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Thermal Inactivation of Viruses and Bacteria with Hot Air Bubbles in Different Electrolyte Solutions

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Abstract. Inactivation of viruses has been an insuperable inhibition to the use of recycled water. Substantial success in solving the problem has recently been achieved using a hot column evaporator (HBCE). Here we extend the technique to inactivate *E. coli* and MS2 viruses in different electrolyte solutions (0.17M NaCl and 0.01M $CaCl_2$). An increase in the inlet air temperature, from 103 to 250 °C, substantially improved the destruction of both pathogenic groups in either solution. *E. coli* proved to be more susceptible than viruses to inactivation in the HBCE. The phenomenon of inhibition of bubble coalescence above 0.17M for the NaCl solutions makes the HBCE process for this solution more efficient than for $CaCl_2$ solutions. In part, this is because of the higher air/water interfacial area with NaCl.

Keywords: virus, *E. coli*, inactivation, coalescence inhibition, hot bubble column evaporator (HBCE), sterilisation.

1. INTRODUCTION

Inactivation of microorganisms, specifically viruses and bacteria is the central problem for the sterilisation of aqueous solutions for almost any application. Pathogens have to be inactivated before the water can be used for industry or drinking water.

Wastewater from human activities usually contains bacteria like *E. coli and* human enteric viruses like hepatitis and rotavirus. If this water is to be reused, even for example in agriculture, it has to be disinfected.

Familiar methods for inactivating viruses, bacteria, and other microorganisms in aqueous media include heat, chemicals (e.g. ozone), irradiation (e.g. ultraviolet treatment), high-pressure treatment, and filtration (e.g. membrane filtration). Only reverse osmosis (RO) and nano-membrane pore scan block viruses. Heat treatments are energy-intensive. Better cheaper technologies are urgently needed. Such a technology has already been developed.[1] It is a surprisingly simple and successful method that kills viruses. It uses carbon dioxide or combustion gases in a bubble column. The process is now being scaled up for major industrial use. However, why and how it kills pathogens

is still obscure. It is to gain insight into the thermal mechanism that inactivates viruses and bacteria at different inlet air temperatures (103, 150, 200, and 250 °C) that is the goal of this work.

For a more detailed account of bubble column evaporators see the first paper in this volume. Bubble column evaporators (BCE) are typically in the form of cylindrical containers. A gas is introduced at the bottom of the column, via a porous frit. A continuous flow of rising bubbles passes through the liquid.[2] A gas-liquid bubble column provides a heat transfer rate between gas and liquid 100 times more efficiently than via bulk.[3] This heat transfer efficiency in a hot bubble column evaporator (HBCE) has been used in this work to inactivate *E. coli* and the MS2 virus for water reuse, without the need for boiling.[4] It does so by transferring heat from the hot air bubbles to the surfaces of pathogens contained in the solution through collisions.[5] The HBCE process requires less thermal energy than solution boiling because of the lower operating temperature of the solution. (the specific heat capacity per unit of weight, C_p, of air is four times lower than that of water).

Following from a previous work[5] two model solutions were chosen. The first one was 0.17 M NaCl. This is the concentration at which bubble coalescence is fully inhibited[6] and so gives an increased hot air/water interfacial area which should enhance the performance of the HBCE. The second solution at 0.01 M $CaCl_2$ is at a concentration less than that for bubble coalescence inhibition. So gas pathogen surface area is much reduced compared with the NaCl solution. But calcium adsorption by proteins should decreases pathogen surface charge (especially for the viruses) so this might be expected to reduce repulsive electrostatic forces with bubbles and so increase the HBCE inactivation efficiency.

The phenomenon of bubble-bubble interactions in electrolytes has remained unquantified until 1993.[6] Gas passing through a frit at the base of a water column produces bubbles. These ascend the column, the bubbles collide, fuse, and become larger. The column stays clear. However, when NaCl is added, and above an effective physiological concentration of 0.17 M, suddenly the bubbles no longer fuse. The column becomes dense with the production of a high-density of bubbles (typically 1–3 mm diameter).[6] The same phenomenon is responsible for the foaminess of the ocean, and the lack of foam production in freshwater.

