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INACTIVATION OF *Bacillus atrophaeus* SPORES IN HEALTHCARE WASTE BY UV LIGHT COUPLED WITH H_2O_2

M. T. Iannotti and R. Pisani Jr.*

University of Ribeirão Preto, Postgraduate Program in Environmental Technology,
Phone: + (55) (16) 9111-0776, Fax: + (55) (16) 3603-6718,
Avenida Costabile Romano 2201, 14096-900, Ribeirânia, Ribeirão Preto - SP, Brazil.
E-mail: pisanijr@terra.com.br

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Abstract - Healthcare waste inoculated with *B. atropheaus* spores was used to evaluate a treatment process using UV light in combination with H_2O_2. First, the influence of the waste mass on the spore inactivation fraction was investigated for a constant radiation exposure time of 10 min and power per unit mass of waste (44-237 W/kg). The degree of inactivation of the spores was then determined as a function of exposure time (5-30 min) and power per mass unit (67-178 W/kg) for a constant waste mass. The experimental results were adjusted according to four kinetic models. The Hom and power law models were the most appropriate for the description of the disinfection process. The maximum experimental inactivation fraction (95%) achieved was obtained with 178 W/kg irradiation for 30 min.
Keywords: *B. atrophaeus* spore; Disinfection, Healthcare waste; Ultraviolet; H_2O_2.

INTRODUCTION

Improper waste disposal has produced environmental passives that may jeopardize natural resources and quality of life for current and future generations. Healthcare waste falls within that context as a potential biological hazard to those involved in the waste management process.

Recent investments in new healthcare waste treatment technologies have extended treatment service and have improved compliance with current environmental regulations, with the intent of improving environmental sustainability and health preservation. In Brazil and abroad, healthcare waste that may contain pathogenic microorganisms must be treated to reduce the pathogen load and reach Level III microbial inactivation, i.e., a reduction of 6·Log10 for vegetative cells (99.9999%) or 4·Log10 for *Bacillus* spores (99.99%) (Brazil, 2004; Prüss *et al*., 1999; STAAT, 1998).

The thermal treatment of wastes is considered to be one of the most effective methods for inactivating microorganisms, and it is one of the most used methods. However, some treatment techniques that use heat, such as incineration, have a high operational cost and generate toxic compounds, such as dioxin, furans and metal emissions when there are precursors in the residue composition (Karademir, 2004; Lee and Huffman, 1996; Li *et al*., 2010).

Processes that are affected by exposure area and require the transfer of physical agents through the mass of the waste, such as ionizing radiation, dielectric heating and autoclaving, encounter difficulties in ensuring the desired levels of inactivation by exposing the entire mass of waste to radiation or heat (Koutchma *et al*., 2001; Lauer *et al*., 1982; Prüss *et al*., 1999). The use of microwaves to disinfect healthcare waste has been increasingly common in several countries (Edlich *et al*., 2006; Oliveira *et al*., 2010). Lee *et al*. (2004) evaluated the costs of

*To whom correspondence should be addressed

treatment and the final disposal of healthcare waste using incineration and microwave techniques. They showed that the cost of the incineration process was US$1.56/kg, which is almost ten times higher than the microwave process, which costs US$0.16/kg. However, Tonuci *et al*. (2008) and Pisani Jr. *et al*. (2008) demonstrated that the microwave inactivation of *E. coli* and *P. aeruginosa* vegetative cells present in healthcare waste may not be effective, depending on the operating conditions. In addition, Oliveira *et al*. (2010) determined the inactivation fraction of artificially inoculated *B. atrophaeus* spores in healthcare waste as a function of microwave exposure time (20-40 min) and power per unit mass of waste (100-200 W/kg). From their experimental results (maximum inactivation of 71.4% of spores inoculated into the waste) and the simulated irradiation times required to inactivate 99.99% of spores at 100 °C (779 min at 100 W/kg and 258 min at 200 W/kg), they concluded that the microwave disinfection technique was ineffective for the inactivation of *B. atrophaeus* spores, and Level III microbial inactivation was not attained when the technique was similarly applied using full scale equipment.

Exposure to UV light is also recognized as an efficient method for microbial inactivation, which is applied mainly to the food industry, water and wastewater disinfection, and surface and packaging sterilization (Devine *et al*., 2001). However, although it is a fast and relatively inexpensive method, the practical use of UV irradiation has been limited because of the possibility of non-uniform exposure of radiation through the medium to be treated.

Spores are 1-50 times more resistant to UV light at 254 nm (which is the most effective wavelength for germicidal effect) than vegetative cells (Nicholson *et al*. 2000; Setlow, 2006). However, research has been conducted to optimize the efficiency of UV for the inactivation of spores and other organisms to diversify its practical applications.

In addition, H_2O_2 is an efficient chemical oxidant that is easy to handle and has a wide application area. It is a bactericidal, fungicidal and sporicidal compound that can also be applied to virus inactivation. It is mainly used as a high-level disinfectant at concentrations up to 10% for spores (Khadre and Yousef, 2001; Labas *et al*., 2008; Raffellini *et al*., 2011).

The use of H_2O_2 and other oxidants in pre-treatments during biological disinfection processes has also shown promise because they increase the susceptibility of the spores to subsequent treatment (Cortezzo *et al*., 2004; Labas, *et al*., 2008; Melly *et al*., 2002; Popham *et al*., 1995; Setlow, 2006; Young and Setlow, 2004).

The combination of H_2O_2/UV for the degradation of contaminants is among the set of processes called advanced oxidation processes (AOP), which consist of oxidation techniques that have been identified as promising and are widely studied as alternatives for the treatment of industrial effluents and water supplies (Klavarioti *et al*., 2009; Matilainen and Sillanpää, 2010; Pera-Titus *et al*., 2004;).

