

Predictive models in the ultrasonic disinfection of hospital-effluents: a review and suggestion for future research

Modelos preditivos na desinfecção ultrassônica de efluentes hospitalares: uma revisão e sugestão para pesquisas futuras

Modelos Predictivos en la Desinfección Ultrasonica de Efluentes Hospitalarios: una revisión y sugerencia para futuras investigaciones

Received: 08/21/2022 | Reviewed: 08/30/2022 | Accept: 09/03/2022 | Published: 09/11/2022

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Abstract

At the end of this review on applications of ultrasonic waves in the inactivation of microorganisms in hospital laundry effluents, the fourth type of predictive model is proposed, based on the application characteristics of the experimental plans. The number of works for the disinfection of wastewater by ultrasound waves (US) associated with other techniques or individuals has been increasing. The use of ultrasound to produce lethal effects on microorganisms is attractive because it is considered a "green" technology in that it involves sound energy and does not require additional chemicals, or a very small amount. Also, the US as a tool for water reuse processes - operates at low pressure and temperature and does not produce toxic or greenhouse gases. Hospital laundry effluents are characterized by different microorganisms, a fact that can hinder the application of conventional physical-chemical technologies. To contribute to future developments in the application of ultrasound (US), this work uses some typical US applications in the disinfection of aqueous effluents. To propose strategies for carrying out new works, comments are made regarding an approach oriented towards new advances based on the need to interrelate the variables involved in the studies, at the same time that these variables are correlated with the rate of inactivation. For this, it is discussed the predictive models and their contributions, according to the number of factors that should be involved for a better understanding of the rate of microbial inactivation.

Keywords: Ultrasonic waves; Hospital laundry; Water disinfection; Experimental planning.

Resumo

Ao final desta revisão sobre aplicações de ondas ultrassônicas na inativação de microrganismos em efluentes de lavanderia hospitalar, é proposto o quarto tipo de modelo preditivo, baseado nas características de aplicação dos planos experimentais. O número de trabalhos para a desinfecção de águas residuais por ondas ultrassônicas (US) associado a outras técnicas ou individuais tem vindo a aumentar. O uso do ultrassom para produzir efeitos letais sobre os microrganismos é atraente porque é considerado uma tecnologia "verde" por envolver energia sonora e não requer produtos químicos adicionais, ou uma quantidade muito pequena. Além disso, o US como ferramenta para processos de reaproveitamento de água - opera em baixa pressão e temperatura e não produz gases tóxicos ou de efeito estufa. Os efluentes de lavanderia hospitalar são caracterizados por diferentes microrganismos, fato que pode dificultar a aplicação de tecnologias físico-químicas convencionais. Para contribuir com futuros desenvolvimentos na aplicação do ultrassom (US), este trabalho utiliza algumas aplicações típicas do US na desinfecção de efluentes aquosos. Para propor estratégias para a realização de novos trabalhos, comenta-se uma abordagem voltada para novos avanços a partir da necessidade de inter-relacionar as variáveis envolvidas nos estudos, ao mesmo tempo em que essas variáveis são correlacionadas com a taxa de inativação. Para isso, são discutidos os modelos preditivos e suas contribuições, de acordo com o número de fatores que devem estar envolvidos para um melhor entendimento da taxa de inativação microbiana.

Palavras-chave: Ondas ultrassônicas; Lavanderia hospitalar; Desinfecção de água; Planejamento experimental.

Resumen

Al final de esta revisión sobre aplicaciones de ondas ultrasónicas en la inactivación de microorganismos en efluentes de lavado hospitalario, se propone el cuarto tipo de modelo predictivo, basado en las características de aplicación de los planes experimentales. El número de trabajos para la desinfección de aguas residuales por ondas de ultrasonido (US) asociado a otras técnicas o particulares ha ido en aumento. El uso de ultrasonido para producir efectos letales en microorganismos es atractivo porque se considera una tecnología "verde" en el sentido de que involucra energía de sonido y no requiere productos químicos adicionales, o una cantidad muy pequeña. Asimismo, el US como herramienta para procesos de reutilización de agua - opera a baja presión y temperatura y no produce gases tóxicos ni de efecto invernadero. Los efluentes de lavandería hospitalaria se caracterizan por la presencia de diferentes microorganismos, hecho que puede dificultar la aplicación de tecnologías físico-químicas convencionales. Para contribuir a futuros desarrollos en la aplicación de ultrasonidos (US), este trabajo utiliza algunas aplicaciones típicas de US en la desinfección de efluentes acuosos. Para proponer estrategias para la realización de nuevos trabajos, se comenta un enfoque orientado hacia nuevos avances basado en la necesidad de interrelacionar las variables involucradas en los estudios, al mismo tiempo que estas variables se correlacionan con la tasa de inactivación. Para ello, se discuten los modelos predictivos y sus aportes, según la cantidad de factores que deben estar involucrados para una mejor comprensión de la tasa de inactivación microbiana.