The phenomenon occurs for a wide variety of electrolytes, always at the same effective concentration 0.17 M or the equivalent Debye length for all salts 1:1 2:1 2:2 3:1… and mixtures. For another class of electrolytes, there is no effect of salt on fusion inhibition.

2. MATERIAL AND METHODS

2.1. Experimental solutions.

Monovalent (1:1) and divalent (2:1) electrolyte solutions of 300 ml were prepared and sterilised by autoclaving in an Aesculap 420 at 15 psi, and 121-124 °C for 15 minutes. A NaCl concentration of 0.17 M NaCl or higher (≥ 99% purity, obtained from Sigma-Aldrich) in Milli-Q water, prevents bubble coalescence and increases the performance of the HBCE process by producing a higher air/water interfacial area. See ref[6] for detailed results.

0.01 M $CaCl_2$ solutions (≥ 99% purity, obtained from Sigma-Aldrich) in Milli-Q water do not reduce bubble coalescence, these solutions were chosen to reduce the virus and bacteria charge, perhaps reducing repulsion between bubbles and pathogens potential of increasing the HBCE inactivation efficiency, without affecting MS2 viability in this salt solution.[5,7]

2.2. Media preparation for experiments with E. coli.

The plate count method is commonly used for the identification of *E. coli* in marine water, treated drinking water, and wastewater. The water quality is assessed based on the ability of *E. coli* colonies to propagate in a layer of agar in the form of colony-forming units (CFU).[26,34]

For each experiment, 1 liter of medium was prepared from two solutions (A and B).

Solution A was composed of 6 g of NaCl, 13 g of tryptone, 1 g of yeast extract, and 1,000 ml of Milli-Q water. A pH value o 6.9 was measured with a Thermos Scientific Orion Star A214 pH meter. This solution was aseptically dispensed into two vessels, broth media, without agar, and the other one containing 1.41% agar (molecular biology-grade from Sigma-Aldrich). To dissolve the agar, the solution was heated to boiling and then sterilized by autoclaving in an Aesculap 420 at 15 psi, and 121-124 °C, for 15 minutes.

Solution B was used to enhance *E. coli* growth. This solution was prepared by adding 0.010 g of thiamine and 1 g of glucose to 50 ml of Milli-Q water and filtered through a 0.22 μm filter for its sterilization and then was aseptically added to solution A (in a proportion of 1:20), once cooled to 50 °C.

The 1.41 % agar solution was poured into 100 × 15 mm Petri dishes which were placed within the sterile field area around the Bunsen burner created by the updraft of the flame.[26]

2.3. Media preparation for experiments with viruses.

A specific optimized Double Layer Plaque Assay technique was used to assess the concentration of active MS2 viruses. This plaque assay method is commonly used for the detection of MS2 in treated drinking water, wastewater, and marine water. The water quality is assessed based on the ability of bacteriophages to kill the host bacteria and allow phages (circular zone of infected cells) to propagate in a confluent lawn of bacterial host cells, immobilized in a layer of agar.[8,9,10,11]

The Double Layer Plaque Assay requires an adequate growth medium to achieve better visibility and higher consistency. [10, 11] The medium is not commercially available. Therefore, it was prepared for each experiment in the form of two solutions, A and B. Solution A contains 15 g of tryptone, 1.5 g of yeast extract, 12 g of NaCl, and 1,425 ml of Milli-Q water. A pH value of 6.9 was measured with a Thermos Scientific Orion Star A214 pH meter. This solution was dispensed aseptically into three vessels with different amounts of agar (1% for the bottom agar, 0.5 % for the top agar, and no agar for the broth media). The agar used in these experiments was molecular biology-grade, obtained from Sigma-Aldrich. These solutions were first heated to boiling to dissolve the agar and then sterilized by autoclaving in an Aesculap 420 at 15 psi, and 121-124 °C, for 15 minutes.

Solution B was used to improve the visibility of the viruses. This solution was prepared by adding 1.5 g of glucose, 0.441 g of $CaCl_2$, and 0.015 g of thiamine to 75 ml of Milli-Q water and filtered through a 0.22 μm filter for its sterilization and then was aseptically added to solutions A (in a proportion of 1:20), once cooled to 50 °C.

