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Water sterilisation using different hot gases in a bubble column reactor.

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Graphical abstract

Highlights

- 3 different gas dependant water sterilisation mechanisms in bubble column evaporator
- Hot CO₂ bubbling at 1 atm. inactivated viruses better than Ar, air, N₂ and O₂
- Hot O₂ bubbling at 1 atm. inactivated E.coli better than Ar, air, N₂ and CO₂
- Hot CO₂ bubbling at 1 atm. can be used to inactivate viruses and bacteria in water
- Reduction in pH due to CO₂ bubbling has not virus and bacterial inactivation effects

Abstract:

This study compares the effect of using a range of different hot gases to sterilise water at atmospheric pressure within a hot bubble column evaporator (HBCE) reactor. Water containing bacteria (E.coli ATCC 15597) and viruses (MS2 ATCC 15597-B1) was sterilized with 6 different gases (CO₂, N₂, O₂, Ar, air and combustion gas) at inlet temperatures of 200°C (for viruses) and 150°C (for bacteria). The results obtained suggest three different gas dependent sterilisation mechanisms: a thermal inactivation mechanism, based on heat transfer between hot gas bubbles for argon, air and nitrogen; a CO₂ specific inactivation mechanism, based on the penetration of CO₂ molecules through bacterial membranes and virus capsids; and an oxidation/combustion mechanism that inactivates only E.coli, essentially by direct contact with oxygen or through reactive oxygen species. CO₂ gas and combustion gases gave

the best inactivation results with viruses and O₂ gas was found to be most effective for the destruction of E.coli.

Keywords: MS2 virus; inactivation; E.coli; water sterilization; water reuse; carbon dioxide; oxidation.

1. Introduction

Bubble column evaporators (BCE) are typically cylindrical containers where a gas introduced via a porous frit at the bottom of the column generates a continuous flow of rising bubbles in the liquid phase [2]. They are used in many biochemical, chemical and petrochemical industries [3]. In the wastewater industry they are used as reactors for chlorination, oxidation and fermentation [4].

BCEs have some key advantages over other systems. For example, they are simple to operate and control and also provide maximum contact for interactions between gases and liquids, and optimal heat and mass transfer [2]. If bubble coalescence inhibition can be induced to control bubble size, these advantages further improve with an enhanced effective interfacial area.

The use of a range of strong electrolytes in a BCE inhibits bubble coalescence and allows the production of high density (1–3 mm diameter) bubbles [5]. Inhibition of bubble coalescence by specific salts at and above physiological concentrations (0.17 M) is a phenomenon much studied since 1993 [5]. It has so far defied explanation, but invites exploitation. Bubbles are inhibited from coalescing in aqueous solutions at concentrations above about 0.17 M NaCl, while there is virtually no coalescence inhibition in water. See ref [5] for detailed

experimental results and analysis. Added salt at 0.17 M NaCl will inhibit bubble coalescence and will increase the performance of the BCE by producing smaller bubbles and a higher gas-water interfacial area [5], which has also been found to increase sterilisation rates [6]. Hence, for comparison between different gases, in the experiments reported here all the studies were carried out in 0.17 M NaCl aqueous solutions.

A gas-liquid bubble column provides a heat transfer rate between liquid and gas 100 times more efficient than single phase flow according to Deckwer [7]. This heat transfer efficiency in a BCE has been used to inactivate waterborne pathogens for water reuse, without the need for boiling [6]. It does so by transferring heat from the hot gas to the bacterial surfaces, through collisions. The hot bubble column evaporator (HBCE) process [6, 8, 9][10] was used to demonstrate this with hot air bubbles in the range 150-250°C, and bubbles of 1-3 mm of diameter, in different electrolyte solutions.

It has been shown that hot, dry gas bubbles do not heat the bulk solution substantially [6, 8]. Whatever the gas inlet temperature, the bulk solution attains a lower steady state temperature, in the range of 42-55°C, due to the effects of evaporative cooling. However, a transient, thin hot layer of water must exist initially at the surface of the rising bubbles. These layers can transfer heat to thermally decompose aqueous solutes, e.g. salts, which are unlikely to penetrate the gas bubbles [11]. The fundamental collision and heat transfer process appears to work for both dispersed bacteria cells and viruses, as we have already shown [6, 8]. However, for the larger microorganisms, the process most likely involves direct collisions and even penetration into the hot gas bubbles [8, 9]. Inactivation of bacteria in the HBCE might be easily understood, since the cloud point (gel-disordered phase change) for lipids that form the

bulk of bacterial membranes, is in the range 45-60°C. Hence, thermal destruction of the membrane is expected at these temperatures [12]. However, for viruses the mechanisms involved are not yet determined.