Gardner and Shama (1998) have investigated the inactivation of *B. subtilis* on filter paper by irradiation with UV light in the presence and absence of H_2O_2. A synergistic action of the UV lethal effect was observed, with an increase of spore inactivation by a factor of 5.8 when they were moistened with a 1% (w/v) H_2O_2 aqueous solution. They also found that the moistened spore inactivation was 6.7 to 10.6 times higher than that obtained with dry spores.

B. subtilis strains are typically used as bioindicators of sterilization process because of their resistance to dry heat and ethylene oxide. Nevertheless, the *B. subtilis* DSM 675 and DSM 2277 control strains, formerly known as *B. subtilis var. niger* and *B. globigii*, respectively, were reclassified as members of the species *B. atrophaeus* (Fritze and Pukall, 2001). Considering the benefits reported in the cited literature, the difficulty of spore inactivation, and the lack of advanced oxidative process studies involving UV light coupled with an H_2O_2 solution in a solid medium, this investigation evaluated the application of this process to healthcare waste treatment through the determination of the level of inactivation of *B. atrophaeus* spores.

MATERIALS AND METHODS

The disinfection experiments were performed in two steps. In the first phase, the influence of healthcare waste mass (405, 540 and 810 g) and UV light power (36, 60 and 96 W) on the inactivation of *B. atrophaeus* spores was investigated with the presence of 1% H_2O_2 in the wet fraction of the waste at the beginning of each test. The exposure time to UV light (10 min), the impeller rotation (8.5 rpm) and the waste moisture (50% on a wet basis) were kept constant. The results of the inactivation fraction of the spores (X) were parameterized according to the UV light power (P) per unit of waste mass (M) (44-237 W/kg). The fluence should not be used because the waste area exposed to radiation is unknown. The waste was mixed to validate the hypothesis of a perfectly stirred batch reactor or to

create conditions that matched the hypothesis as closely as possible. The speed impeller was set to 8.5 rpm, the highest value for which the waste was not shed from the container placed inside the photocatalytic reactor. The tests were done in triplicate, and the fraction of inactivation was calculated by Equation (1):

$$X(\%) = \frac{(N_0 - N)}{N_0}.100 \quad (1)$$

In the second step, a waste mass of 540 g was employed to evaluate the kinetics of the inactivation process in which the dependence of the spore inactivation $\ln(N_0/N)$ on the power of the UV lamp (36, 60 and 96 W) was assessed in the form of the P/M ratio (67-178 W/kg) and the UV exposure time (5-30 min). The incoming waste moisture was adjusted to 50% (wet basis), producing an H_2O_2 concentration of 1% in the wet fraction of the waste. The impeller speed was held constant during these tests at 8.5 rpm.

The study involved the setup of experimental equipment, the preparation of the suspension and the counting of spores, spore inoculation and recovery from the waste mass, adjustment of moisture and H_2O_2 content in the waste, spore inactivation and fitting of the kinetic models.

Experimental Equipment

The waste used was prepared to represent typical healthcare waste, according to the gravimetric composition defined by Oliveira *et al*. (2010) and STAAT (1998). Table 1 shows the composition and gravimetric bulk density of the waste. The preparation procedure involved the weighing of the constituents to result in the desired composition).

Later, the materials were ground so that 100% of the particles were able to pass a sieve with 2 inch openings, and its size distribution was determined by sieving. The waste was autoclaved to ensure sterility of the material. From the size distribution analysis, approximately 90% of the particles mass had sizes between 3.35 mm and 38.1 mm, which is similar to the distribution found by Oliveira *et al*. (2010) and Tonuci *et al*. (2008) for healthcare waste from Ribeirão Preto city, which had a composition of 91.5% by weight for the same size range.

The photocatalytic batch reactor was assembled from a domestic microwave oven in which UV lamps were installed. The pitched blade paddle was also attached to an adjustable speed motor. Two low-pressure mercury germicidal lamps (PHILIPS model TUV PL-L) with nominal powers of 36 W and 60 W were used to produce UV light with a wavelength of 254 nm. The lamps could be turned on independently, resulting in nominal powers of 36 and 60 W or, in combination, a nominal power of 96 W.

Table 1: Gravimetric composition and bulk density of healthcare waste.

Material	% in mass (dry basis)
Paper/Fabric	39.3
Hard plastic	24.7
Plastic film	13.2
Rubber	10.5
Metals	4.4
Glass	2.1
Bone	1.6
Wood	1.1
Polystyrene foam	0.6
Others: sawdust, powder material, cardboard, and other difficult separation materials	2.6
Total	100
Bulk density for 50% moisture content (wet basis)	128 kg.m^{-3}

The intensity of UV radiation is typically represented as irradiance or flux intensity in Wm^{-2} or mW.cm^{-2}. The irradiance measurements of the lamps were performed using a ZED Radiometer (model PRO 11) at a wavelength of 254 nm. The measurements were performed separately, using lamps with nominal powers of 36 W and 60 W. The measurement points were located on the waste surface, 7 cm below the lamps. The points also corresponded to the ends and the central points of each lamp. The mean values for each lamp were found respectively to be 2.66 W.cm^{-2} and 2.82 W.cm^{-2}. These measurements were performed only to characterize the lamps because the intensity of UV radiation, as well as fluence, should not be used because the waste area exposed to radiation is unknown.