The bottom agar was poured into 100 mm x 15 mm Petri dishes which were dried within the sterile field area around the Bunsen burner created by the updraft of the flame, to maintain local environmental sterility, until the agar was not too dry or too moist.[9]

2.4. Bacterial strain Escherichia coli C-3000 (ATCC 15597)

E. coli is a gram-negative bacteria with a straight cylindrical rod shape of 1.0-2.0 μm size.[12] It is found in the gastrointestinal tract of animals and humans. *E. coli* strains can be harmless or pathogenic to the host. As a result of fecal contamination, they can be found in water and soil. Therefore, it was selected as a representative model for bacteria in water[13,14] for the *E. coli* inactivation experiments and also for the virus sterilization experiments, as the MS2 virus-host.[11] *E. coli* C-3000 (ATCC15597) is a biosafety level 1 organism [15] and can be used as the MS2 virus-host.[11]

For a successful plaque assay, for virus inactivation experiments, the *Escherichia coli* C-3000 (ATCC 15597) must be in an exponential growth phase. This was achieved by growing two separate bacterial cultures: an overnight culture and a log phase culture.[10,11,16] The overnight culture was grown in 10 ml of broth media at 37 °C for 18-20 hours in a Labtech digital incubator; model LIB-030M, while shaking at 110 rpm with a PSU-10i orbital shaker. This overnight culture resulted in high numbers of bacteria in the culture and was used as a reference standard.

For a successful plate count, the concentration of *E. coli* from the overnight culture was calculated by serially 10-fold diluting 0.50 ml of *E. coli* overnight culture into a tube containing 4.50 ml sterile saline solution.[10]

To start the log phase *E. coli* culture, 1 ml of the overnight culture was transferred into 25-30 ml of broth media and incubated for 3 h at 37 °C, with gentle shaking at 110 rpm. To prevent loss of F-pill by the cells, they were then quickly cooled in a refrigerator, at 5 °C. A UV-VIS spectrometer, UVmini-1240, was then used to measure the optical density (OD) of the log phase *E. coli* culture. OD readings at 620 nm of between 0.8 and 1.1 indicated that the culture can be used in the plaque assay for the virus experiments and as a standard for the *E. coli* experiments.

2.5. Viral strains MS2 (ATCC 15597-B1)

A freeze-dried vial of MS2 bacteriophage was acquired from the American Type Culture Collection (ATCC). Bacteriophage MS2 (ATCC 15597-B1) was replicated using *Escherichia coli* C-3000 (ATCC 15597) according to the International Standard ISO 10705-1[16] and the Ultraviolet Disinfection Guidance Manual of the United States Environmental Protection Agency.[17] MS2 is a bacteriophage member of a class called group I. Its entire genome has been sequenced. It is a positive-sense, single-stranded RNA molecule of 3,569 nucleotides and it has an icosahedral structure. The virus has a hydrodynamic radius of about 13 nm.[18]

The concentration of the MS2 bacteriophage was calculated by adding 1.0 ml of broth media to the vial and serially 10-fold diluted 10 times by passing 0.50 ml of the bacteriophage into a tube containing 4.50 ml of broth medium.[10] 0.20 ml of the 4 hours host (log phase *E. coli* culture) and 10 mL of 0.5% top agar layer were poured over the Petri dishes with 1% bottom agar, dried around the Bunsen burner, 0.1 mL aliquots of 10^{-6} to 10^{-11} dilutions were inoculated on the surface of 14 Petri dishes.

After overnight incubation, 18-24 hours at 37 °C, individual plaques were countable and the concentration of the MS2 Bacteriophage was calculated using the equation:

Undiluted spiking suspension in PFU / mL = (PFU1 + PFU2… PFUn)/(V1 + V2…. Vn) [1]

Where PFU is the number of plaque-forming units from plates, Vn is the volume (in ml) of each undiluted sample added to the plates containing countable plaques and n is the number of useable counts.

2.6. Hot bubble column evaporator process (HBCE)

In the HBCE process, 27 l/min of ambient air was pumped through a silica gel desiccator, for dehumidification, and from there the dry air was passed through an electrical heater that maintained a hot air temperature, just above the sinter surface, of 103 °C 150 °C, 200 °C or 250 °C, depending on the experiment. The base of the bubble column evaporator was fitted with a 40-100 µm pore size glass sinter (type 2) of 135 mm diameter.

