Artigo

TREATED DOMESTIC SEWAGE: KINETICS OF *Escherichia coli* AND TOTAL COLIFORM INACTIVATION BY OXIDATION WITH HYDROGEN PEROXIDE

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Hydrogen peroxide has been used for decades in developed countries as an oxidizing agent in the treatment of water, domestic sewage and industrial effluents. This study evaluated the influence of the concentration of H_2O_2 and pH on the inactivation of *Escherichia coli* cells and the disinfection of sewage treated. The results showed that the inactivation rate increased with pH and H_2O_2. The presence of other contaminants dissolved in the effluent is probably the cause of these differences, because *E. coli* inactivation in synthetic wastewater was found to be much faster than in the real treated domestic sewage.

Keywords: kinetics; hydrogen peroxide; wastewater.

INTRODUCTION

The reuse of wastewater for less restrictive non-potable purposes, such as agricultural, landscape, industrial, recreational, aquifer recharge, and maintenance of watercourses, can reduce the consumption of water of potable quality. The quality of the wastewater used and the specific purpose of reuse, establish the level of treatment recommended, the safety criteria to be adopted and the capital, operational and maintenance costs. In this context, the study of technologies that reduce contaminants in wastewater is of utmost importance.

Hydrogen peroxide has been the focus of studies seeking its alternative use as a disinfectant because of several advantages offered by this reagent. The main advantage is related to its decomposition, where there is the possibility of complete mineralization of organic compounds, contrary to what occurs with the use of chlorine. Hydrogen peroxide is one of the most versatile oxidants, being superior to chlorine, chlorine dioxide and potassium permanganate. It can be converted into hydroxyl radicals ($^{\bullet}OH$). Given its versatility, hydrogen peroxide can be used for many different purposes both alone and combined with other reagents or treatments.[1]

The reason for its wide application is due to its selectivity when subjected to certain experimental conditions of temperature, concentration, reaction time, and addition of catalysts. The treatment of water, sewage or industrial effluents using H_2O_2 has been a common practice for at least 25 years in developed countries.[1] Applications associated with the use of hydrogen peroxide alone include: control of odors, for example, the inorganic sulfur compounds responsible for bad smells are oxidized by hydrogen peroxide resulting in colloidal sulfur or sulfate ions;[1,2] control of corrosion; destruction of residual chlorine and reduced components, such as thiosulfate, sulfide and sulfites; reduction in chemical and biochemical oxygen demand; oxidation of organic pollutants; oxidation of inorganic components such as cyanides, NO_x/SO_x, nitrites, and hydrazines; oxidation by hydrolysis of organic compounds, such as formaldehyde, carbohydrates, nitrogenous components; destruction of phenols, pesticides, solvents, plasticizers, among others; and control of bioprocesses through disinfection, and inhibition of bacterial growth.[1]

In combined processes, hydrogen peroxide may be used in procedures such as flocculation, precipitation and oxidation of metal complexes, to increase the performance of inorganic flocculants, in treatments involving bioprocesses such as disinfection, or as a source of dissolved oxygen.[1] It can also effectively improve the biodegradability of an effluent, as well as the formation of intermediate compounds such as short-chain carboxylic acids produced from the branching of aliphatic chains oxidized by hydrogen peroxide. These compounds are then easily degraded by microorganisms.[2]

The bactericidal effectiveness of hydrogen peroxide has been demonstrated in treatment systems for water and food, and against those Gram-negative organisms which are more susceptible to the action of this agent. The antimicrobial action stems from the ability of H_2O_2 to form reactive oxygen species such as hydroxyl radicals ($^{\bullet}OH$) and superoxide ($O_2^{\bullet-}$). These radicals can damage microbial DNA, as well as the components of the cell membrane.[3] These species can have both lethal and sublethal effects on the bacterial genome and other intracellular molecules, resulting in physiological alterations, growth delay and oxidative disturbances of bacterial membranes resulting in growth inhibition.[2]