Palabras clave: Ondas ultrasónicas; Lavandería hospitalaria; Desinfección de agua; Planificación experimental.

1. Introduction

There are several sources of contamination of water bodies and one of them may be through the disposal of effluents with high polluting power. These effluents cannot be discharged directly into rivers without any kind of pre-treatment, or in public sewage systems that cannot treat this type of environmental liability. Studies on the possibility of reusing wastewater are essential due to the large amount of water involved in the washing process of industrial laundries (Ciabattia et al., 2009). In

some countries, most of these watercourses, in addition to receiving these effluents, are also sources of collection for the water supply to be consumed by the population, generating risks to the health and well-being of consumers.

Among the sources of contamination are the laundries in the hospital sector, where there is a considerable volume of contaminated effluents. Due to the large generation of effluents, hospital laundry is a sector of great relevance, with an estimated 400 to 1200 L / bed/day (Ashfaq & Qiblawey, 2018). As basic characteristics, less biodegradability can be attributed to the hospital laundry effluent. Also, the composition of the effluent generated in these laundries still contains the presence of pathogenic microorganisms, disinfectants, antibiotics, cleaning agents, surfactants, humectants, among others. According to Nuñez and Moreton (2007), this complex character is what attributes a harmful character to the effluent generated by hospital laundry, causing complications in the biological treatment of treatment plants and presenting risks to the aquatic ecosystems in which this effluent is released.

The bacteriological quality most used for the evaluation of the sanitary conditions of the water is the presence of bacteria of the coliform group (total coliforms, *Escherichia coli*, and Heterotrophic Bacteria). Such bacteria are present in the human intestinal tract and are eliminated by feces, indicating a high probability of the presence of other pathogenic organisms (Verlicchi, et al., 2015). According to studies carried out by Ashfaq and Qiblawey (2018), the treatment of hospital effluents is based on the characteristics of the final effluent generated by these health establishments, taking into account the different sectors of this sector, that is, laboratories, laundry, kitchen, clinics, etc. In this way, the current methods of eliminating bacteria from the water to be reused by the clothes processing unit in a hospital, there is the direct action of chlorine, the application of ozone, and advanced oxidative processes (Souza et al., 2019).

Sewage treatment stations are designed primarily for the removal of organic material (BOD and COD). With rare exceptions, secondary biological treatment fails to efficiently eliminate indicator and pathogenic microorganisms and, therefore, require specific units for disinfection, which becomes an essential barrier for a considerable reduction in public health risks (Zotesso et al., 2017).

The technology of the use of ultrasound (US) has demonstrated good efficacy in the treatment of wastewater [wastewater treatment]. This technique has assumed a prominent role among treatments of a physical nature and, mainly, due to the ease of operation, flexibility, and the ability to vary the necessary intensities of cavitation conditions (Hawrylik, 2019). The ultrasound application aims to avoid the use of chemicals, agents that can be harmful to the health of ecosystems, such as chlorine. This work aims to evaluate predictive models of the inactivation of microorganisms in hospital laundries and to make suggestions for improvements in this area with the help of ultrasound.

2. Methodology

2.1 Ultrasonic Radiations

The ultrasound wave is identified by a mechanical wave and any vibrational wave whose frequency is above the range that the human ear can hear, that is, frequencies above 16 kHz receive this name (Leighton, 2007). Ultrasonic waves can be divided into a high frequency (100 kHz to 1 MHz) and low power (less than 1 W / cm²). There are low frequency (16 kHz to 100 kHz) and high power (10 W / cm² to 1 kW / cm²), which are used to alter the physical and chemical properties of matter, through sonochemistry (Ince, 2018).