In this study we explored the phenomenon further, to better understand the sterilisation mechanisms. The bacteria *E.coli* ATCC 15597 [13] and virus MS2 ATCC 15597-B1 [14, 15] were the chosen pathogens. The hot bubble sterilization rate was measured in 0.17 M NaCl aqueous solutions containing these microorganisms and using hot inlet gases of CO₂, N₂, O₂, Ar, air and combustion gases. The gases were bubbled at different temperatures into a HBCE reactor at close to atmospheric pressure. The results obtained are suggestive of the potential mechanisms involved and allows for some degree of optimisation of the process.

Carbon dioxide has been successfully used for bacterial and viral inactivation when used as a supercritical fluid or at high pressures. Under such conditions it shows better sterilisation properties than other gases [16-18]. Other studies have compared the inactivation rates of baker's yeast (*Saccharomyces cerevisiae*) when using an explosive decompression system with CO₂, N₂, N₂O and Ar under different conditions of high pressure and temperature. These studies found that CO₂ and N₂O achieved higher inactivation rates than the other gases. This was attributed to their higher solubility in water and consequent absorption by the cells [19]. By comparison, the gases used in the HBCE process are used at close to atmospheric pressure.

Using hot inlet O₂ gas in the HBCE process could have an effective oxidizing effect on the dispersed pathogens. Thus, some authors, such as Lucas et al. [20], studied the oxidation

properties of O_3/UV and $O_3/UV/H_2O_2$ in a pilot-scale bubble column reactor to effectively remove chemical oxygen demand (COD) and total organic carbon (TOC) from winery wastewaters. Their work proved that this process is more efficient than ozonation and UV radiation alone. Other sources of oxidation, such as hydrogen peroxide H_2O_2 in solution, can also be used as an oxidizing agent that inactivates bacteria by damaging their proteins and membranes. For example, *E.coli* ATCC 35218 inactivation rates were successfully improved with increasing H_2O_2 concentration [21].

2. Model Water Treatment Viruses and Bacterial Systems.

2.1 MS2 VIRUS

The MS2 bacteriophage (ATCC 15597-B1) [14, 15] was chosen as the model virus to evaluate the efficiency of thermal inactivation of the HBCE process. It was used successfully to demonstrate sterilisation of water contaminated with MS2 viruses using hot (at $150^\circ C$), dry air bubbles [8]. MS2 is usually quantified by counting infectious units via a standard plaque assay that is commonly used for the detection of MS2 in treated drinking water and wastewater [22].

MS2 is used as a surrogate for enteric viruses since it is inactivated only at temperatures above $60^\circ C$, is resistant to high salinity and susceptible only to low pH. This means that it is highly resistant to environmental stress [23].

According to [24], 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 13nm [24].

Thermal inactivation of MS2 viruses dispersed in aqueous solutions was studied using the HBCE process and virus analysis was carried out using the optimized Double Layer Plaque Assay technique [25], that detects only infectious viruses by using E.coli as their host.

2.2 COLIFORM BACTERIA

E.coli is a gram negative bacteria with a straight cylindrical rod shape of 1.1-1.5 μm diameter and 2.0-6.0 μm length [26]. It is found in the gastrointestinal tract of animals and humans. E.coli strains can be harmless or pathogenic to the host. As the result of fecal contamination they can be found in water and soil. Therefore, its presence in water has often been used as an indicator to monitor water quality [27].

Most studies chose E.coli C-3000 (ATCC15597) as a representative model for bacteria in water [28, 29]. E.coli C-3000 (ATCC15597) is a biosafety level 1 organism [13] and can be used as the MS2 virus host [30]. These microorganisms were selected for this study. Size distributions for the E.coli strain (ATCC 15597) were measured in 0.17 M NaCl aqueous solution using a Malvern Zetasizer nano instrument, and a peak intensity of about 1600 nm was observed (see Fig. 1). Which fits within the literature measurements of 1100-1500 nm diameter and 2000-6000 nm length [26] for the cylindrical rod shape coliform.

Figure 1: E.coli size distribution for E.coli ATCC 15597 for 1×10^6 cell/ml in 0.17 M NaCl using DSL at 25°C, peak (mean/area) at 1600 d.nm measured using a Malvern Zetasizer nano instrument.

Spinks et al. demonstrated that pathogenic bacteria are inactivated at a solution temperature range of 55° to 65° C [31]. Other studies found that E.coli presents the first thermal inactivation signs at temperatures over 55° C, achieving high inactivation rates at 60°C [32].

This is expected as the membrane phospholipids have a phase transition and lose their ordered state at this temperature.

3. Material and Methods

i. Experimental solutions.

All electrolyte solutions were prepared and sterilized by autoclaving in an Aesculap 420 at 15 psi, and 121-124°C for 15 minutes [33]. Typically, the aqueous solutions used in this study contained 0.17 M NaCl ($\geq 99\%$ purity, obtained from Sigma-Aldrich) in 300 ml of Milli-Q

water. Salt at such a concentration or higher is necessary to inhibit bubble coalescence and increase the performance of the HBCE by producing finer bubbles and hence a higher gas-water interfacial area [5]. Although NaCl (0.17M) was added in this study to reduce bubble size, wastewater often contains lipids, surfactants and biopolymers, which even at low levels will stabilise bubbles, reduce bubble size and hence improve virus inactivation effects using the HBCE [8].