However, the destruction of pathogens involves a number of factors yet to be elucidated, such as the action of the enzyme catalase synthesized in microbial metabolism, the difficulties involved in diffusive hydroxyl radicals effectively oxidizing the cell membrane, the mutagenic or adaptive capacity of some microorganisms and the influence of the microbial growth phase of the cells, since young cells are more sensitive than those found in the stationary phase of growth.[4-7]

Based on the results obtained by Ksibi,[2] which show that the organic matter present in treated domestic sewage is easily oxidized, we should be able to achieve the purification and disinfection of this type of waste solely with the use of hydrogen peroxide. Thus, this study aims to evaluate the disinfection of treated domestic sewage and a synthetic solution containing cells of *Escherichia coli* using H_2O_2 as an oxidizing agent and bactericide, at different concentrations and over a pH range of 6 to 9.

EXPERIMENTAL

Synthetic wastewater and treated domestic sewage

The synthetic wastewater (SW) used was inoculated with the

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test microorganism *Escherichia coli* - ATCC 25922, and the nutrient broth was prepared according to Watts *et al*..[6] The organisms were then stored in inclined tubes under cooling (4 ºC). The bacteria were removed from the freezer and inoculated into 1-L flasks containing 500 mL of 8 g/L nutrient broth and 1 g/L of glucose, and mixtures were stirred at 150 rpm for 24 h at 35 ºC. The suspended biomass was harvested during the late log phase by centrifugation at 168 g and resuspended in 0.2 L peptone solution (concentration 0.001%), and then held at the dilutions necessary to obtain the desired effluent conditions (similar conditions of the effluent of sanitary sewer Table 1). For the determination of the *E. coli* and total coliforms, 100 mL of wastewater was filtered through a 0.45 µm porosity membrane, introduced into Coligel® bags and incubated for 28 h at 35 ºC. After the incubation period, bacterial colonies were counted and the results calculated as CFU/100 mL.

The treated domestic sewage (TS) was collected from the municipal wastewater treatment plant in Florianópolis (Brazil), after treatment by activated sludge. The main characteristics were determined according to the Standard Methods,[8] and the results are summarized in Table 1.

Table 1. Characteristics of treated domestic sewage and synthetic wastewater

Parameter	Treated domestic sewage*	Synthetic wastewater
pH	6.0-8.5	5.5-7.5
Total solids (mg/L)	655-670	-
Suspended solids (mg/L)	20-75	-
Dissolved solids (mg/L)	645-655	-
BOD_5 (mg O_2/L)*	5-10	-
COD - Chemical Oxygen Demand (mg O_2/L)	20-110	-
Color (mg Pt Co/L)	70-265	-
Turbidity (FTU)	5-150	-
Total coliforms (CFU/100mL)	10^4-10^5	10^4-10^5
Conductivity (µS)	700 -2200	-

*Range of values of 90 samples collected during 90 days after treatment of domestic sewage by activated sludge

H_2O_2

Hydrogen peroxide (50% v/v) was supplied by Degussa (Brazil).

Kinetics of non-catalyzed oxidation - effect of H_2O_2 concentration

Experiments were carried out with contact between the treated domestic sewage effluent and hydrogen peroxide solutions at concentrations of 0 to 300 mg/L with a contact time of 10 min. The kinetic tests involving non-catalyzed oxidation with synthetic sewage and treated domestic sewage, performed using 1 L beakers containing a total volume of 500 mL of sample and H_2O_2, applying H_2O_2 concentrations of 25, 50, 75 and 100 mg/L. Aliquots were removed at regular time intervals for analysis and characterization. The duration of each test was 60 min.

Kinetics of non-catalyzed oxidation – effect of pH

Experiments were performed using 1 L beakers containing a total volume of 500 mL of synthetic wastewater and H_2O_2, applying an H_2O_2 concentration of 100 mg/L. Aliquots were removed at regular time intervals for analysis of *E. coli*. The pH range evaluated in this study was 6 to 9.