Spore Preparation and Count

A suspension of spores with an original concentration of 10^8 spores.mL^{-1} was formed from *B. subtilis var. globigii* ATCC 9372 (*B. atrophaeus* ATCC 9372) from the culture collection of diagnostic Cefar (CCCD).

The spore suspension was subjected to a thermal shock (100 °C for 5 min, followed by an ice bath), which has been suggested to inactivate vegetative forms. Subsequently, a 1 to 10 dilution was made with 0.9% w/v sterile saline to obtain a concentration

of 10^6 spores.mL^{-1}, and this suspension was used for testing. During its storage, it was kept between 2 and 8 °C.

The pour plate technique was used to count the colony-forming units (CFU) of the microorganisms. The suspension was then diluted to 1/10, 1/100, and 1/1000 to count the resulting spores. For the growth and counting of the colonies, 1 mL samples of each dilution were placed in Petri dishes, each of which contained 20 mL of Tryptone soy agar (TSA) culture medium that was previously melted and kept at a controlled temperature of 45 °C. After homogenization and solidification, the Petri dishes were incubated at 35 °C for 48 h. The dishes that had between 10 and 300 colonies were selected for counting. The number of spores per mL was calculated by multiplying the amount seeded (1 mL) by the number of colony forming units counted (CFU) and dividing the result by the dilution performed. The tests were done in triplicate (Hoben and Somasegaran, 1982; Mamane *et al*. 2007; Sawai *et al*., 2009; Uemura *et al*., 2010).

The percentage of sporulated microorganisms was determined from slides prepared by the Gram staining method and by the Wirtz–Conklin method (Demidova and Hamblin, 2005; Golveia *et al*., 2009; Hamouda *et al*., 2002). The number of spores was determined in duplicate by manually counting 1000 spores and vegetative cells observed on the slides. An optical microscope equipped with a reticle eyepiece was used for observation and counting.

Inoculation and Recovery of the Microorganisms

The procedure for the inoculation and recovery of spores from the waste mass was the same as that used by Tonuci *et al*. (2008) and Oliveira *et al*. (2010), but the pour plate technique was used for the growth and counting of colony-forming units instead of the spread plate technique. First, the sterilized healthcare waste was contaminated with 1 mL of suspension containing a known number of spores (N_{0I}) after the waste moisture had been adjusted to 50% wet basis with sterile distilled water. The moist and contaminated waste was homogenized with a glass rod to distribute the spores uniformly. In the next step, this waste mass was immersed in 4 L of sterile saline (0.9% w/v), and the mixture was homogenized again for 10 min. Then, three liquid phase samples of 1 mL volume were collected for spore counting by the pour plate technique described above. Taking into account the initial moisture of the waste and the number of colony-forming units, the dilution allowed for the number of microorganisms recovered from previously inoculated waste (N_{0R}) to be determined. Lastly, the number of spores originally inoculated in the waste (N_{0I}) was compared with the number of spores recovered from it (N_{0R}). The recovery test was performed eight times.

Adjustment of Incoming Moisture and H_2O_2 Content of the Healthcare Waste

The incoming moisture of the healthcare waste was set to a 50% wet basis, which was the lowest value that wet the waste (Oliveira *et al*., 2010). The initial moisture content of the waste sample was obtained by a gravimetric technique. The initial waste moisture content was adjusted by adding distilled water up to 25% (wet basis); then, the humidity was increased to reach the final moisture content of 50% (wet basis), and approximately 1% (w/w) of H_2O_2 in the wet fraction of the waste was reached by adding a 2% (w/v) aqueous solution of hydrogen peroxide at a pH of 5.0. The waste mass was then inoculated with *B. atrophaeus* spores and exposed to UV light for each investigated condition.

Kinetic Models for Disinfection

The basic model to describe microbial inactivation is a first-order kinetic model (Equation (2)) (Chick, 1908):

$$-\frac{dN}{dt} = k.N \tag{2}$$

where N is the instantaneous number of surviving microorganisms (CFU), k is the first-order kinetic constant (s^{-1}, min^{-1}, h^{-1}) and t is time (s, min, h). When the disinfection process by UV/1% H_2O_2 occurs under isothermal conditions, the constant k can be defined and the integration of Equation (2) results in Equation (3):

$$\ln\left(\frac{N_0}{N}\right) = k.t \tag{3}$$

where N_0 is the initial number of microorganisms (CFU).

The typical dose-response curve of UV microbial inactivation may have an initial baseline region (shoulder) where little or no inactivation occurs. This region is attributed to the resilience of the organism against low doses of UV radiation. Above this threshold, the curve follows first-order kinetics with log-linear behavior, which ends with an asymptotic

decay, which is called the tailing effect. The decay is probably caused by shading or dead volumes in the pore surfaces (Chen, *et al*., 2010a; Fenner and Komvuschara, 2005; Marugán *et al*., 2008; Warriner *et al*., 2000).

Lambert and Johnston (2000) proposed the *Intrinsic Quenching* kinetic model to represent the resistance to the inactivation processes that causes the tailing effect in log-survivor curves, which is usually present during chemical disinfection. The model differential equation is given by Equation (4):

$$-\frac{dN}{dt} = k_1.C_0^{\,n}.e^{-k_2 n.t}.N \tag{4}$$

where k_1 is the microbial inactivation kinetic constant, C_0 is the initial concentration of the biocide, k_2 is the kinetic constant of biocide consumption and n is the biocide consumption reaction order. The integration of Equation (3) in excess biocide, in which C_0 can be assumed to be constant and under isothermal conditions, produces Equation (5) (Makky *et al*., 2011):

$$\ln\left(\frac{N_0}{N}\right) = \frac{k_1.C_0^{\,n}}{k_2.n}.(1-e^{-k_2.n.t}) = \frac{k}{Q}.(1-e^{-Q.t}) \tag{5}$$

where $Q = k_2.n$. In the fitting of this model for photocatalytic disinfection, k_1 can be assumed to be constant because it is linked to microbial inactivation. In this case, the isothermal condition can be assumed, and Q should be considered to be variable because it is dependent on biocide consumption. The OH radical is photocatalyzed by UV, which should depend on the UV light power applied.