Acoustic cavitation is one of the effects of ultrasound used in the destruction of membranes that line the cells of pathogenic microorganisms existing in a liquid medium. Ultrasonic waves through a liquid medium generate compressions and rarefactions (Patil & Pandit, 2007). The ultrasonic field compresses the molecular structure of the medium with an increase in acoustic pressure and then expands them by a decrease in pressure, dispersing the molecules. The rarefaction produces microbubbles or cavities containing the liquid itself in the form of vapor or the gases dissolved in the liquid. In the

compression stage, there is a violent collapse of these microbubbles, completing a cycle. When these microbubbles reach this rarefaction cycle, where negative sound pressure is large enough to separate the water molecules from each other. The critical molecular distance achieved, R , for molecules it is on the order of 10^{-8} m. As a result, 'voids' are created in the liquid (Zupanc et al., 2019).

The speed of an ultrasound wave through a medium varies with the physical property of the medium. In low-density media, such as air and other gases, molecules can move relatively long distances before they influence neighboring molecules (Yusof et al., 2016). Within these media, the speed of an ultrasound wave is relatively low. In solids, molecules are limited in their movements and the ultrasound speed is relatively high. Liquids exhibit intermediate ultrasound speeds between those of gases and solids.

In liquids and gases, such oscillations propagate with greater intensity in the wave direction, producing longitudinal waves (Shabir et al., 2021). When US vibrations propagate through a liquid medium, they generate regions of compression and rarefaction (de Andrade Filho et al., 2022). Ultrasound combine with WRS (Water resonance system) was able to separate bacteria effectively from the surface of the skin (Vetchapitak et al., 2020).

2.2 Effect of Ultrasound on Inactivation of Microorganisms

When the liquid is under an ultrasonic field, microbubbles formed by large pressure oscillations (500 to 10,000 atm) implode, giving rise to high temperatures (3000 to 5000 K) inside these microbubbles, associating such conditions to the production of active species as free radicals. Studies have proven that the mechanisms of inactivation of microorganisms by ultrasound derive from these ultrasonic effects, called acoustic cavitation, and their consequences as shear forces and microjets, leading to a complete rupture of the cell encapsulation membrane (Li et al., 2016). Sonication, the name given to the combination of these effects, can also destroy clusters of microorganisms and flakes in effluents.

3. Results and Discussion

3.1 Predictive Models

The prediction of the inactivation rate plays an important role in the control of disinfection processes and is therefore an essential tool in the use of existing techniques and the development of new techniques (Amin et al., 2013). The variability in the responses of microorganisms, given the different conditions imposed by the disinfection technique, justifies the need to select appropriate models for a precise interpretation in the validation of the results obtained.

The classification of predictive models in primary, secondary, and tertiary was proposed by (Whiting, 1993). The so-called primary models consider only the variation in cell concentration over time. Such models also do not take into account variables of the environment such as pH, temperature, among others (Rodriguez-Martinez et al., 2020). The works that are based on these models show mortality or growth curves of microorganisms. Among the primary predictive models, the most used are the logistical curve (Chatterjee et al., 2015), the Gompertz model (Belda-Galbis et al., 2014), and the Baranyi and Roberts model (Huang, 2013). In Madge and Jensen (2002) applied 20 kHz ultrasonic waves for the disinfection of domestic sewage. The disinfection efficiency was evaluated using fecal coliforms, with the aid of a filtering membrane. The disinfection data were adjusted to first-order kinetics, constant k , which was determined by a primary model through linear regression of the type $-\log(N/N_0)$ as a function of time. N is the concentration of fecal coliforms at a time t and N_0 is the initial concentration of fecal coliforms.

Secondary predictive models in which variations in environmental factors are considered. In polynomial secondary models, the effects caused by environmental conditions on the growth or mortality of microorganisms are described by a polynomial function. Thus, from the growth rates of primary models, the coefficients of the second model can be determined

by regression. However, the use of prediction models obtained with the application of factorial, fractional factorial, or central composite design (CCD) design can also obtain the coefficients of these models (Ansari et al., 2016).

In predictive models of the square root model and cardinal model types, the effect, T_{max} , for example, is described by separate equations (Ratkowsky et al., 1983). Based on the argument that the model proposed by Ratkowsky et al. (1983) is restricted to a single parameter (Rosso, Lobry & Flandrois, 1993), they proposed an empirically constructed model to describe microbial growth or mortality. With justifications for improving the quality of the predictions, with the aid of a more accurate description of variability and uncertainties, stochastic models were proposed by Baranyi and Roberts (1995).

The application of models based on the approach of metabolic flow networks to the area of predictive microbiology is on the rise (Vercammen, et al., 2014). The metabolic flow analysis technique is applied in steady-state situations and the dynamic metabolic flow balance analysis technique is applied in non-steady-state conditions. Both are used to predict intracellular flows.

