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Mineral water disinfection using ultrasound.

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Abstract

The objective of this study is to evaluate the effects of ultrasound waves on the viability of different bacteria in mineral waters such as spa thermal waters. The application of ultrasound for the disinfection of water can be an alternative to other methods, such as, chlorination, ozonation, UV irradiation. Sonication was performed using a commercial ultrasonic bath at constant frequency of 40 kHz for 10 to 20 minutes. Experiments were carried out at a concentration of 10^6 CFU mL^{-1} and a constant temperature of 309.65 K. The effects of the ultrasounds waves on bacterial growth were analyzed using a Calvet microcalorimeter. By plotting heat voltage difference versus time, we are able to obtain the graphs of *Staphylococcus epidermidis* and *Escherichia coli*, with and without ultrasound treatment. Furthermore, the heat released by microorganisms in the treated and untreated samples sonicated for 24 hours was calculated. The ultrasound treatment appears to be effective in inhibiting the growth of *S. epidermidis*, but not in the case of *E. coli* which presented greater resistance at the same experimental conditions. This study also showed microcalorimetry as an efficient technique to determine the effect of ultrasound waves on bacterial growth.

1 Introduction

Water has been used as a source of health and wellness since time immemorial; all cultures have and make use of it for different purposes: hygienic, therapeutic, recreational and industrial (Mourelle [1]).

In our country, there are abundant spas with great tradition. The use of these facilities could lead, in the case of not caring water quality and environment, a possible health risk to people.

Disinfection is a process used to eliminate pathogens in mineral water and protect the health of users (Naddeo [2]).

Choosing the best system for mineral water disinfection is not an easy task. Ideally, a method that provides water free of pathogenic microorganisms without losing its mineral properties and at the same time is comfortable for the user.

There are a variety of chemical and physical techniques for water disinfection including chlorination, ozonation, UV irradiation (Chowdhury [3]; Donofrio [4]; Hijnen [5]; Nelson [6]; Zwiener [7]. However, these disinfection methods suffer from severe drawbacks. Chemical techniques, like chlorination and ozonation, are often not environmental friendly. Also, chemical disinfection can lead to the formation of mutagenic and carcinogenic agents in the treated water (Chowdhury [3]; Antoniadis [8]; Lee [9]; Kim [10]; Zwiener [7]. Furthermore, chlorination has been causing the appearance of resistant microorganisms (Furuta [11]). The potency of certain physical techniques, such as UV irradiation, is limited in highly light scattering or when microorganisms are capable of photoreactivation (self-repair). Due to the inherent disadvantages of traditional water disinfection techniques, there is still a need for alternative disinfection methods (Hulsmans [12]).

In this study, we investigated the potential of ultrasound as an alternative for more traditional techniques for the disinfection of water.

Ultrasound refers to longitudinal, mechanical and scalar (pressure) waves that have a frequency which is higher than 20 kHz, which represents the upper audibility threshold of the human ear (Leighton [13]).

The powerful effect induced by sonication in water is due to the phenomenon of acoustic cavitation that is the formation, growth and collapse of microscopic bubbles or cavities within very small timescales (milliseconds) (Foladori [14]).

The exact mechanism by which the ultrasonic waves inactivate microorganisms has not been clearly established. However, it is recognized that the antimicrobial effect of ultrasound is caused by a combination of the following simultaneously acting mechanisms: mechanical effects, chemical effects including generation of active free radicals and heat effects (generation of local hot spots). It has been generally observed that the mechanical effects are more responsible for the microbial disinfection and the chemical and heat effects play only a supporting role (Gogate [15]).

In this work, we present the results obtained after ultrasonic treatment of two bacterial species in mineral water. The effects of the ultrasounds waves on bacterial growth were analyzed using a microcalorimetric analysis.

Microcalorimetry is an analytical technique that can be used to measure the heat flow produced as a result of biological activities (Braissant [16]). Like all living beings which exchange heat as a consequence of their metabolism, the heat rate is an appropriate measure of the metabolic activity of the organisms. Microorganisms produce small amounts of heat, in the order of 1-3 pW per cell. Despite the low levels of heat produced by the bacteria, their exponential replication in culture medium allows their detection within a few hours, even from samples with a low concentration, e. g., 10 colony forming units (CFU) mL^{-1} (Lago [17]).