The Hom model is suitable for the representation of one of two behaviors in the disinfection curve, shoulder or tailing effects. This model is not able to adjust the two shapes simultaneously, and it is described by Equation (6) (Chen *et al*., 2010a; Chong *et al*., 2011; Dalrymple *et al*., 2010; Facile *et al*., 2000; Marugán *et al*., 2008; Retta and Sagripanti, 2008):

$$-\frac{dN}{dt} = k.C^n.N.t^{m-1} \tag{6}$$

where k is the kinetic constant for microbial inactivation, C is the concentration of biocide, n is the reaction order of biocide consumption, t is the UV exposure time, and m is the exponent that takes into account the temporal dependence in the model. The integration of Equation (6) produces Equation (7):

$$\ln\left(\frac{N_0}{N}\right) = \frac{k.k'.t^m}{m} \tag{7}$$

where $k' = C^n$. In the Hom model, the product kk' should be dependent on P/M because it is associated with biocide consumption, and m can be assumed to be constant because it is bound to a dependence on the temporal order in the model.

Unlike the other models, the power law model is not first-order with respect to the decay of the microorganism concentration. The kinetic equation that represents this model is Equation (8) (Dalrymple *et al*., 2010; Haas and Kaymak, 2003; Majumdar *et al*., 1973):

$$-\frac{dN}{dt} = k.C^n.N^x \tag{8}$$

where x is the microbial inactivation order. The integrated equation of this model, in the condition of excess biocide and constant temperature, is given by Equation (9):

$$\ln\left(\frac{N_0}{N}\right) = \frac{1}{(x-1)}.\ln\left[1+(x-1).K.t.N_0^{\,(x-1)}\right] \tag{9}$$

with $K = k.C^n$. In this model, K must be variable with respect to P/M because it is associated with the biocide consumption, and *x* can be an adopted constant because it represents the order of microbial inactivation.

Kinetic Model Fit

The experimental inactivation levels ($\ln(N_0/N)$) were adjusted by the least squares method based on Chick's Law, intrinsic quenching, Hom and power law kinetic models to obtain the kinetic constants and assess the ability of the models to adequately describe the process studied. The errors were defined as the differences between the experimental and calculated inactivation levels for each test condition. The fit quality was evaluated from the comparison between the calculated and experimental inactivation levels with the bisector of the first quadrant as well as by the Root-Mean-Square Error (RMSE) and coefficient of determination (R^2) values present in Equations (10) and (11) (Kreyenschmidt *et al*., 2010):

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}\left[\ln\left(\frac{N_0}{N}\right)_{exp_i} - \ln\left(\frac{N_0}{N}\right)_{calc_i}\right]^2}{n}} \quad (10)$$

$$R^2 = 1 - \frac{\sum_{i=1}^{n}\left[\ln\left(\frac{N_0}{N}\right)_{exp_i} - \ln\left(\frac{N_0}{N}\right)_{calc_i}\right]^2}{\sum_{i=1}^{n}\left[\ln\left(\frac{N_0}{N}\right)_{exp_i} - \ln\left(\frac{N_0}{N}\right)_{mean}\right]^2} \quad (11)$$

RESULTS AND DISCUSSION

The initial percentage of sporulated microorganisms was evaluated, which resulted in 95% spores counted over 1000 elements (spores and vegetative cells), confirming that the *B. atrophaeus* used was predominantly in the spore form.

The recovery procedure for the spores inoculated in the waste mass was intended to recover, as near as possible, the same number of spores from the sterile waste as that recovered from the waste that was not exposed to the inactivation process. Table 2 shows the number of spores inoculated (N_{0I}) and subsequently recovered (N_{0R}) in this step.

The procedure for the recovery of the spores from the waste mass was satisfactory because it produced numbers on the same order of magnitude (10^6 spores) for spores inoculated and spores recovered and an average recovery percentage of 96% (Table 2). The recovery percentages obtained (87-104%) were considerably higher than those found by Oliveira *et al*. (2010) (72-93%) using the same saline solution immersion microorganism recovery procedure, but by counting the colony-forming units by the spread plate technique. However, for the healthcare waste mass of 0.540 kg used in the disinfection kinetic assays, the recovery fraction was nearly 100%; therefore, this method is valid for the analysis of inactivation efficiency during UV/H_2O_2 tests. The recovery levels obtained in the present study are much higher than the typical values presented in the literature (Brown *et al*., 2007; Hodges *et al*., 2006; Nicholson and Law, 1999; Perez *et al*., 2005), and this improvement can be attributed to the differences in the extraction methodology and the microorganism species involved. In the present case, the dispersion of the inoculated waste in a large volume of saline solution and the surface properties of *B. atrophaeus* fostered improved spore extraction (Chen *et al*., 2010b; Parkar *et al*., 2001; Seale *et al*., 2008).

Table 2: Results of recovery tests of the spores in the waste mass.

