



Improved virus inactivation using a hot bubble column evaporator (HBCE)

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ABSTRACT

An improved hot bubble column evaporator (HBCE) was used to study virus inactivation rates using hot bubble-virus interactions in two different conditions: (1) using the bubble coalescence inhibition phenomenon of monovalent electrolytes and (2) with reducing the electrostatic repulsive forces between virus and bubble, by the addition of divalent electrolytes. It is shown that the continuous flow of (dry) air, even at 150–250 °C, only heats the aqueous solution in the bubble column to about 45°–55 °C and it was also established that viruses are not significantly affected by even long term exposure to this solution temperature, as confirmed separately from water bath experiments. Hence, the effects observed appeared to be caused entirely by collisions between the hot air bubbles and the virus organisms. It was also established that the use of high air inlet temperatures, for short periods of time, can reduce the thermal energy requirement to only about 25% (about 114 kJ/L) of that required for boiling (about 450 kJ/L).

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1. Introduction

Water is a vital resource and so throughout history all human societies have ensured their continuous access to drinking water. When a population is large enough water treatment and sanitation become essential urban issues. Many of our modern water technologies evolve from three thousand years old ancient technologies. There are very good examples like aqueducts, dams, wells and various water purification devices [1]. Water purification devices have evolved from the bronze era to the present with the recent emergence of the hot bubble column evaporator (HBCE) desalination and sterilization process, which is based on the surprising discovery that a continuous flow of hot, dry bubbles does not cause the column of water to boil.

In Crete during the Minoan Era (3000–2000 BC), a water clarifying device similar to a terracotta pipe was used to filter domestic water through its pores. After the settlement of the solids the device was regularly cleaned. A similar device was found in the tombs of the Egyptian Pharaohs Rameses II and Amenophis II (1500 B.C.) [2].

In the Tylissos houses during the Minoan Era, conical terracotta infiltration devices were filled with charcoal for the removal of inor-

ganic and organic impurities. This technology was transferred to the Mycenaeans in continental Greece and later to the Romans [1].

In the Arab world during the 11th century, the Persian physician Avicenna (considered by many as the Father of Medicine) recommended travellers to filter the water through a cloth or boil it [1]. During the 17th century, Francis Bacon studied and published experimental results on water purification techniques like distillation, percolation and coagulation. The Italian Lu Antonio Porzio designed the first multi-pass filter with sand filtration [1]. In the 18th century, James Peacock claimed a patent for a back-washing sand filter. In 1804 in Scotland the first filter facility for the entire town of Paisley was produced. It is interesting that the widespread adoption of tea drinking, imported from China, led to substantial health improvements across England due to the boiling of water. During the 20th century ozone and chlorine water treatment became popular in the USA and Europe [1].

In recent studies, Garrido A. et al. [3] found that the model MS2 waterborne virus can be inactivated by heat exchange with hot air bubbles at 150 °C, during hot bubble-virus collisions in the HBCE process. The interaction of hot bubbles with viruses is considered the basic mechanism for this study. In these studies, the MS2 virus survival factors were correlated with the surface forces acting between bubbles and viruses in different solutions. It was shown that surface forces between viruses and hot air bubbles (at 150 °C) could be controlled by using added electrolytes, designed

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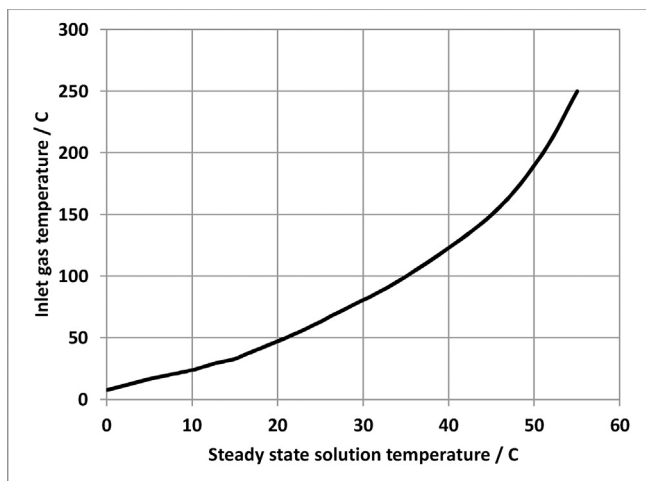


Fig. 1. Bubble column evaporator equilibrium temperatures calculated for a range of inlet air temperatures for water columns [23].

to reduce electrostatic repulsive forces, resulting in more effective and efficient virus inactivation.

In this study [3] total electronic charges per virus in NaCl 0.17 M (−65) and CaCl₂ 0.01 M (−17) solutions were calculated and used as an input of a classical DLVO model. Then for the Derjaguin approximation viruses were assumed to be 13 nm radius spheres interacting with a flat surface (the bubbles). This approach can be used as a consistent and scientifically based argument to understand virus interfacial properties in the current work and explains why different salts and solutions can change the interaction forces between virus and bubbles.