Once the solutions with known concentrations of virus and *E. coli* were prepared, two rounds of experiments (one for viruses and the other one for bacteria) were conducted in the HBCE. The temperature of the hot air (103 °C, 150 °C, 200 °C, 250 °C for this study) was measured, before the introduction of the solutions in the column, with a thermocouple above the sinter in the center of the dry column. The hot air passed through the sinter, in the form of bubbles, into the 300 ml solutions (0.17 M NaCl and 0.01 M $CaCl_2$). This inactivated the pathogens (MS2 viruses and *E. coli)* and heated the solution to a steady-state temperature (of 34 to 55 °C, depending on the inlet air temperature).

2.7. Disinfection experiments

A total of 18 viral and bacterial sterilization experiments were conducted in two solutions: 0.17 M NaCl and 0.01 M $CaCl_2$ at 4 different inlet air temperatures (103 °C, 150 °C, 200 °C, and 250 °C) in the HBCE.

The evaluation of bacteriophage and *E. coli* results was performed by the plaque assay and plate count methods (respectively).[8,10,19]

For each experiment, the target number of pathogens per 0.1 ml aliquot was 290. The following equation [4] was used to determine the spiking volume (that is, the volume that was taken from the virus/bacteria stock):

$$S = \frac{T * B}{C} \quad [2]$$

where S is the virus/bacteria stock volume (ml) added to 300 ml of solution, T is the target number of pathogen per sample, B is the total number of samples in 300 ml and C is the concentration (pathogen/ml) in the virus/bacteria dilution to be used for spiking.[17]

Once the solutions with the known concentration of pathogens were prepared the inactivation experiments at different temperatures were conducted in the HBCE. 1 ml samples were collected from 10 to 15 mm above the central area of the sinter. For each sample, 0.1 ml was spotted in triplicate following the double layer or the plaque count technique,[17] the same procedure that was used to determine the concentration of the MS2 bacteriophage or *E. coli*.

2.8. Zeta potential measurements

Zeta potential measurements were performed using a nano zeta sizer (Zetasizer Nano ZS Malvern Instruments Ltd.)[20] to study the MS2 virus and *E. coli* surface charge in various electrolyte solutions containing 10^8 MS2 viruses/ml or 10^8 *E. coli* C-3000/ml. Trapped air bubbles in the zeta cells were carefully avoided in the disposable zeta cells used to conduct the zeta measurements.

2.9. Data analysis

The linear decay model was used to study the time dependence of inactivation of pathogens (MS2 virus or *E. coli*). Plate counts were performed for all 18–21 plates from each of the experiments. The mean and standard deviation of each triplicated sample were obtained using bacterial survival factor, $\log_{10}(N_t/N_0)$, where N_0 is the initial number of pathogens per sample and N_t is the number of pathogens after a set exposure time in minutes.[21]

The decimal reduction time (D-value) was used to measure the temperature impact on both pathogenic groups (virus and bacteria). This can also be defined as the time needed to inactivate 90% (i.e. 1-log) of *E. coli* or virus (heat resistance). The Z-value is the temperature change required to change the D-value by a factor of 10.

D-values and Z-values were calculated using a linear exponential decay model or Thermal Death Model.[22]

$$\log(N_t) = \log(N_0) - \frac{t}{D} \quad [3]$$

$$\log\left(\frac{N_t}{N_0}\right) = -\frac{t}{D} \quad [4]$$

Where, D = the decimal reduction time, -(1/D) = the slope of the curve.

The Z-value is the increase in temperature needed to reduce the D-value by 1-log. It measures the impact of

a change in temperature on *E. coli* or virus inactivation. Thus:

$$Z = \frac{T_1 - T_2}{logD_1 - logD_2} \quad [5]$$

Where, T_1 is the first temperature of the interval, T_2 second temperature of the interval, and D_1 and D_2 are the D-values at T_1 and T_2.