Test	Waste Mass (kg)	N_{0I} (CFU)	N_{0R} (CFU)	Recovery[a] (%)
1	0.405	1.0×10^6	1.1×10^6	104
2	0.405	1.0×10^6	9.7×10^5	95
3	0.405	1.0×10^6	8.9×10^5	87
4	0.405	1.2×10^6	1.1×10^6	91
5	0.405	1.5×10^6	1.4×10^6	93
6	0.540	9.9×10^5	1.0×10^6	101
7	0.540	1.2×10^6	1.2×10^6	100
8	0.810	1.5×10^6	1.4×10^6	93
		mean value		96

[a]Defined as N_{0R}/N_{0I}*100%. Tests were run in duplicate with an incoming waste moisture of 50 %.
*SD: standard deviation.

Figure 1 shows the influence of P/M on the inactivation fraction of spores (X) in the presence of 1% H_2O_2 in the wet fraction of the waste at the beginning of each test.

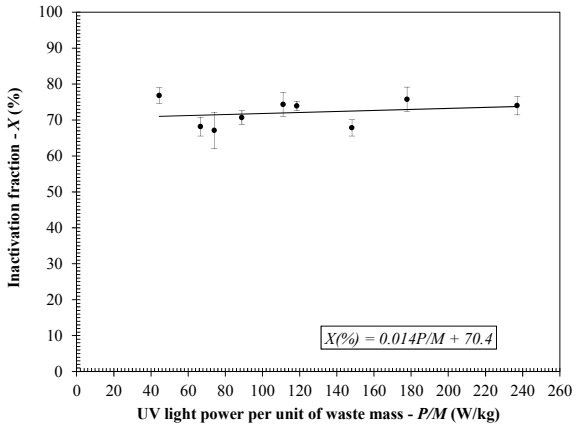

Figure 1: Spore inactivation fraction as a function of P/M for 50% moisture (wet basis), an H_2O_2 content of 1% (w/v) in the waste wet fraction and an exposure time of 10 min: (●) experimental data and (-) linear regression.

Figure 1 indicates that the spore inactivation fraction was essentially constant as a function of the UV light power per unit of waste mass (P/M). The increase in waste mass from 405 g to 810 g for the same power of UV light did not cause the expected decrease in the inactivation fraction. This decrease is expected because increasing the waste mass decreases the contact probability between the OH

radicals and the spores, which is one of the conditions required for the inactivation of the microorganism. The OH radicals originate from H_2O_2 and are photo-catalyzed by UV light. Thus, the greater the number of particles, greater the possibility of shadowing in the wet portion of the waste, which could prevent the UV light from forming OH radicals in some portions of the waste mass. Nevertheless, the available amount of H_2O_2, the impeller rotation and the degree of agitation produced conditions that validate the hypothesis of the completely mixed batch reactor in the solid medium. In Fig. 1, the increase in P/M from 44 to 237 W/kg (439% compared to the initial value) produced a slight upward trend in the inactivation fraction, which increased from 70 to 74% (only 6% of its initial value). These X values are fairly close to the average of all of the experimental values (72%). Consequently, the completely mixed batch reactor model was assumed to be representative of the disinfection system under study.

In the second phase, the inactivation degree of *B. atrophaeus* spores was experimentally obtained as a function of exposure time and P/M for a constant waste mass (540 g). Table 3 shows the experimental spore inactivation fraction after UV/1% H_2O_2 treatment (from 68 to 95%) and the experimental results obtained by Oliveira *et al*. (2010) for healthcare waste of the same gravimetric composition and incoming moisture, which was treated with microwaves. The table also shows the calculated inactivation fractions from the kinetic parameters of Oliveira *et al*. (2010) for microwave processing under the same conditions of the present study.

The inactivation fractions obtained using UV/1% H_2O_2 and microwave treatments suggest that exposure of healthcare waste to UV/1% H_2O_2 is significantly more efficient (72-95%) for spore inactivation than microwave processing (7-58%) for the same operational conditions, even for the maximum temperature for microwave processing at atmospheric pressure, 100 °C (Table 3). Nevertheless, the values obtained with UV/1% H_2O_2 are significantly below 99.99%, which is equivalent to Level III microbial inactivation.

Kinetic models were fitted to the experimental results to obtain the kinetic parameters and to identify the models that are able to represent the observed behavior. Figures 2-4 show the agreement between the experimental data and the fitted curves for the inactivation level of spores as a function of exposure time and P/M.

Table 3: Spore inactivation fraction by UV/1% H_2O_2 and by microwave.

	Time (min)	X (%) - *Present study*		
		67 W/kg	111 W/kg	178 W/kg
UV/H_2O_2 at room temperature	5	70	72	80
	10	68	74	76
	15	78	73	75
	20	78	88	89
	25	89	88	94
	30	90	88	95
	Time (min)	**X (%) - Oliveira *et al.* (2010)**		
		100 W/kg	**150 W/kg**	**200 W/kg**
Microwave in non-isothermal condition	20	15	34	36
	25	20	39	53
	30	22	43	60
	35	25	46	70
	40	33	49	71
	Time (min)	**X (%)[a] - Present study**		
		67 W/kg[b]	**111 W/kg**	**178 W/kg**
Microwave in isothermal condition at 100 °C	5	-	7	14
	10	-	13	25
	15	-	19	35
	20	-	24	44
	25	-	29	52
	30	-	34	58

[a]Based on the kinetic parameters of Oliveira *et al*. (2010). [b]Condition not covered by Oliveira *et al*. (2010).

Figures 2-4 show that the first-order kinetic model was not able to represent the experimental data because the model underestimated the inactivation level at the beginning and overestimated the level at the end of the process. The intrinsic quenching, Hom, and power law models were able to describe the tailing effect at the end of the inactivation curve, which was probably caused by spores that were not exposed to UV light because of pores and roughness in the solid material (Gardner and Shama, 1998; Warriner *et al*., 2000). However, the disinfection process did not exhibit a shoulder effect, which can be attributed to the spore resilience to the low UV dose. In contrast, a high level of inactivation was observed in the first 10 min, possibly indicating the preferential consumption of hydrogen peroxide in this period.