From our previous work [3] two model solutions have been identified for the present research. The first one was 0.17 M NaCl, which prevents bubble coalescence and increases the performance of the HBCE by producing a higher hot air/water interfacial area. The second one, 0.01 M CaCl₂, according to our proposed theory reduces the virus charge, so that the repulsive electrostatic forces with bubbles are reduced, thus increasing the HBCE inactivation efficiency, but without increasing the air/water interfacial area.

The novelty of this new work is to identify which mechanism, bubble coalescence inhibition in NaCl solutions (there is not an explanation for this phenomenon) or electrostatic bubble-virus repulsive force reduction (in CaCl₂ solutions), is more effective at inactivating viruses, in a bubble column with increasing inlet air temperatures (i.e. from 150 to 250 °C).

The HBCE process requires less thermal energy than solution boiling because the heat capacity of air is much lower than water and the hot inlet gases equilibrate with the column solution to produce a steady state temperature of only about 55 °C (see Fig. 1) [4].

The HBCE reaches dynamic equilibrium and hence a steady state column temperature is attained, when the heat supplied to the column by each new (hot) gas bubble is precisely balanced by the heat required to vaporise water to saturate the bubble at the equilibrium vapour pressure, at the steady-state temperature of the column. Hence, it follows that at steady state equilibrium the energy balance, in Joules per m³ of gas (leaving the top of the column) is given by the equation:

$$[\Delta T \times C_p(T_e)] + \Delta P = \rho^v(T_e) \times \Delta H_v(T_e) \quad (\text{in units of J/m}^3) \quad (1)$$

Where $C_p(T_e)$ is the specific heat capacity of the gas at constant pressure, T_e is the steady state equilibrium temperature of the column, $\rho^v(T_e)$ is the water vapour density at T_e , ΔH_v is the heat of

vaporization of the solution and ΔT is the temperature difference between the gas entering and leaving the column [5]. The additional correction term ΔP is equal to the hydrostatic pressure difference between the gas inlet into the sinter and atmospheric pressure at the top of the column. It represents the work done on the column by the gas flowing into the base of the column, at this increased pressure [5].

The model MS2 bacteriophage [6,7] was selected to evaluate the efficiency of thermal inactivation by the HBCE process, at air inlet temperatures of 150 °C, 200 °C and 250 °C. This bacteriophage is widely used as a surrogate organism to estimate infectious virus survival in various environments, and it is usually quantified by plaque assay. Bacteriophage MS2 is a group I male-specific RNA coliphage that infects *Escherichia coli* [8]. Its structure is similar to many pathogenic enteric viruses and is resistant to conditions in natural water environments and water treatment processes. MS2 bacteriophage can be enumerated via plaque assay technique. Visibility and consistency of the current plaque assay was ensured by using the spread plate technique, instead of the pour plate technique, which is commonly used in existing methods [9].

Valegard et al., [10] classified MS2 as a bacteriophage member of group I, its entire genome has been sequenced, is a positive-sense single-stranded RNA molecule of 3569 nucleotides and has an icosahedral structure with a hydrodynamic radius of about 13 nm [10].

In the HBCE treatment process inactivation appears to be due to the collisions of viruses with the hot gas bubbles [3]. Therefore, the HBCE process can be included within thermal water treatments.

The double layer plaque assay technique [11], detects only pathogenic viruses. It has been used here for monitoring thermal inactivation of MS2 viruses using the HBCE process at 3 different inlet air temperatures (150 °C, 200 °C and 250 °C) and 2 different electrolyte solutions (NaCl and CaCl₂) for water reuse applications.

2. Material and methods

2.1. Experimental solutions

Monovalent (1:1) and divalent (2:1) electrolyte solutions of 250 ml were prepared and sterilised by autoclaving in an Aesculap 420 at 15 psi, and 121–124 °C for 15 min. A NaCl concentration of 0.17 M NaCl or higher ($\geq 99\%$ 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. Some salts inhibit bubble coalescence and same no. NaCl at 0.17 M, what is the salt level in the human body, reaches at this value its maximum bubble inactivation effect [12]. There is not clear explanation for this phenomenon. The MS2 virus (ATCC 15597 B1) is unaffected by high salinity, and is stable in the presence of 1–2 M of NaCl [13], therefore the concentration of NaCl should not have contributed to the inactivation of the virus.

0.01 M CaCl₂ solutions ($\geq 99\%$ purity, obtained from Sigma-Aldrich) in Milli-Q water were used to reduce the virus charge, so that the repulsive electrostatic forces between bubbles and viruses was reduced, with the potential of increasing the HBCE inactivation efficiency, without affecting MS2 viability in this salt solution [3,14].

2.2. Media preparation

A specific optimised Double Layer Plaque Assay technique was developed to assess the concentration of active MS2 viruses. The plaque assay method is commonly used for detection of MS2 in treated drinking water, wastewater and marine water. The water quality is assessed based on the capability of bacteriophages to kill the host bacteria and allow phages to propagate in a confluent lawn of bacterial host cells immobilised in a layer of agar [11,15–17].

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