Rico et al [34] found that bubble coalescence in water depends on gas type and pH. In that study it was reported that CO₂ bubbles inhibited coalescence, whereas other gases like Ar, N₂ and air readily coalesced. For this reason, 0.17 M NaCl aqueous solutions were chosen for this study, to ensure that bubble coalescence was the same for all the gases, eliminating a possible confounding variable.

Related to this, some initial experiments were carried out using CO₂ in low salt concentrations. It was observed that the effect of CO₂ on virus and bacterial inactivation in 0.17 M and 0.001 M NaCl solution were similar. Therefore, it appears that the salt concentration did not play an important role on pathogen inactivation, when using CO₂ gas in the HBCE process.

The MS2 virus is resistant to high salinity and is stable in the presence of 1-2 M NaCl [35]. Hence, the concentration of NaCl should also not be a direct factor in the inactivation of the virus or bacteria.

ii. Media preparation for the virus experiments.

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 to propagate in a confluent lawn of bacterial host cells, immobilized in a layer of agar. [25, 36] [30, 37].

In this technique it is essential to use an adequate growth media to achieve better visibility and higher consistency [30, 37]. The medium is not commercially available. So it was prepared before each experiment in the form of two solutions, A and B. The preparation of solution A was obtained using 15 g. of tryptone, 1.5 g. of yeast extract, 12 g. of NaCl and 1425 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 aseptically dispensed into 3 vessels with different amounts of agar (1% for the bottom agar, 0.5% for the top agar and no agar for the media). The agar used in these experiments was of molecular biology-grade, obtained from Sigma-Aldrich. These solutions were heated to boiling to dissolve the agar and were 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 sterilisation and then was aseptically added to each of the 3 solutions A, once they cooled to 50°C.

The bottom agar was poured into 100mm x 15 mm petri dishes and dried around a lighted Bunsen burner, to maintain local environmental sterility, until the agar was not too dry or too moist [36].

iii. Media preparation for the E.coli experiments.

The plaque assay method is commonly used for detection of E.coli in treated drinking water, wastewater and marine water. The water quality is assessed based on the ability of bacteria to propagate in a layer of agar [26, 34].

For each experiment 1 L was prepared from two solutions (A and B). Solution A was composed of 13 g. of tryptone, 1 g. of yeast extract, 6 g. of NaCl and 1000 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 aseptically dispensed into 2 vessels with different amounts of agar (1.41% agar and no agar for the media), the agar used in the experiments was of molecular biology-grade from Sigma-Aldrich. These solutions were heated to boiling to dissolve the agar and sterilized by autoclaving in an Aesculap 420 at 15 psi, and 121-124°C, for 15 minutes.

Solution B was used to improve the growth of the bacteria. This solution was prepared by adding 1 g. of glucose and 0.010 g. of thiamine to 50 ml of Milli-Q water and filtered through a 0.22µm filter for its sterilisation and then was aseptically added to each of the 2 solutions A, once they cooled to 50°C.

The 1.41% agar solution was poured into 100mm x 15 mm petri dishes and dried around a lighted Bunsen burner, to maintain local environmental sterility, until the agar was not too dry or too moist [26].

iv. Production and characterisation of the Bacterial strain

Escherichia coli C-3000 (ATCC15597) was used as a representative model for general bacterias in water [28, 29] for the *E.coli* inactivation experiments and also for the virus experiments, as the MS2 virus host [30]. The size and the zeta-potential of the *E.coli* was measured in 0.17 M NaCl aqueous solution, equilibrated with the different gases used in the HBCE experiments, using a Malvern Zetasizer nano series instrument.

For a successful plaque assay, the *Escherichia coli* C-3000 (ATCC 15597) must be in the exponential phase of growth. This was achieved by growing two separate bacterial cultures: an overnight culture and the log phase culture [30, 33, 37]. The overnight culture was grown in 10 ml of the media without agar 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. The overnight culture resulted in high numbers of bacteria in the culture and this was used as reference standard.

To start the log phase *E.coli* culture, 1 ml of the overnight culture was transferred into 25-30 ml of broth without agar 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 520nm of between 0.8 and 1.1 indicated that the culture could be used in the plaque assay for the virus experiments and also as a standard for the E.coli experiments.

v. Production and characterisation of the Viral strains.

A freeze-dried vial of MS2 bacteriophage was acquired from the American Type Culture Collection. Bacteriophage MS2 (ATCC 15597-B1) was replicated using Escherichia coli C-3000 (ATCC 15597) according to the International Standard ISO 10705-1 [33] and the Ultraviolet disinfection guidance manual of the United States Environmental Protection Agency [38]. The zeta-potential of the MS2 viruses in 0.17 M NaCl aqueous solution, equilibrated with different gases, was measured using a Malvern Zetasizer nano series instrument.

vi. Viral and bacterial dilutions.