Table 4 shows the kinetic constants adjusted for the intrinsic quenching, Hom, and power law models. Table A1 contains the experimental and calculated levels of inactivation, the standard deviations between the triplicate experimental data and the standard deviations between the predicted and the mean values of $\ln(N_0/N)$.

Table 4: Kinetic parameters for the first-order kinetic, intrinsic quenching, Hom, and power law models.

P/M		67 W/kg	111 W/kg	178 W/kg	RMSE	R^2
Kinetic model	**Parameter**	**Value**				
First-order kinetic	k (min^{-1})	0.09	0.09	0.11	0.454	0.84
Intrinsic Quenching	k (min^{-1}) Q (min^{-1})	0.18 0.08	0.20 0.08	0.20 0.06	0.324	0.87
Hom	kk' (min^{-m}) m (-)	0.21 0.47	0.23 0.38	0.25 0.54	0.266	0.91
Hom but m equal to a constant	kk' ($min^{-0,46}$) m (-)	0.21 0.47	0.22	0.26	0.271	0.85
Power Law	K ($CFU^{1-x}.min^{-1}$) x	6.9E-06 1.72	0.9E-06 1.88	3.1E-05 1.64	0.249	0.92
Power Law but x equal to a constant	K ($CFU^{-0,80}.min^{-1}$) x	2.6E-06 1.80	2.4E-06	4.1E-06	0.256	0.92

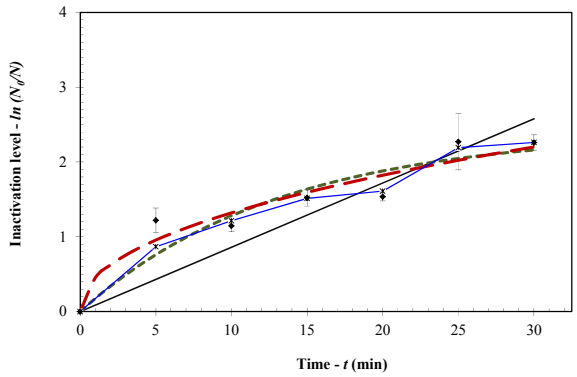

Figure 2: Levels of spore inactivation as a function of time for a P/M of 67 W/kg for 50% moisture (wet basis) and an H_2O_2 content of 1% (w/v) in the waste wet fraction: (♦) experimental data, (—) first-order kinetic model, (--) Intrinsic Quenching model, (— —) Hom model and (—✳—) Power Law model.

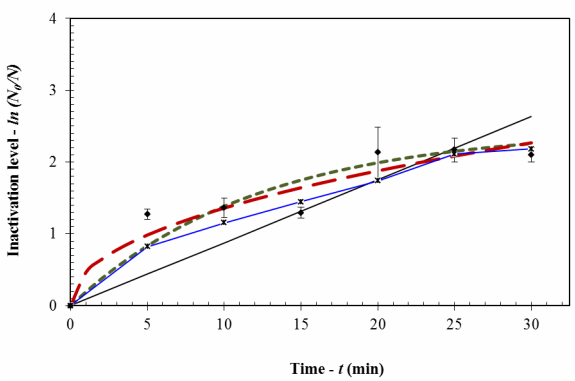

Figure 3: Levels of spore inactivation as a function of time for a P/M of 111 W/kg for 50% moisture (wet basis) and an H_2O_2 content of 1% (w/v) in the waste wet fraction: (♦) experimental data, (—) first-order kinetic model, (--) Intrinsic Quenching model, (— —) Hom model and (—✳—) Power Law model.

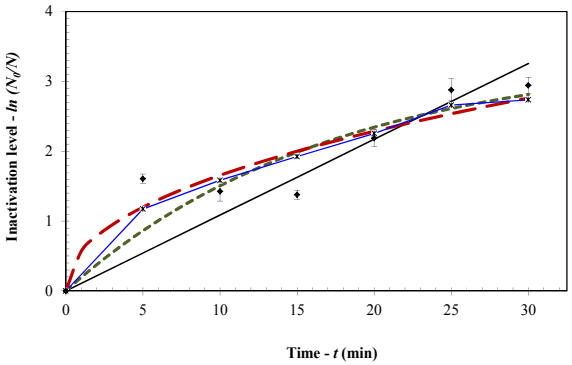

Figure 4: Levels of spore inactivation as a function of time for a P/M of 178 W/kg for 50% moisture (wet basis) and an H_2O_2 content of 1% (w/v) in the waste wet fraction: (♦) experimental data, (—) first-order kinetic model, (--) Intrinsic Quenching model, (— —) Hom model and (—✳—) Power Law model.