RESULTS

Synthetic wastewater

Kinetics of Escherichia coli inactivation by oxidation with H_2O_2 – effect of H_2O_2 concentration

The death of bacterial cells in the presence of hydrogen peroxide is due to oxidation of intracellular constituents.[2] The active forms of the oxidant are the hydroxyl (•OH) and perhydroxyl (HO_2•) radicals generated from the decomposition of H_2O_2. These active oxygen species can have both lethal and sublethal effects on the bacterial genome and other intracellular molecules, resulting in physiological alterations, growth delay and oxidative disturbances of bacterial membranes resulting in growth inhibition.[2] The inactivation kinetics of *E. coli* cells present in synthetic wastewater can be modeled using a modified version of the Hom model (1972) (Equation 1), which considers the variation in the concentration of hydrogen peroxide over time.

$$dN/dt = -k_1 N t^m [C^n(t)] \quad (1)$$

where: $C_{(t)}$ = concentration of H_2O_2 at time t; N = number of coliforms at time t; k_1 = rate constant of bacterial inactivation; n = coefficient of dilution or concentration constant; and m = decomposition constant of the oxidant agent.

Hydrogen peroxide in aqueous solutions is in steady-state, but its decomposition can be induced and accelerated substantially in the presence of metallic ions frequently found in domestic sewage, such as iron, and manganese.[9] However, these ions are not present in the synthetic effluent. The decrease in hydrogen peroxide concentration over time has been described according to a second-order reaction, which was also considered by Wagner *et al*.,[9] (Equation 2).

$$C(t) = 1/((1/C_0) + k_2 t) \quad (2)$$

where: k_2 = second-order rate constant of the decomposition of H_2O_2 (L/mg min).

The combination of Equations 1 and 2 results in Equation 3, which can be used in this study to describe the inactivation of *E. coli* by hydrogen peroxide in the synthetic effluent.

$$N = N_0 \left(k_2 C_0 t + 1\right)^{-\frac{k_1}{k_2}} \quad (3)$$

where: N_o = initial number of bacterial colonies (CFU/100 mL); N = number of bacterial colonies at time t (CFU/100 mL); k_1 = pseudo-first-order rate constant for bacterial inactivation (min^{-1}); k_2 = second-order rate constant for H_2O_2 decomposition (L/mg min); and C_o = initial H_2O_2 concentration (mg/L).

The kinetic rate constants k_1 and k_2 were evaluated by fitting Equation 3 to the experimental data and resulted in $k_1 = 0.043 min^{-1}$ and $k_2 = 0.058$ L/mg min.

Figure 1 shows the toxic effect of hydrogen peroxide when used at low concentrations, these results proved similar to those obtained by Watts *et al*.[6] who worked with initial hydrogen peroxide concentrations in the range 50 to 470 mg/L. The modified Hom model can adequately describe the inactivation of *E. coli* in the synthetic effluent.

Kinetics of Escherichia coli inactivation by oxidation with H_2O_2 – effect of pH

The effect of pH in the range 6 to 9 on the inactivation of *E. coli*

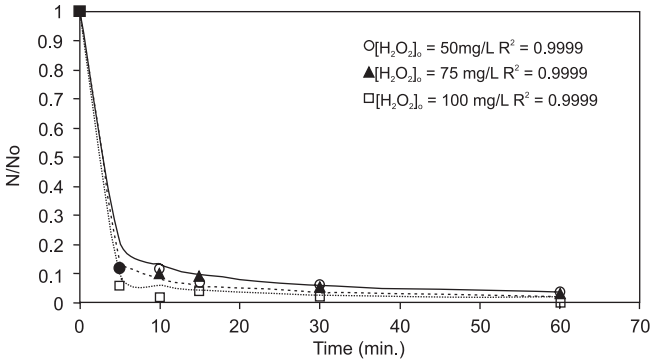

Figure 1. *Kinetics of inactivation of Escherichia coli using different initial H_2O_2 concentrations, pH = 5.6 (○ 50 mg/L, ▲ 75mg/L, ■ 100 mg/L; symbols: experimental results; lines: simulation)*

in the synthetic effluent was evaluated, using an initial hydrogen peroxide concentration of 100 mg/L, and the modified Hom model was fitted to the experimental data (Figure 2). The results obtained can be explained considering that the pH range suitable for the microbial growth of *E. coli* lies between pH 4 and 9.[10]