Software such as MicroFit, MicroHibro, NeuralWorks, FANN (Fast Neural Networks), and COMMGEN / Matlab is part of predictive microbiology as tertiary type models. This software for predicting the growth/mortality of microorganisms has become popular in recent years using more elaborate logic (Oscar, 2020).

3.2 Relevant Parameters

In general, the rate of inactivation of microorganisms has shown to be dependent on parameters associated with the technique and operational conditions, such as duration of sonication, ultrasonic energy level, frequency, the temperature of the medium, pH of the medium, properties of the microorganisms, including the cell size and shape, stage of development and species, among others such as Amabilis-Sosa et al. (2018) and Gao et al. (2014).

Using ultrasonic radiation from a generating unit with a frequency of 20 kHz, Madge and Jensen (2002) studied the disinfection of domestic sewage. Fecal coliform bacteria were used as a microorganism to indicate the rate of disinfection. For this, an ultrasonic reactor Misonix, Inc. (Farmingdale, New York), Sonicador of the XL series equipped with a conical probe of 13 mm was used. The factors controlled during sonication were frequency, sonication time, ultrasound power and intensity, pH, alkalinity, and total suspended solids. The disinfection efficiency increased with the increase of the power transferred to the ultrasound, from 0.003 log (deaths) / min at 70 W / L to 1.8 log (deaths) / min at 1250 W / L.

To assess the scientific and economic potentials and optimize the disinfection of wastewater (*E. coli* and fecal streptococci), Blume and Neis (2004) applied a combination of US with UV in the pre-treatment stage. The application of ultrasound for 20 s at a low density of 30 W / L reduced the particle size distribution of the samples, varying the average particle diameter from 70 to 11 μ m. The ultrasound reactor was a "Branson Sonifier W-450", a horn sonotrode equipped with a 1.3 cm diameter horn tip that is operated at 20 kHz. As independent variables stand out the electrical energy used, in the range of 41-154 W, at ultrasonic intensities of 1.7 to 60.8 W / cm², and densities of 10 to 400 W / L. As a UV source, a low pressure mercury arc lamp (manufacturer: "Pureflow Ultraviolet Inc.", Nominal length: 20 cm, diameter: 1.3 cm) was installed inside a tubular floating chamber (volume: 300 mL). A thin surrounding layer of quartz protected the glass of the sample lamp which flowed parallel to the orientation of the lamp. The energy consumption was 14 W, of which 3 W are emitted at 254 nm (37 mW / cm² @ 1 m), the relevant wavelength for bacteria inactivation.

Ultrasonic irradiation of bacterial suspensions, in the presence of t-butanol as a free radical scavenger, was performed by Koda et al. (2009) to investigate the effect of these radicals on the inactivation of microorganisms. The experiments were carried out with the aid of two individual frequencies, 20 and 500 kHz, using two types of reactors. The frequency of 20 kHz was used as a reference. US at 500 kHz was used to inactivate the microorganisms *Escherichia coli* and *Streptococcus mutans* as a function of the sonication time and the power supplied. For sonication at 500 kHz, a sonochemical reactor of the HSR-01

type (Honda Electronic Co. Ltd.) was used. The diameter and height of the irradiation glass cell were 43 and 112 mm, respectively. For sonication at 20 kHz, a Sonifier 450D (Branson Ultrasonics Co.) was used. In this case, a double-walled glass cell 55 mm in diameter and 80 mm high, with a volume of 50 cm³. To avoid simultaneous thermal disinfection, the temperature was set to 10 ° C. The sonication of bacterial suspensions was performed in four different ultrasonic potencies, ranging from about 1 to 14 W. The logarithm of the survival rate decreased linearly with the irradiation time, except for *E. coli*, in the higher band range of power. It was observed that the speed constants increase with the ultrasonic power supplied to the solution and decrease dramatically with the increase in the concentration of t-butanol as a scavenger of free radicals in the bacterial suspension.

Drakopoulou et al. (2009) studied the effect of ultrasound on the disinfection of gram-negative and gram-positive bacteria, in the presence and absence of solid titanium dioxide particles. For high energy US (5400 W / L) of 24 kHz, the average deactivation rate of gram-negative bacteria, such as total coliforms, fecal coliforms, and *Pseudomonas* spp. was 99.5%, 99.2%, and 99.7%, respectively. However, the gram-positive bacteria *Clostridium perfringens* and fecal *streptococci* were resistant to deactivation with an average removal of 66% and 84%. The statistical analysis applied to the experimental data demonstrated a great concern of the authors to validate the preliminary data for later application, although important concepts on how to calculate their uncertainties have not been discussed.