3. RESULTS AND DISCUSSION

3.1. Theoretical model estimate of thickness and temperature of the transient hot water layer around the hot bubbles used in the inactivation of viruses and bacteria

The HBCE process successfully sterilised water contaminated with *E. coli* and MS2 viruses using hot air bubbles in two different solutions (0.17 M NaCl and 0.01 M $CaCl_2$). This inactivation process appears to be triggered by collisions between the bacteria and viruses with the hot air bubbles.[5,23,24] In the HBCE, a thin layer of heated water must also be formed transiently around the surface of the hot air bubbles, once they are released into the column. The thickness and the temperature of this thin, transient layer can be roughly estimated and it appears that this may be an important parameter to understand inactivation rates of virus and *E. coli* occurring at the HBCE. The collisions between these heated water layers, as well as the hot air in the bubbles themselves, is considered as the fundamental mechanism proposed for pathogen inactivation (*E. coli* and virus).[24,5]

During the HBCE experiments the inlet air temperature was always between 103 and 250 °C and the estimated average temperature of the heated water layers was in a range of 66 to 70 °C (see Table 1). By comparison, the column solution temperatures were always significantly lower (i.e. from 32 to 55 °C) (Fig. 1).

In the theoretical model, the temperature and the thickness of the hot water layer around the surface of a 1 mm diameter air bubble were estimated roughly for a range of inlet air temperatures using the formula:

$$T_{avg} = \frac{100 + T_c}{2} \quad [6]$$

where T_{avg} (in °C) is the average (transient) temperature of the hot water layer surrounding the air bubble and T_c (°C) is the steady-state temperature of the solution in the HBCE, with the assumption that the hot air bubbles had cooled from their initial inlet temperature to 100 °C when they first enter the bubble column.

The thickness of the transient has been estimated by balancing the heat supplied by the cooling bubble with

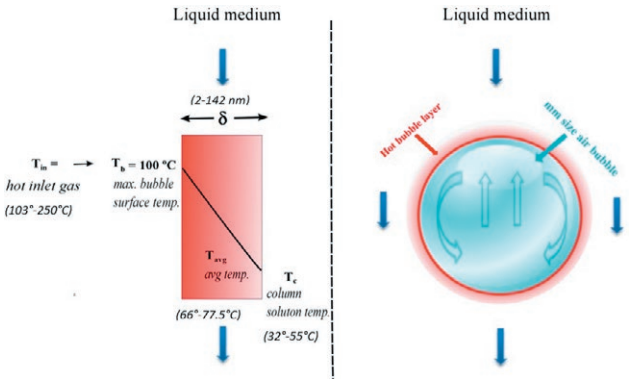

Figure 1. Model used to estimate the thickness and temperature of the hot layer around the air bubble at different inlet air temperatures.

Table 1. Estimated thickness and temperature of the transiently heated water layer around the air bubbles at different inlet air temperatures.

Inlet Gas Temperature T_{in} (°C)	Column Solution Temperature T_c (°C)	Density of Water (g/cm^3)	Hot Layer Thickness δ (nm)	Average Temperature of Hot Layer T_{avg} (°C)
103	32	0.99053	2	66
150	45	0.99022	39	72.5
200	51	0.98758	87	75.5
250	55	0.98569	142	77.5

the heat required to raise the film to this average temperature (see Table 1). Thus, since the volume of the film V is given by:

$$V = 4\pi r^2 z \quad [7]$$

where r is the bubble radius with a constant value of 0.001 m, z the layer thickness around the bubble and $r >> z$, this thermal energy balance is given by.[25]

$$C_p \Delta T V = C_{water} \Delta t 4\pi r^2 \rho_w z \quad [8]$$

where C_p air heat capacities per unit volume, C_{water} is the air heat capacity per unit weight, is the liquid water mass density, ΔT is the cooling of the air bubble (from inlet temperature to 100 °C) and Δt is the transient temperature increase in the water layer, relative to the column solution temperature.

In practice, likely, at least half of the heat supplied by the cooling bubble will be used in evaporating water into the bubble, and hence the calculated, roughly estimated, film thicknesses should be about halved.

Table 2. D and Z values for *E. coli* and MS2 virus in 2 different solutions (NaCl and $CaCl_2$).