The concentration of the MS2 bacteriophage and E.coli was calculated by adding 1.0 ml of medium without agar to the vial and serially 10-fold diluted 12 times, by passing 0.50 ml of the bacteriophage and the E.coli (in a separate experiment) into tubes containing 4.50 ml of medium without agar [37]. 0.1 ml of dilutions from 10^{-6} to 10^{-12} were spotted on the surface of 14 petri dishes and spread with a bacterial spreader hockey stick.

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

Undiluted spiking suspension in PFU / mL = (PFU1 + PFU2... PFUn)/(V1 + V2.... Vn) [7]

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

vii. Hot bubble column evaporator (HBCE) process.

The HBCE process used in these experiments, was used with 6 different gases: N_2 , CO_2 , Ar, air, O_2 and combustion gas, which were pumped through the electrical heater that maintained the gas temperature just above the sinter surface in the range of 150° to 200° 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 (Fig.2).

Once the experimental solutions were poured into the column, the temperature of the solution was measured with a thermocouple in the centre of the column solution. Gases (from 22° to $200^\circ C$ for this study) travel through the sinter into the 300 ml solution inactivating MS2 viruses or E.coli in separate experiments.

When using combustion gases, the exhaust pipe of a generator (Honda EM2200) was attached to an isolated metal pipe with a valve that provides an exhaust gas flow rate of 27 l/min through a bubble column (see Fig. 5).

Figure 2: Bubble column evaporator [Extracted from Shahid et al., 2016, Review Paper [1]]

viii. Bacteria and virus disinfection experiments

A total of 16 HBCE experiments were performed using a 0.17 M NaCl aqueous solution, with inlet gases of CO₂, argon, nitrogen, air, oxygen and combustion gas mixtures, at temperatures of 150° and 200°C and, for comparison, in a water bath at 22.5° C and pH 4.2.

The enumeration of bacteriophage and E.coli viability was, in each case, obtained by the plaque assay method [25, 36, 37].

Once the solutions with known concentration of coliphage and E.coli were prepared, 2 rounds of experiments were conducted in the HBCE (Fig. 3) to study the inactivation of the coliphage with different hot gases: first at 200° C inlet gas temperature for viruses and then at 150°C for E.coli. In each experiment, 1.3 ml samples were collected from 10 to 15 mm above the central area of the sinter (Fig. 3) every 6 minutes during 36 minutes for viruses and every 2 min for E.coli. 0.07 ml of each sample was spotted in triplicate, following the double layer plaque assay technique [38].

Figure 3: Hot bubble column evaporator experiments. Hot gas

To evaluate the effect of solution pH on the inactivation of MS2 bacteriophage and E.coli experiments, comparison tests using 0.17 M NaCl aqueous solution at pH 4.1, were carried out in a separate beaker, within a water bath. During these experiments, samples were regularly taken from the beaker and analysed, as before.

ix. Data analysis

Plaque counts were performed for all 19 - 21 plates from each of the experiments. The mean of each triplicated sample was used to calculate the virus or bacteria survival factor: $\text{Log}_{10}(\text{PFU}/\text{PFU}_0)$, where PFU_0 is the initial number of PFU per sample and PFU is the PFU per sample after an exposure time in minutes. When plotted against the time survival curves were obtained [23].

4. Results and Discussion

i. pH effect on virus and bacteria inactivation when bubbling CO_2 in the HBCE.

When using CO_2 gas in the bubble column the pH of the water typically dropped from 5.9 to 4.2 in less than 45 seconds. When CO_2 dissolves in water 99% stays as the dissolved molecular gas and less than 1% exists as carbonic acid (H_2CO_3). This reduces the pH of the water to around 4 (equation 2). Carbonic acid dissociates into bicarbonate ion (HCO_3^-) and carbonate ion (CO_3^{2-}) (see equations 3 and 4) [39].

In order to test if the reduction in pH, due to dissolved CO₂ (via equations 1, 2, 3 and 4), was related to its disinfectant effects, two experiments with carbonic acid (H₂CO₃) at pH 4.2 were conducted in a separate series of experiments using a continuous stirred beaker, one for viruses and another one for bacteria.

Carbonic acid (H₂CO₃). was produced by bubbling 27 l/ min. of pure CO₂ through a 0.17 M NaCl aqueous solution for 10 minutes at 22° C. The solution was continuously stirred in the beaker and a sample was taken every 3 minutes. The pH was found to be about 4.2 and a log virus reduction of only about 0.002 was observed. For E.coli, only a 0.08-log removal was observed, which suggests that the reduced pH had only a slight inactivation effect on E.coli and no effect on viruses. Some authors describe a small, low pH inactivation effect on microbial cells, since membranes stop protons from penetration but low pH makes membrane more permeable to other substances like CO₂, due to the chemical modification on the phospholipid bilayer of membranes [40, 41]. X.Cheng et al [16] observed almost no inactivation change under different pH conditions (pH 4, 4.5, 5 and 5.5) for 3 different viruses (MS2, Qβ and φX174). They believed that H⁺ ions could not enter the capsid as easily as CO₂ molecules.