The sonication effect at frequencies of 20, 40, and 580 kHz and approximately the same acoustic intensity on the viability and decline of two microorganisms (*Escherichia coli* and *Klebsiella pneumonia*) were investigated by Joyce, et al., (2011). The biocidal effect of sonication was verified using plaque counts. The results recorded at 20 and 40 kHz indicate a decline in the number of cells after 15 minutes of sonication. The results at frequencies of 580 kHz, but an intensity similar to 20 and 40 kHz, generally resulted in a half log reduction of viable bacteria after 15 min of sonication. Sonication can cause inactivation or decline, depending on the intensity and frequency. Flow cytometry provides a method for distinguishing and quantifying effects by looking at two subpopulations: (i) live / viable cells and (ii) dead bacterial cells.

Gao et al. (2014) demonstrate that the main reason for bacterial resistance to ultrasonic deactivation is due to the properties of the bacterial capsule. Microbes with thicker and "softer" capsules are highly resistant to the process of ultrasonic deactivation. *Enterobacter aerogenes*, *Bacillus subtilis*, *Staphylococcus epidermidis*, *S. epidermidis* SK and *Staphylococcus pseudintermedius* were chosen for this study due to their variable physical and biological properties. This study determined the effects of treatment with low-frequency US (20 kHz) for a constant sonication time of 20 min, but with a variation in US power up to an intensity of 13 W. More specifically, they investigated the relationship between the efficiency of deactivation and physical properties (size, hydrophobicity) and biological (growth phase) microbes.

Kumar et al. (2014) irradiated wastewater with US at two different frequencies (35 kHz, 130 kHz) to remove *E. coli*, for different periods (5, 10, 20, and 30 min). The sonication experiments were conducted according to the following conditions: Treatment time of 5, 10, 20, and 30 min; Sample volume of 100 ml; Frequencies: 35 kHz and 130 kHz; US power of 250 W; Power intensity provided per transducer area of 50.95 watts per cm²; Energy density supplied per sample volume of 2500 W / L. With the increase in frequency and time, the population of bacteria has decreased. It was observed that the frequency of 130 kHz was more effective than 35 kHz.

Ganesan et al. (2015) investigated the effects of high-intensity ultrasound on the inactivation of wild bacteria in the pasteurized bed (*Bacillus atrophaeus* spores inoculated in sterile milk and *Saccharomyces cerevisiae* inoculated in sterile orange juice). They used an experimental design to correlate the independent variables: sonication temperature (range 0 to 84 ° C), amplitude (range 0 to 216 μm), and time (range 0.17 to 5 min). As a response variable, the logarithmic reduction of microorganisms was monitored. Optimization of microorganism inactivation was found at 84.8 ° C, the amplitude of 216 μm and the time of 5.8 min. The authors did not refer to previous studies to define the factors used in the definition of optimized

operational conditions of the process.

The absence of fecal coliforms in treated wastewater is a safety indicator for use in activities such as green irrigation areas. Based on this premise, total and fecal coliforms were used as standard indicators by Amabilis-Sosa et al. (2018), aiming at reducing water stress, with the reuse of municipal wastewater recovered by treatment using US in the inactivation of these bacteria. Inactivation tests were performed on pure cultures of *Escherichia coli* (EC) ATCC 11229 and *Bacillus subtilis* (BS) ATCC 6633. During experimentation, amplitude (35%) and frequency (20 kHz) were kept constant. Thus, the statistical treatment was based on two factors: (1) The type of microorganism was evaluated with two levels of EC and BS; (2) The sonication time was evaluated with four levels of 15, 30, 45, and 60 min. All combinations were performed in triplicate with temperature monitoring. Thus, during the experiments, the amplitude and frequency were kept constant. Thus, the experiments were based on two factors: (1) The type of microorganism, evaluated with two levels - EC and BS; (2) The sonication time was evaluated with four levels of 15, 30, 45, and 60 min. Total inactivation of fecal coliform bacteria was obtained by combining 30 min of sonication, frequency of 20 kHz, and amplitude of 35%.