Pathogen	Solutions	D-values at different inlet air temperatures (min.)					Z-values (°C)
		102 °C	150 °C	195 °C	205 °C	250 °C	
Virus	0.17M NaCl		121.95		29.41	6.15	77
	0.01M CaCl2		75.19		23.75	15.5	145
E. coli	0.17M NaCl	65.36	9.31	3.87			76
	0.01M CaCl2	33.00	18.73		3.51		105

Table 3. Zeta potential and bubble coalescence values for virus and bacteria in NaCl and $CaCl_2$ solutions.

Pathogen	Solution	% Coalescence	Zeta Potential (mV)
Virus	0.17 M NaCl	0	-6.32
	0.01 M $CaCl_2$	94	-2.55
E. coli	0.17 M NaCl	0	-12.50
	0.01 M $CaCl_2$	94	-12.20

When the inlet gas temperature increases, so does the thickness of the surface hot water layer around the bubble surface (Fig. 1). Consequently, the volume of the inactivation area is increased and this should make the inactivation rate more effective. Typical results from this model are given above (Table 1). Therefore, the inlet air temperature has a direct relation with pathogen inactivation as will be shown.

3.2. Temperature effects on pathogen inactivation in the HBCE in two different solutions

The impact of temperature for MS2 viruses and *E. coli* inactivation in the HBCE process at 103 °C, 150 °C, 200 °C and 250 °C inlet air temperatures was studied using two solutions: 0.17 M of NaCl and 0.01 M $CaCl_2$ (Table 2).

3.3. Solution comparison.

A 0.17 M solution of NaCl will inhibit bubble coalescence and so will increase the interfacial area of the bubbles within a bubble column [6], whereas the other solutions of 0.01 M $CaCl_2$ do not produce bubble coalescence inhibition [4, 26] (Table 3). Higher bubble interfacial areas were expected to increase will increase the chances of a collision between pathogens and hot air bubbles so improving virus and bacteria inactivation rates.

The Zeta potential for viruses presented lower values for $CaCl_2$ than for NaCl solutions. Presumably, this can be attributed to the absorption of calcium on the MS2 virus. *E. coli* did not present this selective absorption so that a similar Zeta potential was observed for both electrolyte solutions for this pathogenic group (see Table 3).

To understand the effect of hot air on different solutions (0.17 M NaCl vs 0.01 M $CaCl_2$) when thermally inactivating pathogens, such as MS2 virus and *E. coli*, decimal reduction times (D-values) at four inlet air temperatures, 50 °C intervals, were obtained (Table 2). The correlation between the log of the D-values and the corresponding temperature was represented in Fig. 2 and 3. A D-value is the time needed to inactivate 90% (i.e. 1-log) of the pathogens. To measure the heat resistance of these pathogens, Z-values have been calculated. The Z-value gives the temperature required to change the D-value by a factor of 10 and reflects the temperature impact on a pathogen (*E. coli* and MS2 virus in our study). The smaller the Z-value, the greater the sensitivity to heat.

Figures 2 and 3 show the minimum air bubbling times, at different temperatures, needed to achieve 1-log pathogen (virus and bacteria) inactivation in 0.17 M NaCl and 0.01 M $CaCl_2$ solutions. Above and to the right of the lines the pathogens will be sterilised by 1-log.

At low inlet air temperatures, between 100 and 150 °C, both pathogenic groups MS2 viruses and *E. coli* present higher inactivation rates in $CaCl_2$ than in NaCl solutions with D-values of 33.00 and 75.19 min for *E. coli* and virus in $CaCl_2$ solutions and 121.95 and 65.36 min for NaCl solutions (see Table 2 and Figures 2 and 3). For an intermediate range of temperatures, 150 to 200 °C NaCl solutions show better inactivation rates for *E. coli* with D-values of 9.31 min. than do $CaCl_2$ solutions do for viruses with D-values of 23.75 min. At higher temperatures, 200 to 250 °C, both solutions presented similar inactivation rates for *E. coli* with D-values in a range of 3.5 to 3.8 min. For viruses, NaCl solutions inactivated almost 3 times faster than $CaCl_2$ solutions with a D-value of just 6.15 min. (see Table 2 and Figures 2, and 3).

Virus (Z-value= 145 °C) and *E. coli* (Z-value= 105 °C) inactivation in $CaCl_2$ solutions is less temperature dependent than in NaCl solutions with Z-values of 77 °C for virus and 76 °C for *E. coli* See Table 2.