Microcalorimetry is a non destructive method, with high sensitivity, accuracy and simplicity, which has been extensively applied in physics, chemistry, life sciences and other fields. Also, it provides us the real-time the curves of each bacterium behaving like a “thermal fingerprint”. However, this technique presents drawbacks, which are its lack of specificity and that it requires an initial equilibration time of approximately 2 hours (Braissant [18]).

2 Material and methods

2.1 Mineral water

For this study, it has been used a medicinal mineral water sulphur, bicarbonate-sodium, silicate and fluoride. This mineral water is used with the following therapeutic indications: prevention and treatment of psychological disorders (stress, anxiety, depression and of nervous system), chronic rheumatism, chronic respiratory diseases (chronic obstructive pulmonary disease, asthma and bronchiectasis), skin diseases and arthritis problems.

2.2 Bacterial strains and sample preparation

The bacteria were supplied by the American Type Culture Collection (ATCC): *Staphylococcus epidermidis* (ATCC 35983) and *Escherichia coli* (ATCC 25922). These bacterial strains were streaked onto blood agar plates and incubated at 309.65 K for 24 hours. The blood agar plates with multiple bacterial colonies were then used to prepare a bacterial suspension with the mineral water mentioned above, whose concentration was adjusted to the corresponding 0.5 on the McFarland scale, using an optical densitometer. This solution was then used to prepare further dilutions with the mineral water to obtain final concentrations of 10^6 CFU mL^{-1}.

2.3 Experimental equipment

The ultrasound treatment was carried out using a Brason® 3510 ultrasonic bath operating at the constant frequency of 40 kHz and maximum power of 130 W during 10 and 20 minutes. The ultrasonic bath temperature was controlled and did not change during the experiment.

Bacterial growth curves were obtained using Calvet microcalorimeter equipment (Calvet and Prat [19]) (see Figure 1). It is equipped with a device allowing operation in the absence of vapour phase, and has two Teflon® screw capped stainless steel cells of approximately 10 cm^3. One of these cells contains the reference solution, named the reference cell, and the other one the sample, named the experimental cell (see Figure 2). A Philips PM2535 multimeter and a data acquisition system were linked to the microcalorimeter. Calibration was performed electrically using a Setaram EJP30 stabilised current source. The precision in calorimetric signal was ±1 μV (Lago [17]); Rivero [20]).