The best model to represent the healthcare waste disinfection process by UV/1% H_2O_2 was the power law model, which exhibited the lowest RMSE (0.249-0.256) and R^2 equal to 0.92, followed by the Hom model (RMSE in the range of 0.266-0.271 and R^2 of 0.85-0.91), under the studied conditions. However, the application of the power law model is not feasible during the design phase of a disinfection system with UV/H_2O_2 because it requires knowledge of the initial number of microorganisms that con-

taminate the waste, which is not always available. Table 4 shows that, for the intrinsic quenching model, k ($k_1.C_0^n$) is almost constant as a function of P/M because it is a parameter directly associated with microbial inactivation (k_1) and the biocide concentration (C_0), which was in excess. In this model, the biocide is inactivated or quenched; however, its initial quantity is assumed to be in large excess over the number of microorganisms in the waste. The effect of biocide consumption (by OH radicals) is expressed by the Q parameter. The Q values (k_2.n) decreased as a function of P/M, which suggests an increase in the biocide consumption rate, indicating an increase in the photocleavage of H_2O_2 by UV light, which was reflected in increased levels of spore inactivation. In the IQ model, as the k value increases, the final inactivation level also increases, and the Q value is reduced, resulting in a stronger trend toward first-order kinetics (Lambert and Johnston, 2000). Therefore, the increase in P/M caused the inactivation to obey a trend similar to first-order kinetics, but distinct from first-order behavior because first-order kinetics were not able to represent the behavior of the results in Figure 4 (higher P/M).

Similar to the IQ model, the Hom model uses two kinetic constants to represent the complex process of photocatalytic disinfection. The Hom model attempts to describe the initial shoulder and the tailing effects that appear in some disinfection processes, but it does not describe both behaviors simultaneously. The k constant represents the kinetics of the microbial inactivation, and k' represents the concentration of biocide (C^n). The product k.k' became dependent on the P/M ratio because it was related to the consumption rate of biocide, and m was adopted as a constant because it is linked to the temporal dependence in the model. Values of m higher than 1 describe a shoulder behavior in the inactivation level curves, and m values less than 1 represent the tailing end in the same curves. In fact, the m value was found to be equal to 0.47; therefore, it was compatible with the tailing behavior observed in the microbial inactivation curves (Figures 2-4). The kinetic parameter kk' tended to increase as a function of P/M, probably because the higher UV power provided more biocide (OH radicals) from H_2O_2 photocleavage to inactivate the spores, resulting in higher inactivation rates in relation to P/M because of the increase of k' (C^n) (Table 4).

In the power law model, the inactivation rate is assumed not to show first-order kinetics (x order) with respect to the concentration or number of microorganisms. In the case of biocide in excess, the kinetic constant K can also involve the intrinsic death kinetic constant (k) and the biocide concentration (C^n). Analogously to the Hom model, this model can fit shoulder ($x < 1$) or tailing behavior ($x > 1$) in the inactivation level curve. The model was first applied to each data set with respect to P/M, for which x was in the range of 1.6 to 1.9, and K increased as a function of P/M. Because x is the inactivation reaction order with respect to the number or concentration of microorganisms (N), it was assumed to be constant in relation to P/M. Again, the tailing effect was demonstrated because x was found to be equal to 1.8 ($x > 1$), and K was found to increase in the range of 2.4×10^{-6} to 4.1×10^{-6} $CFU^{-0,80}.min^{-1}$ as a function of P/M. This trend probably occurred because the UV light provided more biocide (OH radicals) in the medium to inactivate the spores (Table 4).

The fit quality was also evaluated from the comparison between the calculated and experimental inactivation levels with the bisector of the first quadrant (Figure 5).

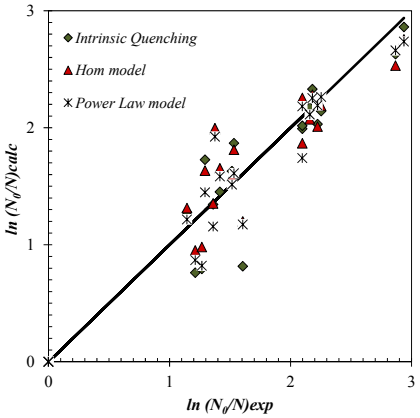

Figure 5: Inactivation levels in relation to the bisector of the first-quadrant: (♦) Intrisic Quenching model, (▲) Hom model and (✱) Power Law model.

In Figure 5, the scattering of the points from the diagonal line was very similar, which indicates that the models equivalently represent the disinfection process of healthcare waste by UV/1% H_2O_2 under the studied conditions. Although the power law model produced lower RMSE values when compared with the results from the Hom model, the Hom model was applied to estimate the time required to reach Level III microbial inactivation (Table 5).

The inactivation times from Table 5 confirm that healthcare waste treatment by UV/1% H_2O_2 is more efficient for the inactivation of spores than exposure to microwaves (Table 5), but the time required to

reach Level III inactivation is still quite large for use as an independent treatment process.

Table 5: *B. atrophaeus* spore inactivation time to reach Level III.

Process	P/M (W/kg)	Inactivation time (min)
UV/H_2O_2 at room temperature	67	687
	100	582
	111	552
	178	411
Microwave in isothermal condition at 100°C[a]	67	-[b]
	100	779
	111	665
	178	317

[a]Based on the kinetic parameters of Oliveira *et al*. (2010).
[b]Condition not covered by Oliveira *et al*. (2010).

CONCLUSIONS

The procedure tested for recovery of the spores inoculated in the waste mass (by immersion in saline and counting the colony-forming units by the pour plate technique) provided a recovery percentage sufficiently close to 100% and higher than the results obtained by other procedures described in the literature.

The constancy of the spore inactivation fraction (X) with variation of the waste mass (M) and UV power per waste mass unit (P/M) showed that the impeller rotation and agitation degree produced conditions that validated the hypothesis of a completely mixed batch reactor in the solid medium.

The spore inactivation fractions in the range of 68 to 95% obtained in the study confirmed that healthcare waste treatment by UV/1% H_2O_2 is significantly more effective than exposure to microwaves under similar conditions.

The first-order kinetic model was not able to represent the treatment process by UV/1% H_2O_2 under the conditions studied. The intrinsic quenching model, despite describing the tailing effect of the experimental levels of spore inactivation, did not provide the best representation of the process studied. The power law model offered the best description of the experimental spore inactivation level. However, the use of the Hom model provided an equivalent representation.