The effect of 0.17 M NaCl on the inhibition of bubble coalescence makes the HBCE process more temperature-dependent (reduced Z-values of 77 °C) than when

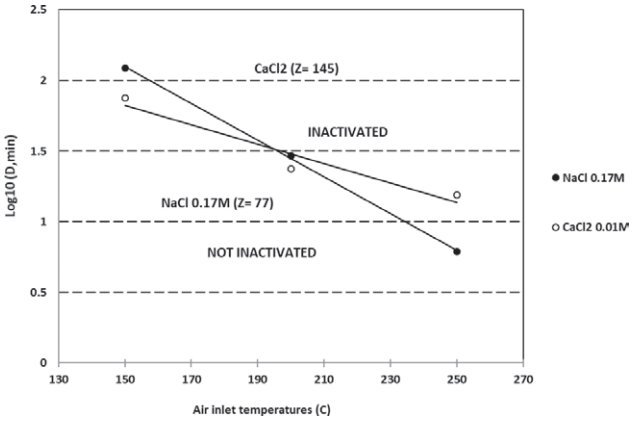

Figure 2. Comparison of the impact of temperature on MS2 virus inactivation between 0.17M NaCl and 0.01M $CaCl_2$ solutions.

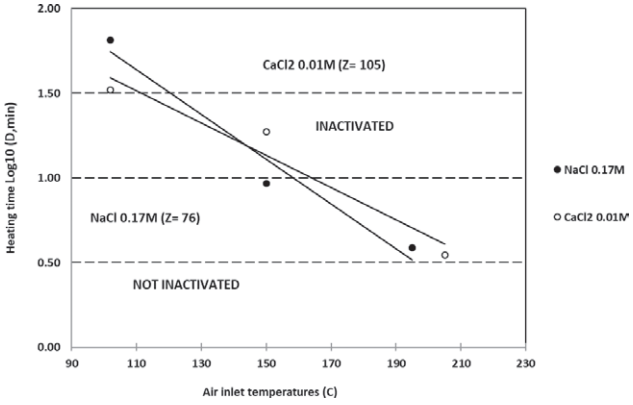

Figure 3. Comparison of the impact of temperature on *E. coli* inactivation between 0.17M NaCl and 0.01M $CaCl_2$ solutions.

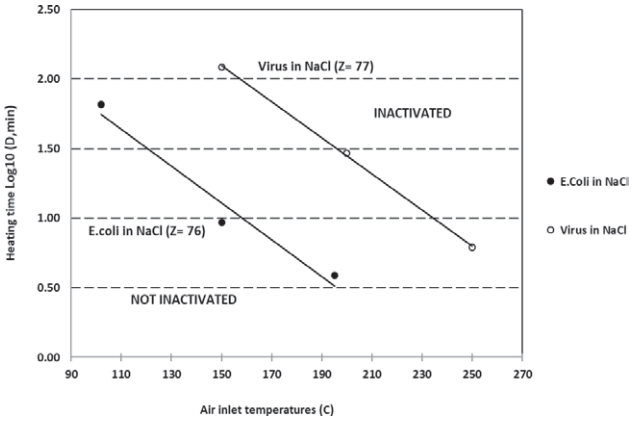

Figure 4. Comparison of the impact of temperature on *E. coli* and MS2 virus in 0.17M NaCl solutions.

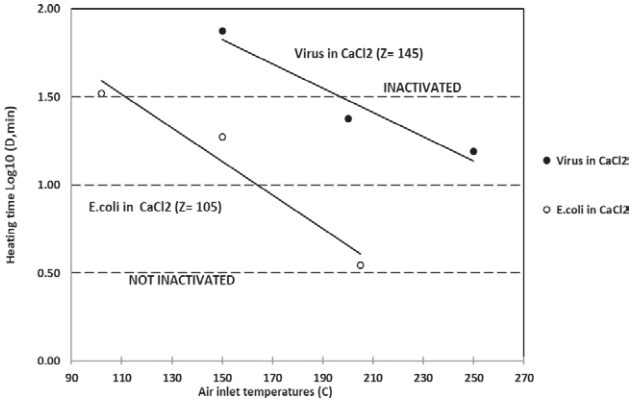

Figure 5. Comparison of the impact of temperature on *E. coli* and MS2 virus in 0.17M $CaCl_2$ solutions.

using $CaCl_2$ solutions. It can reasonably be assumed that this is due to the higher air/water interfacial bubble area produced. Consequently, there will be an increase in the volume of the transiently heated layer around the bubbles (see Table 1). When this volume increases, the chances of collision between pathogens and hot air bubbles will also increase with the consequent improvement in heat transfer.