These results indicate that the reduced pH was clearly not responsible for the high virus and bacterial inactivation effects that were observed when bubbling hot CO₂ into the HBCE. Most likely, when using the HBCE, high CO₂ solubility in water played an important role in virus and bacteria inactivation. This will be studied in detail in a succeeding paper.

ii. Effect of different gases on virus and bacterial inactivation.

In these experiments, MS2 virus (ATCC 15597-B1) and E.coli (ATCC 15597) inactivation rates were determined when bubbling air at 200 °C (for virus) and 150 °C (for E.coli) in the HBCE through 0.17 M NaCl aqueous solutions. The same experiments were carried out with different gases, present in air. 99% of air is a mixture of nitrogen and oxygen gases, the other 1% contains argon and CO₂ (among others). Lastly, combustion gases from a generator at 60°C were used to inactivate first MS2 viruses and later E.coli.

Inactivation rates for MS2 virus with gases at 200° C were found to be quite similar, except for CO₂ and combustion gases. When inactivating E.coli with gases at 150°C, oxygen produced the highest inactivation rates, followed by CO₂ and combustion gas.

When bubbling CO₂, Ar, N₂ and air through water in previous studies [34] [19] it was demonstrated that CO₂ behaved differently due to its solubility in water, changes in pH and the zeta potential of its bubbles.

Enomoto et al. [19] found that when using an explosive decomposition system, highly soluble gases in water like, CO₂ and N₂O, achieved greater inactivation rates of *Saccharomyces cerevisiae* than when using other gases, like Ar or N₂, that are less soluble in water.

Tabor et al [34] reported that CO₂ prevents bubble coalescence much better than other gases. Since bubble coalescence inhibition is a very important variable in the performance of the HBCE, since it increases the gas/water interfacial area, all of the experiments were conducted in a 0.17 M NaCl aqueous solution [5], to ensure that all the gases presented the same degree of inhibition of bubble coalescence in the column solution.

Carbon dioxide is also different because it has a higher water solubility, produces a lower magnitude of coliform and virus Zeta potentials and lower pH values (see Table 1) compared with the other gases used in these experiments.

Gas	Solubility *	Zeta potential values	pH	Cp (Kj/(kg K))	Water Temperature C	Heated water Layer Thickness z (nm)	Average Temperature of the Layer. C
<i>Escherichia coli</i> C-3000 (ATCC15597) with gases at 150°C and solution at 43 C.							
Oxygen	0.030	-11.8	6.1	0.92	45.2	38.6	72.6
Nitrogen	0.014	-12.2	5.7	1.04	43.4	37	71.7
Carbon dioxide	0.950	-9.4	4.0	0.84	43.3	30	71.7
Argon	0.040	-12.4	6.0	0.52	38.6	24.3	69.3
Air	0.017	-12.5	5.7	1.01	44.7	38.1	72.4

Combustion Gas		-9.1	4.1		39.5		
MS2 bacteriophage (ATCC 15597-B1) with gases at 200°C							
Oxygen	0.027	-12.6	6	0.92	49.5	83.8	74.8
Nitrogen	0.013	-10.8	5.6	1.04	53.7	90.5	76.9
Carbon dioxide	0.800	-6.2	4.0	0.84	46.5	99.9	73.2
Argon	0.035	-12.2	6.0	0.52	46	55.3	73
Air	0.016	-14.3	5.8	1.01	52	87.2	76
Combustion Gas		-5.1	4.1		30		

*Solubility of gases in Water at 43 °C for E.coli and 52° C for virus. Grams of gas dissolved in 1 Kg of water at 1 atm.

Table 1: Solubility, microorganism Zeta potential, pH, heat capacity, equilibrium solution temperature, thickness of the heated layer around the gas bubble and average temperature of this layer for the gases in the study with viruses and bacteria.

The solubility at 52°C (for viruses) and 43°C (for E.coli) of the different gases used were obtained from literature values (<http://www.engineeringtoolbox.com>). The pH values were measured during the experiments and the Zeta potential values were measured during the experiments at a virus concentration of 10^8 per ml and E.coli concentration of 10^7 per ml.

In Table 1 (virus section) the CO₂ inlet gas data gave a lower column solution temperature, of only 46.5°C, than expected from steady state conditions, according to its heat capacity. This is due to the fact that only the average temperature of the water for the first 4 min. was used, since this was the time needed to achieve total virus inactivation, when using CO₂. For the rest of the gases the average temperature after 37.5 min was used, as this was the time needed to achieve 1.5 log virus inactivation. For bacteria, the average temperature was obtained after 10 min., which was the time needed to achieve an average of 1.5 log of E.coli inactivation.