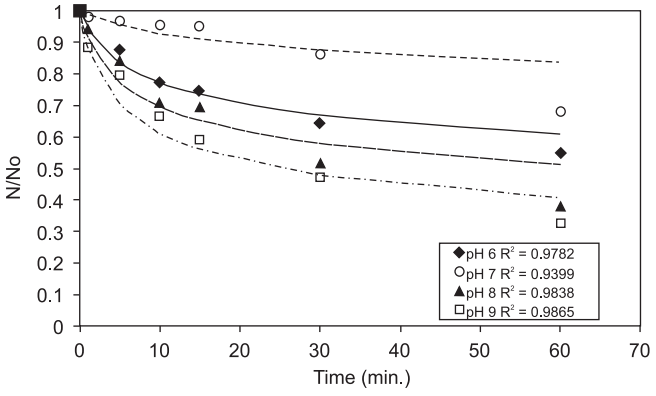

Figure 2. *Kinetics of Escherichia coli inactivation using different initial pH values ($[H_2O_2]_o$ = 100 mg/L; ◆ pH 6, ○ pH 7, ▲ pH 8, □ pH 9; symbols: experimental; lines: simulation)*

Thus, pH 9 is at the limit for the maintenance of the bacterium, while neutral pH is well within the growth limits. Table 2 shows the fitting of the model to the results for the parameters considered in *Escherichia coli* ATCC-25922 inactivation by hydrogen peroxide, under different pH conditions. The results show that the inactivation rate is greater at pH 8-9 than under neutral conditions.

Table 2. Rate constants for the inactivation of *Escherichia coli* under different pH conditions obtained using the modified Hom model

pH	k_1 (min^{-1})	k_2 (L/mg min)
6	7.00×10^{-4}	4.80×10^{-3}
7	1.30×10^{-4}	1.80×10^{-3}
8	1.12×10^{-3}	6.10×10^{-3}
9	1.65×10^{-3}	6.90×10^{-3}

Treated domestic sewage

Effect of H_2O_2 concentration on the destruction of total coliforms

The effect of initial hydrogen peroxide concentration on the inactivation of coliforms in the batch reactor was evaluated for initial concentrations ranging from 0 to 300 mg/L. Figure 3 shows the results after the sewage effluent treatment in the presence of different concentrations of hydrogen peroxide. With a contact time of 10 min, hydrogen peroxide was toxic to the coliform bacteria at all concentrations studied, as reported by Watts *et al.*,[6] in their study with initial hydrogen peroxide concentrations in the range 50 to 470 mg/L. Similar results for the effect of hydrogen peroxide concentration can also be observed.

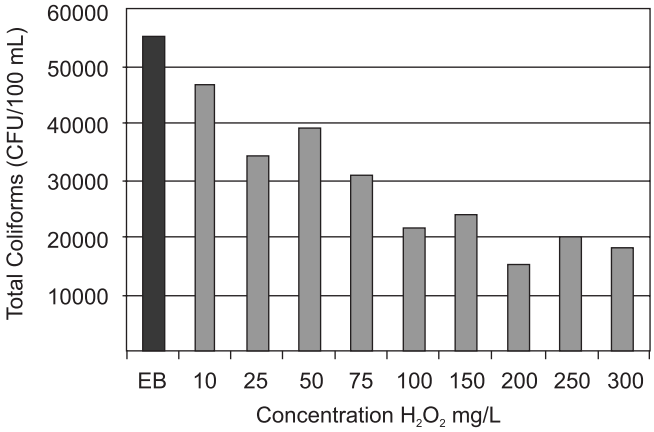

Figure 3. *Inactivation of total coliforms using different initial H_2O_2 concentrations with a contact time of 10 min (TDS = treated domestic sewage with* higher coliform count*)*

Concentrations above 100 mg/L of hydrogen peroxide are more efficient in the inactivation of bacteria, but these are not effective from an economic point of view, in addition to showing little variation when concentrations above 100 mg/L are used. Decomposition in sewage treatment can be induced and accelerated significantly in the presence of metal ions, particularly iron, copper, manganese, nickel, and chromium.[9] Thus, the combined use of hydrogen peroxide as an oxidizing agent and a process of filtration using granular coal coated with iron oxide (heterogeneous catalyst) may be an alternative for the use of lower hydrogen peroxide concentrations.