3.4. Pathogenic group comparison

The World Health Organisation (WHO) studied the sterilization properties of hot liquids for thermal inactivation of bacteria and viruses in their guidelines for drinking-water quality.[27] WHO concluded that water temperatures above 60 °C effectively inactivate both pathogenic groups and when the temperature range lies between 60 and 65 °C, the inactivation of bacteria

For *E. coli* and MS2 virus inactivation in the HBCE was improved by increasing the inlet air temperatures from 103 to 250 °C. The thermal inactivation effect is more effective when the inlet air temperature increases. This is probably because of a thicker and hotter transient heated water layers created around the rising air bubble surface[28] (see Table 1). *E. coli* and viruses will be thermally inactivated by the collisions with this layer.

To understand the temperature effect of inlet air for thermal inactivation of pathogens (MS2 virus and *E. coli*) decimal reduction times (D-Values) at four different inlet gas temperatures, at intervals of 50 °C, were obtained and the correlation between log of the D-values and the corresponding temperature was represented in Figures 4 and 5. Again, D-values and Z-values have been calculated to assess the temperature impact on the pathogens.

Figures 4 and 5 show the minimum hot air bubbling times at different temperatures to achieve 1-log pathogen

(virus and bacteria) inactivation in 0.17 M NaCl and 0.01 M $CaCl_2$ solutions. Above and to the right of the lines the pathogens will be sterilised by 1-log.

For both solutions and for the entire range of temperatures *E. coli* was inactivated much faster than MS2 virus, therefore, *E. coli* proved to be more sensitive to hot air bubbles than MS2 virus (Fig. 4).

At 150 °C inlet air temperatures *E. coli* presented D-values of 9.31 min. in NaCl solutions and 18.73 min. in $CaCl_2$ solutions while the D-values for MS2 virus at the same temperature were 121.75 min. in NaCl and 75.19 min in $CaCl_2$ (Figures 4 and 5, Table 2). When inlet air temperature raised to 200 °C D-values for *E. coli* were 3.87 min in NaCl and 3.51 min in $CaCl_2$ for virus these values were much higher with 29.41 min for NaCl solutions and 23.75 in $CaCl_2$.

We can infer then that in the HBCE process, viruses and *E. coli* are inactivated when they are immersed in the hot water layer around the hot air bubbles or the bubbles themselves. The results agree with WHO with a faster inactivation for bacteria than for viruses, at similar water temperature [27]. But this phenomenon is considerably enhanced by using the HBCE for both solutions. Thus, the inactivation rates for viruses are much slower than for bacteria even though the gas temperature range for the virus (150 to 250 °C) was higher than for the bacteria (103 to 205 °C). This difference, might in part, due to the large size/momentum effect of *E. coli* compared with viruses. In a turbulent regime within the HBCE, the significantly larger momentum of *E. coli* cells is more likely to enable direct contact with the hot air phase what will increase their inactivation. A more likely contributor is the destruction of phospholipid bilayers of the bacteria with high temperatures.

4. CONCLUSIONS

This work has shown that viral and bacterial inactivation in the HBCE process can be substantially improved by raising the inlet air temperature, from 103 °C to 250 °C. According to the hot water layer theoretical model, when the inlet gas temperature increases, so will the thickness and the temperature of the transient heated water layer around the rising air bubble surface, and this appears to facilitate pathogen inactivation.

When thermal processes are used for water sterilization faster inactivation is always observed for bacteria than for viruses, at similar water temperatures. This phenomenon is considerably enhanced when using the HBCE independently of the solution.

The effect of 0.17 M NaCl on the inhibition of bubble coalescence makes the HBCE process more temperature-dependent than with $CaCl_2$ solutions by producing a higher air/water interfacial area and a better heat transfer.

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