The temperature and the thickness of the transient hot water layer around the surface of a 1 mm diameter gas bubble was roughly estimated for 200°C and 150°C inlet gas temperatures from the following analysis, where it is assumed that the hot gas bubbles initially cool to 100°C. Hence:

$T_1 = \frac{100 + T_{we}}{2}$, where T_1 (in °C) is the average (transient) temperature of the hot water layer surrounding the gas bubble and T_{we} (°C) is the equilibrium temperature of the solution in the BCE, assuming that the hot gas bubble cooled from 200°C to 100 °C or from 150°C to 100 °C.

The thickness of the corresponding transient, heated layer can then be roughly estimated by balancing the heat supplied by the cooling bubble with the heat required to raise the film to this average temperature.

Thus, the volume of the transiently heated water film, V , is given by:

$V = 4\pi r^2 z$, where the layer thickness around the bubble is z , when $r \gg z$.

The corresponding thermal energy balance is therefore given by:

$$C_p \Delta T V = C_{water} \Delta t 4 \pi r^2 \rho_w z \quad [7]$$

where C_p , C_{water} are the gas and water heat capacities, respectively, ρ_w is the liquid water mass density, ΔT is the initial cooling of the air bubble (to 100°C) and Δt is the transient temperature increase in the water layer, caused by this cooling.

Typical results from this crude model are given in Table 1. The average temperature of the transient water layer would be expected around 70°C and the corresponding thickness of the heated layer would be around 30nm for 150°C and 80-100nm at 200°C.

i. MS2 virus inactivation with different gases at 200°C.

Thermal inactivation mechanism

Collisions between the hot gas bubbles and the transient hot layers around the rising hot gas bubbles and the MS2 viruses are assumed to be the fundamental mechanism of thermal virus inactivation. The MS2 virus is rapidly inactivated at temperatures above 60° C [23, 42], so hot gas bubbles will effectively inactivate viruses if the temperature of the hot layer around the bubbles is greater than 60° C. It was previously shown (Garrido et al [8]) that the MS2 virus can be inactivated by heat exchange with hot air bubbles at 150°C during collisions in the HBCE. This mechanism appears to be related to how closely the viruses and the bubbles approach on collision. In the earlier experiments MS2 virus survival factors were tested and correlated with the interaction surface forces between virus and bubbles. The results reported indicate that at 150°C added electrolytes can control the surface forces between the virus and the hot surface of the bubbles and so influence deactivation rates.

Figure 4: MS2 virus inactivation when bubbling different pure gases at 200° C and combustion gas from a generator at 60° C in a 0.17 M NaCl solution.

MS2 virus survival factors for several different gases at 200°C and combustion gas at 60°C, were compared using the HBCE process (see Fig. 4). Air, oxygen, nitrogen and argon were found to have very similar virus inactivation rates, of between 1.33 and 1.67 – log reduction after about 37.5 min of treatment.

CO₂ inactivation mechanism

By comparison, when CO₂ (100%) and combustion gas (with a 12.5 to 14 % of CO₂, according to the text book: Combustion Fundamentals, Chapter 2 [43]) at 200° C and 62°C, respectively, were used in these experiments, the collisions between viruses and the hot CO₂ bubbles appears to be not the only mechanism that caused the fast virus inactivation rates, of 2.2 log for combustion gas and 2.7 log for pure CO₂, in just 3.5 minutes (Fig. 4), observed in these studies.

When using combustion gas (Fig.5) other combustion products than CO₂, H₂O and N₂ could be present due to minor components and impurities in the fuel and different fuel/air ratios. These gases are: carbon monoxide (CO), hydrogen (H₂), sulphur oxide (SO₂) and mono-nitrogen oxides (NO_x) like NO and NO₂ [43]. The presence of these gases in very small amounts could explain why we obtain similar results when using pure CO₂ at 200° C and the combustion gas was only used at an inlet temperature of 62° C. For example, Richard et al [44] investigated the significant viral inactivation properties of SO₂ and its high solubility in water.

Figure 5: Bubble column evaporator with exhaust gas from a generator.

Francis et al. [43] studied the latent heat of vaporization in a BCE with different aqueous salt solutions and estimated for a dry nitrogen inlet gas temperature of 62°C , an equilibrium temperature of 22°C . However, combustion gases are saturated with water and therefore heat is not used to evaporate water vapour into the bubbles; this explains why when the combustion gas inlet temperature was only 62°C , the equilibrium temperature of the water stayed high at 42.5°C , rather than 22°C , which would have been expected if the gas were dry. Therefore, if much of the heat in the hot gas bubbles was not used to evaporate water, but to heat the water surrounding the rising bubbles, this would assist in the thermal inactivation of the viruses.