The coliform inactivation kinetics of the treated domestic sewage was studied using different initial hydrogen peroxide concentrations (Figure 4).

The results show that initially the disinfection is rapid where the rate of inactivation of coliforms decreases with reaction time. One

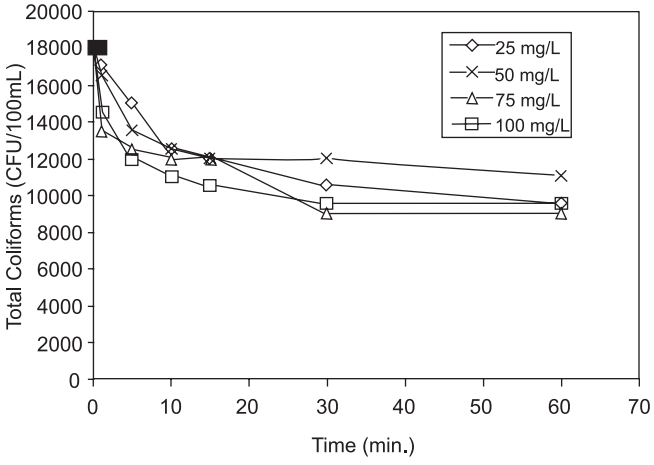

Figure 4. *Inactivation kinetics of total coliforms in treated domestic sewage using different initial H_2O_2 concentrations at pH = 7.5 (◇ 25 mg/L, × 50 mg/L, △ 75 mg/L, □ 100 mg/L)*

reason for this is a decrease in the hydrogen peroxide concentration, due to its decomposition. However, these results found the inactivation kinetics of *E. coli* and total coliforms is much slower than those reported by Wagner *et al*.[9] and faster than those reported by Yamagiwa *et al*..[11] The presence of other contaminants dissolved in the effluent is probably the cause of these differences.

Few studies on the kinetics of non-catalyzed disinfection with hydrogen peroxide are reported in the literature. In general, the rate of disinfection is proportional to the hydrogen peroxide concentration to the high n-th power. It has been reported that the order of the reaction (n) is mainly dependent on the type of disinfectant agent and is less sensitive to the type of microorganism.[11,12] The value of n for the disinfection of *L. pneumophila* by hydrogen peroxide reported by Yamagiwa *et al*. [11] is 0.68 and for the disinfection of *Salmonella typhi* by H_2O_2 is 0.5.[12] This study used the method of initial rates to propose a kinetic model of microbial inactivation (Equation 4) that fits the experimental data, as presented in Figures 4 and 5.

$$-r_o = -dC/dt\big|_{t=o} = f\left[H_2O_2\right]_o[C]_o \quad (4)$$

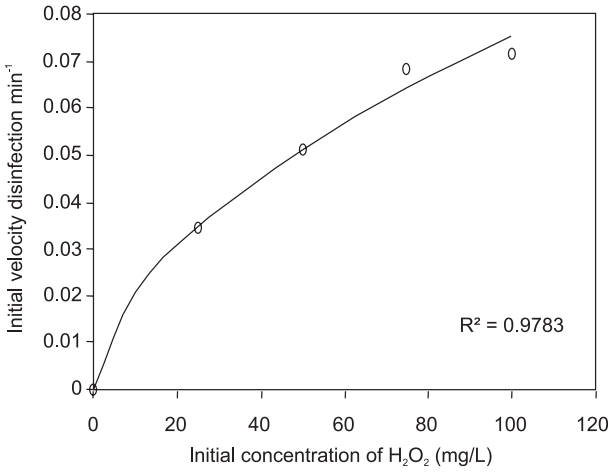

Figure 5. *Initial inactivation rate of coliforms versus initial concentration of H_2O_2. (Points: experimental, line: fit of the dependence of the initial concentration of hydrogen peroxide to the n-th power, n = 0.56)*

Note that there is a considerable increase in the inactivation rate when higher concentrations of H_2O_2 are used. The dependence of the initial disinfection rate on the initial hydrogen peroxide concentration resulted in n = 0.56 ± 0.06. However, the concentration of hydrogen peroxide varies during disinfection, not only because of the reactions that lead to the inactivation of microorganisms, but also due to decomposition reactions, as described above.