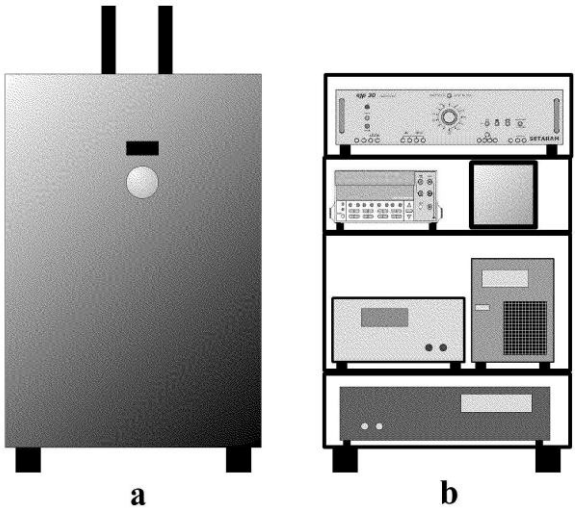

Figure 1. a) Calvet microcalorimeter and b) system control and data acquisition

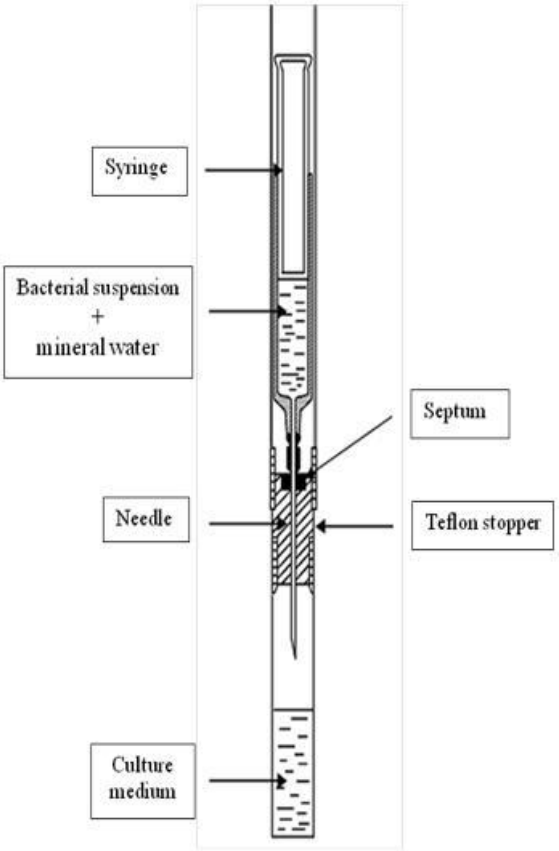

Figure 2. Experimental cell

The external media of the calorimeter was maintained at a constant temperature of 309.65 K. The reference cell was filled with 7 mL of culture medium + 1 mL of mineral water while the experimental cell was injected with 7 mL of culture medium + 1 mL of bacterial suspension. The culture medium used was a liquid enriched with digested soy-casein (Becton, Dickinson and Company, USA), which is a mixture of processed water (40 mL), soybean-casein digest broth (2.75 % w/v), yeast extract (0.25 % w/v), animal tissue digest (0.10 % w/v), sodium pyruvate (0.10 % w/v), dextrose (0.06 % w/v), sucrose (0.08 % w/v), hemin (0.0005 % w/v), menadione (0.00005 % w/v), sodium polyanetholsulfonate (0.020 % w/v) and pyridoxal HCl (0.001 %w/v). Both cells were then introduced, from the upper part of the calorimeter in the internal thermopile chamber through two cylindrical holes aligned in parallel. The large distance that separates the cells from the entrance ensures the minimisation of heat flow to the exterior (Lago [17]) (see Figure 3).

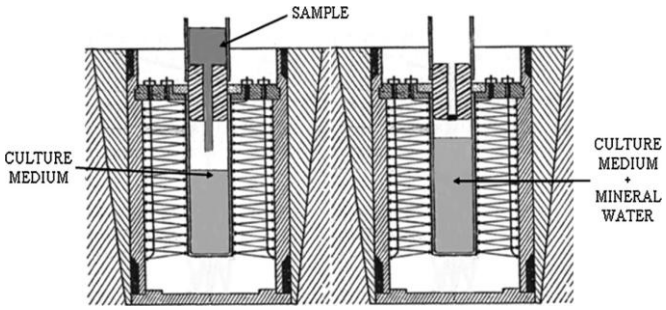

Figure 3. Schematic diagram of the Calvet microcalorimeter internal block

The experiment was also carried out with a sample not containing any bacteria (control). All experiments were realized in triplicate.

Both cells were cleaned and sterilized by autoclaving before using (20 minutes at 394.15 K).

A data collection and processing system were used to record the electrical signal at intervals of 15 seconds throughout the duration of the experiment.

3 Results

Using the experimental difference of voltage generated between the sample and reference cells over time, the growth curves of *S. epidermidis* and *E. coli* at 10^6 CFU mL^{-1} have been obtained. The growth profiles of bacteria with and without sonication at different times (10 and 20 minutes) have been compared.

The curve of *S. epidermidis* (Rivero [20]) presented a unique energetic characteristic phase, and the signal is recorded for about 15 h, returning to minimum levels after this time (see Figure 4a). However, when we apply ultrasound for 10 minutes in the sample we saw that the shape of the curve of the bacteria is repeated but with less intensity (see Figure 4b). On the other hand, when we increase the sonication time 20 minutes, we did not detect growth throughout duration of the experiment (see Figure 4c).

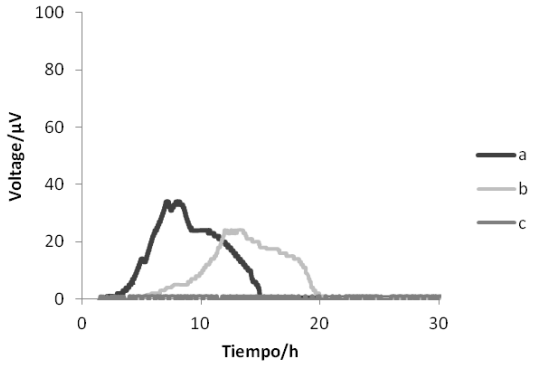

Figure 4. Calorimetric bacterial growth curves of the *S. epidermidis*: a) without previous sonication, b) with previous sonication 10 minutes, c) with previous sonication 20 minutes

The graph of *E. coli* (see Figure 5a) showed two metabolic phases, the first with greater energy, but shorter duration, followed by a period of latency that precedes a second of lower energy that is prolonged over time (Rivero [21]). In this case, the shape of curve with sonication is very similar to that without sonication. The main differences between the thermograms are the potential difference between the two peaks of maximum voltage, being less intense in the sample with ultrasound, and the displacement of the curve with sonication to bigger time values (see Figure 5bc).