When CO_2 gas is bubbled through the sinter area a large CO_2 -liquid contact surface is continually produced. This increases the amount of CO_2 dissolved in the solution perhaps producing a similar effect to what can be achieved by raising the pressure in dense phase carbon dioxide (DPCD) processes, even though in the bubble column the pressure remained

around 1 atm. Many authors have demonstrated the inactivation of microorganisms when using pressurized CO₂ [16-18]. The mechanism used to explain this inactivation effect is related to its high solubility in water.

Cheng et al [16] proposed an inactivation mechanism for bacteriophages MS2 and Q β based on the penetration of CO₂ inside the capsid under high pressure and later expansion when depressurized, so damaging the capsid. CO₂-protein binding could also damage the capsid inactivating the virus.

ii. E.coli (ATCC 15597) inactivation results in the HBCE with gases at 150°C.

Thermal inactivation mechanism

When inactivating E.coli (ATCC 15597) with air and nitrogen at 150° C in the HBCE, similar E.coli inactivation results (Fig. 6) were obtained to those reported by Shahid [10], when using natural lake water samples with hot air. The collisions between the hot rising bubbles and coliforms was assumed to be the fundamental mechanism for coliform inactivation in their work [9]. This was also assumed here for the fundamental coliform inactivation mechanism in this work when using the hot gases: CO₂, N₂, Air, Ar, combustion gas, and O₂.

Bubbling over a 10 min period, the E.coli survival factors for several different gases at inlet temperatures of 150° C, 58° C and 20° C were compared for the HBCE in Fig. 6. At 150° C, air and nitrogen presented very similar E.coli inactivation rates after 10 min of treatment, with 1.43 and 1.74 – log reduction, respectively. No inactivation was detected with air at 20° C with only 0.03 log reduction. Argon presented lower inactivation rates with just 0.40 log

reduction after 10 minutes, probably due to its low heat capacity, of only 0.52 kJ/kgK, which is half of the heat capacity of nitrogen and air (Table 1). For these 3 gases, when the hot gas bubbles form on the surface of the sinter a thin layer of heated water is transiently formed around the surface of the bubbles and this thin layer will have a similar average temperature, with values from 69.3°C to 72.4°C (Table 1). E.coli is rapidly inactivated at temperatures above 60° C [31], so hot gas bubbles will effectively thermally inactivate if the temperature of the bubbles (and the transient water film) is greater than 60° C, hence when bubbling air at 20°C no E.coli inactivation was observed (0.03 log reduction), which supports the thermal collision hypothesis and establishes a base line.

CO₂ inactivation mechanism

With CO₂ (100%) and combustion gas (with a 12.5 to 14 % of CO₂ according to text book Combustion Fundamentals, Chapter 2 [43]) at 150° C and 60°C, respectively, the collision between E.coli and the hot CO₂ bubbles appears to be not the only mechanism that explains the higher inactivation rates of 2.63 log for combustion gas and 2.84 log for pure CO₂, after 10 minutes (see Fig. 6).

Different mechanisms have been suggested to explain the antibacterial effect of dissolved CO₂. In Chapter 4 of the book 'Dense Phase Carbon Dioxide' [40] Osman describes, in great detail, different steps of the bacterial inactivation mechanism for pressurized CO₂. When pressurized CO₂ first dissolves into the solution its pH decreases. This acidification of the solution increases the penetration of CO₂ through the cell membranes. The CO₂ inside of the cell will then produce an intracellular pH decrease what will exceed the cell's buffering capacity resulting in cell inactivation [40, 41]. However, it also possible that bicarbonate ions

bind to lipid headgroups, changing their hydration and head group curvature, pore structure and therefore viability.

When using combustion gases other combustion products than CO₂, H₂O and N₂ could be present due to minor components and impurities in the fuel and different fuel/air ratios. These gases are typically: carbon monoxide (CO), hydrogen (H₂), sulphur oxide (SO₂) and mono-nitrogen oxides (NO_x) like NO and NO₂ ⁴³. The presence of these gases in very small amounts could explain why similar results were obtained when using pure CO₂ at 150° C and combustion gas at only 60° C.

Oxidation/combustion mechanism

Hot oxygen bubbles at 150° C in the HBCE with 0.17 M NaCl aqueous solution containing E.coli produced the best inactivation results, with 2.90 log reduction after just 3.5 minutes. Pure hot oxygen could oxidize the E.coli by direct contact or through reactive oxygen species produced at 150°C. It is known that the oxidative properties of H₂O₂ can inactivate E.coli, and this inactivation effect increases with the concentration of H₂O₂ ⁴⁵. Proteins, lipids and nucleic acids can also be oxidised by active oxygen species from incomplete reduction of O₂ during respiration ⁴⁶. These E.coli cells are straight cylindrical rod shapes of 1.1-1.5 µm diameter and 2.0-6.0 µm length ²⁶. Compared with the size of the bubbles, of 1-3 mm, these cells could easily get through the thin heated water layer around the oxygen bubbles (Table 1) and directly contact the hot oxygen gas within the bubbles.