The kinetic constants associated with microbial inactivation increased with increasing P/M for both the Hom and Power Law models. This behavior was attributed to the catalytic effects of UV light on H_2O_2 photocleavage and the consequent increase in the concentration of the biocide in the medium.

The simulated times of spore inactivation in the range of 411-687 min by the Hom model show that the tailing effect excludes this process from consideration as a sole treatment alternative for obtaining Level III spore inactivation.

The inactivation fractions in the range of 70% to 80% for exposure times in the range of 5 to 10 min and P/M values of 67 W/kg to 178 W/kg showed the potential of this technique as a promising pre-treatment process for healthcare waste.

ACKNOWLEDGEMENTS

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NOMENCLATURE

C	concentration of the biocide	M
C_0	initial concentration of the biocide	M
K	kinetic parameter ($K = k.C^n$) in the Power Law model	$CFU^{1-x}.min^{-1}$
k	first-order kinetic constant, $k_1.C_0^n$ in the Intrinsic Quenching model	$CFU^{1-x}.min^{-1}$
	(s^{-1}, min^{-1}, h^{-1}), kinetic constant of microbial inactivation in the Hom model ($M^{-n}.min^{-m}$) or kinetic constant in the Power Law model	
k_1	microbial inactivation kinetic constant in the Intrinsic Quenching model	$min^{-1}.M^{-n}$
k_2	kinetic constant of biocide consumption in the Intrinsic Quenching model	s^{-1}, min^{-1}, h^{-1}
k'	$k' = C^n$ in the Hom model	M^{+n}
M	healthcare waste mass	kg
m	temporal order in the Hom model	(-)
N	instantaneous number of surviving microorganisms	CFU
N_0	initial number of spores inoculated in the waste	CFU
N_{0I}	number of spores inoculated in the waste at recovery tests	CFU
N_{0R}	number of spores recovered from previously inoculated waste at recovery test	CFU

n	reaction order of biocide consumption	(-)
P	UV light power	W
Q	parameter in the Intrinsic Quenching model	$k_2.n\ s^{-1}$, s^{-1}, min^{-1}, h^{-1}
RMSE	Root-Mean-Square Error	(-)
R^2	coefficient of determination	(-)
t	time	s, min. h
X	inactivation fraction of the spores	%
x	microbial inactivation order in the Power Law model	(-)

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APPENDIX

Table A1 contains the experimental and calculated levels of inactivation, the standard deviations between the triplicate experimental data and the standard deviations between the predicted and the mean values of $\ln(N_0/N)$.

Table A1: Levels of inactivation and the values of the standard deviations obtained during the fitting of the kinetic models.

P/M (W/kg)	time (min)	N_0 (CFU)	Experiment		First-order kinetic model		Intrinsic Quenching model		Hom model*		Power Law model**	
			$\ln(N_0/N)_{exp}$	SD***	$\ln(N_0/N)_{calc}$	SD	$\ln(N_0/N)_{calc}$	SD	$\ln(N_0/N)_{calc}$	SD	$\ln(N_0/N)_{calc}$	SD
67	5	1.7E+06	1.22	0.16	0.43	0.56	0.76	0.33	0.96	0.19	0.87	0.25
	10	1.3E+06	1.15	0.08	0.86	0.20	1.28	0.10	1.32	0.12	1.21	0.05
	15	1.2E+06	1.52	0.11	1.29	0.16	1.64	0.08	1.59	0.05	1.51	0.00
	20	9.9E+05	1.53	0.05	1.72	0.13	1.88	0.24	1.82	0.20	1.61	0.05
	25	1.6E+06	2.27	0.38	2.15	0.09	2.05	0.16	2.02	0.17	2.19	0.06
	30	1.4E+06	2.26	0.11	2.58	0.23	2.16	0.07	2.20	0.04	2.26	0.00
111	5	1.7E+06	1.27	0.07	0.44	0.59	0.83	0.31	0.99	0.20	0.82	0.32
	10	1.3E+06	1.37	0.14	0.88	0.35	1.38	0.01	1.36	0.00	1.16	0.15
	15	1.2E+06	1.30	0.08	1.32	0.01	1.75	0.32	1.64	0.25	1.45	0.11
	20	1.3E+06	2.14	0.35	1.75	0.27	1.99	0.10	1.88	0.18	1.74	0.28
	25	1.6E+06	2.17	0.16	2.19	0.02	2.15	0.01	2.08	0.06	2.11	0.04
	30	1.4E+06	2.10	0.10	2.63	0.37	2.04	0.05	2.27	0.12	2.18	0.06
178	5	1.7E+06	1.61	0.07	0.54	0.75	0.86	0.53	1.20	0.29	1.17	0.31
	10	1.3E+06	1.42	0.14	1.09	0.24	1.51	0.06	1.66	0.16	1.58	0.11
	15	1.2E+06	1.38	0.06	1.63	0.18	1.99	0.43	2.00	0.44	1.92	0.39
	20	1.3E+06	2.19	0.12	2.17	0.01	2.34	0.11	2.29	0.07	2.25	0.05
	25	1.6E+06	2.88	0.16	2.71	0.12	2.61	0.19	2.54	0.24	2.66	0.15
	30	1.4E+06	2.94	0.12	3.26	0.22	2.81	0.09	2.76	0.13	2.74	0.15

* Data from *Hom* model assuming *m* equal to a constant.
** Data from *Power Law* model assuming *x* equal to a constant.
***SD: standard deviation