the model organism. The results of HP as the only disinfectant agent are shown in Figure 3, in which the good fit between the model results and the experimental data obtained for the inactivation of the *E. coli* can be observed. Since peroxide is a weaker oxidizing agent than PAA, the lag phase can be appreciated in Figure 3.

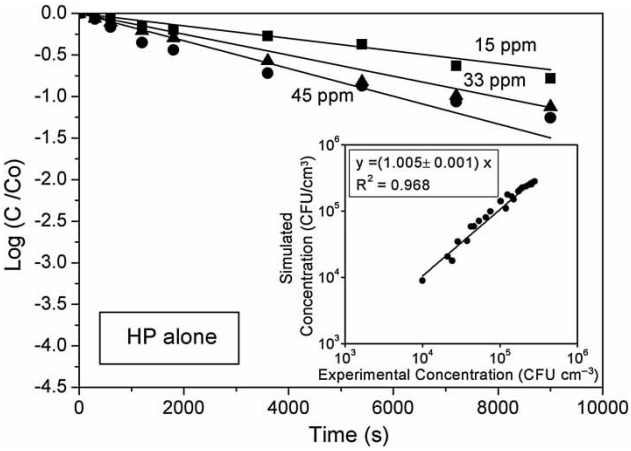


**Figure 3** | Experimental and simulated concentrations of *E. coli* vs. time for different initial concentrations of hydrogen peroxide alone (experimental data: symbols; model simulation results: solid lines). In the box: simulated concentrations of *E. coli* vs. experimental values, for all experiments, employing hydrogen peroxide as the only disinfectant agent.

The first kinetic parameter obtained in the study of HP as a biocidal agent was $K_{HP,1} = (4.93 \pm 0.60) \times 10^{-1}$ mM$^{-1}$ s$^{-1}$, which was of the same order as parameter $K_{PAA,1} = (4.86 \pm 0.25) \times 10^{-1}$ mM$^{-1}$ s$^{-1}$ when PAA was the only disinfectant agent.

Since PAA is a very strong oxidizing agent (1.81 eV) and has a stronger oxidation potential than HP, one would expect a different value of $K$. However, there can be several explanations in this respect:

(i) Both are peroxygens compounds and the initial interaction with the bacterial cell is in the bacterial membrane, the first site of disinfectant attack. The mechanism action against bacterial cells by peroxygens is mainly based on active oxygen and the ability to form radical species; this has been previously reported in the literature by several authors (Powers & Jackson 2008; Park *et al.* 2009; Galban *et al.* 2010).

(ii) HP is stable and permeable to membranes; it reacts with the bioavailable metals in the cell membrane. The cytotoxicity of HP primarily occurs through its ability to generate a hydroxyl radical through metal-catalyzed reactions, such as the Fenton reaction (Fernando & Othman 2006; Romero *et al.* 2011).

(iii) The bacterial cell exhibits the same defense mechanisms to both biocides; also, in unicellular organisms, $H_2O_2$ mainly stimulates the production of antioxidants and repairing enzymes (Vlasits *et al.* 2010).

The $K_{HP,2} = (3.94 \pm 0.19) \times 10^{-4}$ mM$^{-1}$ s$^{-1}$ parameter has the lowest value; therefore, it is the controlling step of the proposed reaction scheme. This stage was represented by two predominant reactions: *sensitization* and *inactivation*, both occurring at special sites of reactivity, the inner membrane and catalase enzyme active site. HP can cause cell inner membrane sensitization. It has been proposed that exposed sulfhydryl groups and double bonds are particularly targeted by HP (McDonnell & Russell 1999); HP loses its effectiveness when reacting with catalase, releasing water and oxygen. With a large concentration of HP and in the absence of an exogenous electron donor, the catalase enzyme is deactivated with time.

The $K_{HP,3} = (8.04 \pm 3.66) \times 10^{-2}$ mM$^{-1}$ s$^{-1}$ parameter represents the reaction leading to the state of irreversibly damaged bacteria. The loss of viability of bacteria would occur when the principal constituents of the cell undergo a certain level of irreversible damage due to the reaction with disinfectant (Flores *et al.* 2012, 2014). In order to compare the values of both estimated parameters, $K_{HP,3}$ and $K_{PAA,3}$, a simple explanation could be proposed: after the defense mechanism in bacteria cells has been defeated, both disinfectants exhibit different disinfection rates. It is suggested that this stage is characterized by a reaction of the cell inner membrane sensitization and the bacterial catalase deactivation reaction.