The results given in Figures 4 and 5 show that the use of hydrogen peroxide alone in the treatment of water and wastewater disinfection results in slow rates, as reported by Wagner *et al*.[9] and Yamagiwa *et al*..[11] Thus, the combined use of H_2O_2 with a heterogeneous catalyst can improve efficiency considerably in relation to microbial inactivation and removal.

Effect of hydrogen peroxide concentration on the inactivation kinetics of total coliforms in the treated domestic sewage

The inactivation kinetics of coliforms was studied using different initial hydrogen peroxide concentrations, as shown in Figure 6.

The disinfection kinetics of the treated domestic sewage can also be modeled using a modified version of the Hom model (1972), which considers the variation in the hydrogen peroxide concentration over time. The Hom equation given in Equation 1, when combined with Equation 2, developed by Wagner[9] to describe the decay in the

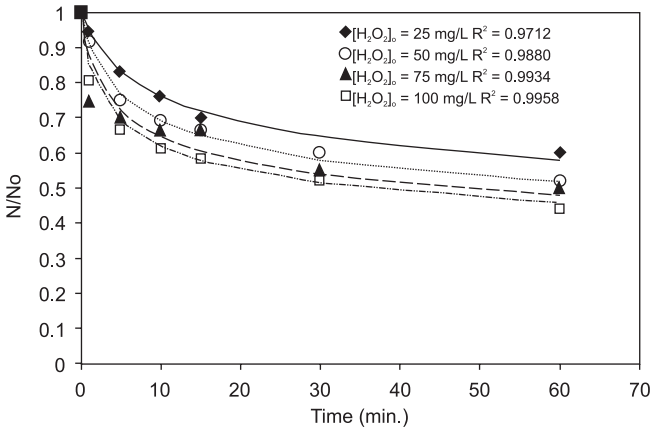

Figure 6. *Inactivation kinetics of total coliforms in treated domestic sewage using different initial H_2O_2 concentrations (◆ 25mg/L, ○ 50 mg/L, ▲ 75 mg/L, □ 100 mg/L; symbols: experimental; lines: simulation)*

concentration of hydrogen peroxide over time, results in Equation 3, and this was used to describe the inactivation of total coliforms using hydrogen peroxide.

The k_1 and k_2 rate constants were determined through the fitting of Equation 3 to the experimental data and resulted in $k_1 = 0.0026$ min^{-1} and $k_2 = 0.015$ (L/mg min). These results show that the inactivation kinetics of *E. coli* and total coliforms are much slower than those reported by Wagner *et al*.[9] but faster than those reported by Yamagiwa *et al*..[11]

The presence of other contaminants dissolved in the effluent is probably the cause of these differences, given that *E. coli* inactivation in synthetic wastewater (Figures 1 and 2) was found to be much faster than in the treated domestic sewage. Similar results were reported by Raffellini *et al*.,[13] who reported that the cytotoxic effect caused by H_2O_2 depends on cellular characteristics, physiologic state, contact time, pH and temperature.

Recently, Ronen *et al*.[14] demonstrated that H_2O_2 or Cl_2 are effective for disinfecting greywater using 125 and 10 mg/L, respectively, with contact times of 120 and 30 min, respectively. They also reported that the costs of treating up to 5 m^3/day using H_2O_2 or Cl_2 are very similar at around 0.16 USD/y m^3, with an advantage using H_2O_2 of no chlorinated by-products formed.

CONCLUSIONS

The modified version of the Hom model successfully described the inactivation kinetics of coliforms in both treated domestic sewage and synthetic sewage. Total coliforms and *Escherichia coli* were susceptible to the oxidizing action of hydrogen peroxide. However, it should be emphasized that the disinfection of treated sewage was slower than that of synthetic sewage, possibly due to the presence of other pollutants that could be oxidized by the hydrogen peroxide. Thus, studies involving this oxidizing agent are extremely important given its oxidation potential, which can be expanded with the concomitant use of other technologies, and allow the reuse of wastewater.

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