By comparison, the results obtained for E.coli using hot oxygen (at 150°C) as inlet gas were found to be much more effective than for the MS2 virus (even with hot oxygen at 200°C). However, the virus has a much smaller hydrodynamic radius of about 13nm ²⁴ and so may not

be able to contact the hot gas within the bubbles, having insufficient momentum in typical collisions. Thus, the inactivation times of viruses (37 min) compared with E.coli (10 min) and the 0.65 log E.coli reduction when using oxygen at 20° C inlet gas temperature (Fig. 6), supports the hypothesis of an oxidative mechanism for E.coli inactivation through direct contact with the gas phase, which is unlikely to occur with the much smaller viruses.

Figure 6: E.coli inactivation in HBCE with different gases in a 0.17 M NaCl solution.

iii. Comparison of the thermal inactivation mechanisms for E.coli and viruses.

The World Health Organisation (WHO) in their guidelines for drinking-water quality⁴² compared thermal inactivation rates for different types of bacteria and viruses in hot liquids. They concluded that water temperatures above 60°C effectively inactivate both viruses and bacteria and when the temperature range lies between 60°C and 65°C, bacterial inactivation occurs faster than viral inactivation. These studies showed that at 60° C water temperature E.coli needs 600 seconds to reach a 3 log reduction compared with 1350 seconds for viruses like enterovirus, echovirus 6, coxsackievirus B4, coxsackievirus B5 to reach 3 log reduction.

In our experiments, E.coli reached 1.5 log inactivation after 10 minutes (Fig. 6) when bubbling gases at 150°C (solution temperature at 43°C) and MS2 virus reached 1.5 log inactivation after 37 min with gases at 200°C (solution temperature at 50°C) (Fig. 4). It is proposed that in the HBCE process, viruses and bacteria are inactivated when they get into the hot water layer around the hot bubbles or into the bubbles themselves⁴². Our results agree with the WHO data with a faster bacterial inactivation than for viruses, at similar water temperature. But this phenomena is considerably enhanced when using a HBCE. Thus, the inactivation rates for viruses are much slower than for bacteria even though the gas temperature for the virus was higher than for the bacteria. This difference may be, in part, due to a 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 gas, increasing the inactivation rate and the oxidation rate observed with O₂ at 150°C and 22°C (Fig.6).

5. Conclusions

Bubbling hot gases such as CO₂, air, combustion gases, N₂, argon and O₂, into a high temperature bubble column evaporator, at atmospheric pressure, can be used to effectively inactivate typical waterborne pathogens (e.g. viruses and bacteria) through 3 different mechanisms that depend on the type of gas used and the temperature of the inlet gas.

A thermal inactivation mechanism based on the heat transfer between hot gas bubbles and pathogens during collisions has been identified as the main driver for E.coli inactivation when using Ar, air and N₂, and for MS2 virus inactivation with Ar, air, N₂ and O₂. For both pathogens Ar gas gives lower inactivation rates than the other gases, apparently due to its lower heat capacity.

With CO₂ gas, the inactivation mechanism appears to be based on the penetration of CO₂ molecules through bacterial membranes and virus capsids. HBCE produces a high density of CO₂ gas at atmospheric pressure that can be effectively used to inactivate viruses and bacteria in water, where other gases like air, nitrogen and argon produce only a limited inactivation effect. Reduction in pH due to CO₂ bubbling was found not to be responsible for the high virus and bacterial inactivation effects obtained when using hot CO₂ bubbling. It appears likely that the HBCE method, which produces a high liquid-gas interfacial area combined with the high CO₂ solubility in water, plays an important role in virus and bacteria inactivation. This will be studied in detail in future work.

The use of O₂ at high temperatures and even at room temperature seems to produce an oxidative mechanism, which is particularly effective for E.coli inactivation, presumably via direct contact with the oxygen bubbles. However, this oxidation effect was absent for viruses. The different responses may be due to a difference in collision momentum with the O₂

bubbles, with the much larger E.coli cells more easily reaching contact with the oxygen within the bubbles.

The inactivation rates for MS2 viruses with different inlet gases at 200° C were found to be quite similar, except for CO₂ and combustion gases, which both produced faster inactivation rates, by about an order of magnitude. When E.coli was inactivated with different gases at 150°C, O₂ gas produced the highest efficiency, 3 times faster than air and N₂, followed by CO₂ and combustion gas.

6. Future work

In this work we have identified hot CO₂ as the best gas to inactivate viruses and bacteria in a HBCE reactor at temperatures between 150°C to 200°C and atmospheric pressure. In future work we will study the effect of CO₂ on virus and bacterial inactivation in an HBCE with a range of different electrolyte solutions.

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