To identify the ideal water disinfection process that can satisfy the national drinking water quality standard in the USA, a new pilot continuous flow system has been proposed by Zou and Tang (2019). This system combines the effects of chlorination and ultrasound. *Escherichia coli* (*E. coli*), *Bacillus subtilis* (*B. subtilis*), and *Staphylococcus aureus* (*S. aureus*) were the microorganisms selected as indicators of the disinfection effects in this treatment system. The authors themselves designed and built the US-chlorination system in stainless steel with components connected by tubes. Different disinfection processes can be selected through valves, including treatment of isolated US, disinfection with isolated chlorine, or a combination of US and chlorine. Each ultrasonic reactor was equipped with ultrasonic transducers of two different frequencies (reactor I: 17 and 33 kHz, reactor II: 70 and 100 kHz). Each group of ultrasonic transducers mounted on the reaction reactor wall is controlled independently by electric actuation switches. Thus, the ultrasonic reactor could operate at single or double frequencies. The input power of the ultrasonic transducers was adjustable in the range of 0 to 350 W. The results showed that the dual-frequency US radiation had better inactivation effects compared to the single frequency US, although it was unable to reach a level of optimal disinfection (complete disinfection). Besides, the 17 + 33 kHz dual-frequency US pretreatment had an obvious increase in disinfection efficiency, with logarithmic reductions of 3, 85 (*E. coli*), 3.65 (*S. aureus*), and 3.52 (*B. subtilis*), when 8 mg / L of NaClO was used for 10 minutes. All three bacteria achieved a reduction of 4 log after 30 minutes. The treated water met the Chinese national standard for drinking quality water, where the residual chlorine concentration was less than 4 mg / L.

4. Conclusion

The action of ultrasonic radiation in a biological environment is attributed to physical mechanisms such as thermal effect, cavitation, acoustic microflows, among others. Cavitation can result in electrical and chemical phenomena and, as a consequence, the destruction of microbial cell walls. Due to the very high frequency of this type of radiation, collapses of the cavities or microbubbles occur, or the gas bubbles grow until they are big enough to vibrate in resonance with the sound waves. Cavitation generated by the US produces intense shock waves, instantaneous increases in temperature and pressure, and chemical effects in the environment, which are generated by the collapse of cavities or microbubbles. The percentage of mortality and changes in molecular structures increases with the duration of exposure to US, with the level of intensity generated by the US, and with the formation of free radicals (Fang et al., 2018).

The different ways of measuring the effectiveness of lethality rates give rise to predictive models, for which attention is drawn. When the individual and interactive contributions of the different independent variables are known, predictive modeling can be practiced without major concerns (Gil et al., 2017). If the technique is in the preliminary phase of studies,

each predictive model must be analyzed and supported by the analyst's degree of knowledge about both the environmental and operational conditions of the experiments concerning the microbial inactivation rate. After this preliminary phase of identifying the contributions of the relevant parameters to microbial growth rates, the use of primary predictive models, secondary or tertiary will depend on the degree of information that you want to obtain about the inactivation process through the response variable. In this way, all models are important, as long as the appropriate working conditions are properly established before the experiments are carried out.

The application of the response surface methodology (RSM) has led researchers to define relevant variables for defining products and processes in their preliminary studies and for modifications or optimizations (Cervantes-Elizarrará et al., 2017). It is therefore recommended that a sequence of procedures be followed in this direction. Preliminarily, fractional factorial planning, or the use of multivariate statistical tools such as principal component analysis (Arhouma & Hassan, 2016), can be used to establish relevant parameters and their respective contributions to the variability of the disinfection phenomenon. In a later step, it is recommended to search for the probability of the existence of regions where maximum disinfection rates are found, using full 3-level factorial planning in this type of survey. Finally, after locating the vicinity of possible regions of maximum efficiency, a central composite design (CCD) could be applied in search of optimized conditions for each set of two factors. Additionally, these individual conditions can be used with the aid of a desirability function in search of global optimized conditions (da Silva, et al., 2019).

Based on what was seen in this manuscript, it is suggested that in future works, predictive models can be better used, considering the correct application of each model aiming at statistically more relevant results. It is also suggested the use of the response surface method and other statistical tools that can contribute to the development of parameters that improve the use of ultrasound in the disinfection of hospital laundry effluents.

Acknowledgments

This study was funded by the Research and Development Program of the National Agency of Electrical Energy (ANEEL) and Thermoelectric Termocabo SA, the Foundation for the Support of Science and Technology of the State of Pernambuco (FACEPE), the National Council for Scientific and Technological Development (CNPq), and the Coordination for the Advancement of Higher Education Personnel (CAPES). The authors are grateful to the Center of Sciences and Technology of the Catholic University of Pernambuco and the Advanced Institute of Technology and Innovation (IATI), Brazil.

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