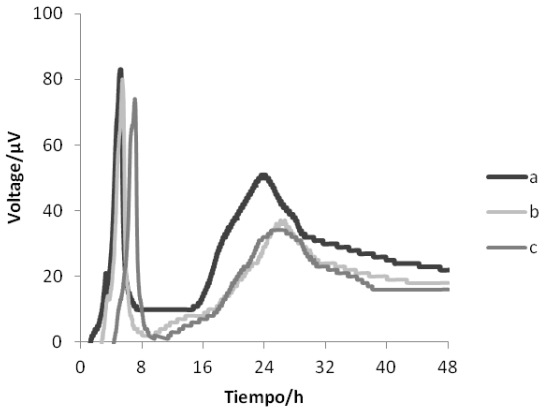

Figure 5. Calorimetric bacterial growth curves of the *E. coli*: a) without previous sonication, b) with previous sonication 10 minutes, c) with previous sonication 20 minutes

By means of the first derivative of the polynomial equations we determine the value of the maximum thermal power (V_{max}) and the time of its registration (t_{max}) in the thermograms of bacteria with and without ultrasounds (see Table 1).

The detection time of the signal (t_d) of *S. epidermidis* and *E. coli* are presented in Table 1. As can be seen, all samples sonicated showed higher t_d than the samples without sonication. We also saw that the t_d increased with sonication time (see Table 1).

From the thermograms, we can calculate the amount of heat released (Q) during the culture time:

$$Q = K \cdot A$$

where A (µV h) is the area, calculated by the trapezoidal method, and K represents a constant whose value, 23.8 J μV^{-1} h^{-1}, was calculated from the electric calibration performed by the Joule effect on the equipment.

This parameter is important because it allows one to know the heat output of microorganisms.

In Table 2 the values of heat released by bacteria with and without ultrasound during the first 24 hours, Q_{24}, are compared. In all cases, values of Q_{24} for bacterial suspensions treated with sonication are smaller than those for the samples not exposed to sonication.

Table 1 Detection time (t_d), maximum voltage peak (V_{max}) and time registration of maximum peak (t_{max}) of *S. epidermidis* and *E. coli* at 10^6 CFU mL^{-1}

Microorganism	t_d/h	t_{max}/h	V_{max}/μV
Without ultrasounds			
S. epidermidis	2.74	7.15	34
E. coli	1.46	5.16	83
With ultrasounds			
S. epidermidis at 10 minutes	4.79	11.97	24
E. coli at 10 minutes	2.79	5.39	80
S. epidermidis at 20 minutes	-	-	-
E. coli at 20 minutes	4.43	7.01	74

Table 2. Area under the curve, AUC_{24}, and heat, Q_{24}, of *S. epidermidis* and *E. coli* at 10^6 CFU mL^{-1}, during 24 hours

Microorganism	AUC_{24}/μV h	Q_{24}/KJ
Without ultrasounds		
S. epidermidis	210.374	5.006
E. coli	521.856	12.211
With ultrasounds		
S. epidermidis at 10 minutes	79.290	1.887
E. coli at 10 minutes	432.866	10.302
S. epidermidis at 20 minutes	-	-
E. coli at 20 minutes	418.516	9.960

4 Conclusions

Ultrasound treatment at 40 kHz and an exposure time of 20 min appeared to be effective in inhibiting the growth of *S. epidermidis* in the mineral water. However, *E. coli* seem to be more resistant to the ultrasound treatment in the same experimental conditions.

Future trends in research should focus on the study of the inhibition of these bacteria under different experimental conditions, that is, considering different ultrasound frequencies, powers, culture medium, exposure times and concentrations of bacteria. A systematic study in this direction would improve the efficiency of ultrasonic treatment techniques that allow select the experimental conditions according to the microorganism that is inhibited.

Acknowledgments

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