This result unravels the problem posed before. It is at this stage where the PAA acts according to its very particular chain mechanism (exposed in our previous work) to explain the significant reactivity differences of PAA as compared with other oxidizing agents such as HP (Flores *et al.* 2014).

## Commercial PAA

Experimental methodologies have enabled the selection of effective commercial PAA concentrations (Flores *et al.* 2014). As already stated, the chosen concentrations were 5, 6 and 8 ppm of PAA in the commercial mixture.

Figure 4 shows the good fit between the experimental data with the proposal model. The synergism exhibited by the commercial solution can be observed in Figure 4, when compared with Figures 2 and 3, where the disinfection rate is slow.

The kinetic parameters obtained in the study were: $K_{PAA,4} = (1.00 \pm 0.07)$ mM$^{-1}$ s$^{-1}$; $K_{PAA,5} = (2.46 \pm 0.51)$ mM$^{-1}$ s$^{-1}$; $K_{HP,4} = (5.17 \pm 2.35) \times 10^3$ mM$^{-1}$ s$^{-1}$.

The results of the synergistic action of the commercial mixture were quite significant, in particular those represented by $K_{HP,4}$. The $K_{HP,4} = (5.1731 \pm 2.3537) \times 10^3$ mM$^{-1}$ s$^{-1}$ value shows that both HP and PAA treatment greatly enhanced the efficiency of disinfection. It is

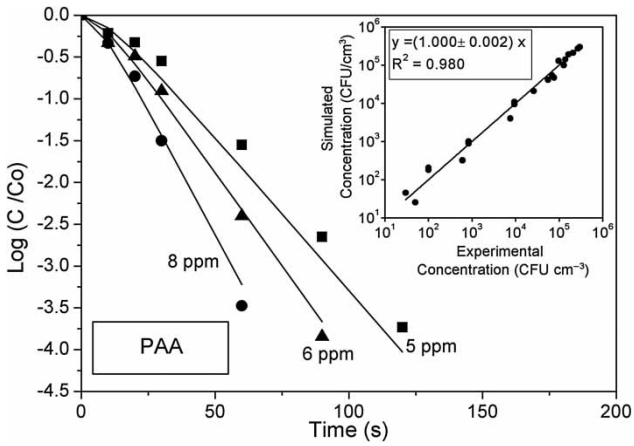


**Figure 4** | Experimental and simulated concentrations of *E. coli* vs. time for different initial concentrations of commercial peracetic acid (experimental data: symbols; model simulation results: solid lines). In the box: simulated concentrations of *E. coli* vs. experimental values for all experiments, employing commercial peracetic acid.

suggested that once peracetic deactivated the bacterial catalase enzyme, HP exhibited a high disinfection rate, thus oxidizing the multiple cellular targets (free radical oxidation), changing the energy processes and alternating the synthesis of proteins, finally culminating in cell death (Block 2001; Wagner *et al.* 2002; Galban *et al.* 2010).

The $K_{PAA,5}$ and $K_{HP,2}$ parameters represented the passage from sensitized to damaged bacteria ($B_{SEM} \rightarrow B_{DAM}$). The analysis of these results showed that with HP alone, the $B_{SEM} \rightarrow B_{DAM}$ step was the slowest stage of all the disinfection processes; however, when the commercial mixture was used, the disinfection rate of this stage became faster by three orders of magnitude.

The $K_{HP,4}$ and $K_{PAA,3}$ parameters corresponded to the $B_{CIN} \rightarrow B_{ID}$ passage. A comparison of the values $K_{HP,4} = (5.17 \pm 2.35) \times 10^3\ mM^{-1}\ s^{-1}$ versus $K_{PAA,3} = (8.34 \pm 2.02) \times 10^2\ mM^{-1}\ s^{-1}$ shows that although PAA alone was very effective as an inactivating agent, when the commercial mixture was used, the bacteria–peroxide interaction, which culminated in lethal bacterial damage, was still faster (6.2 times).

## CONCLUSIONS

A kinetic model for bacterial inactivation with commercial PAA was developed. It was validated with experiments using *E. coli* as a model bacterium.

The proposed scheme includes four stages for both agents, PAA and HP: sensitization, the attack on catalase, the irreversible damage that ends in lysis (final stage).

The model was able to incorporate all the essential features of experimental data and achieved a detailed and comprehensive description of the kinetics of water disinfection with two disinfectant agents.

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