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(Article begins on next page)

# Water disinfection by orifice-induced hydrodynamic cavitation\*

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## Abstract

Hydrodynamic Cavitation (HC) is considered as a promising water-disinfection technique. Due to the enormous complexity of the physical and chemical processes at play, research on HC reactors is usually carried out following an empirical approach. Surprisingly, past experimental studies have never been designed on dimensional-analysis principles, which makes it difficult to identify the key processes controlling the problem, isolate their effects and scale up the results from laboratory to full-scale scenarios.

The present paper overcomes this issue and applies the principles of dimensional analysis to identify the major non-dimensional parameters controlling disinfection efficacy in classical HC reactors, namely orifice plates. On the basis of this this analysis, it presents results from a new set of experiments, which were designed to isolate mainly the effects of the so-called cavitation number  $(\sigma_v)$ . Experimental data confirm that the disinfection efficacy of orifice plates increases with decreasing  $\sigma_v$ . Finally, in order to discuss the significance of the results presented herein and frame the scope of future research, the present paper provides an overview of the drawbacks associated with dimensional analysis within the context of HC.

Keywords: hydrodynamic cavitation; water disinfection; E. Coli; dimensional

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analysis; orifice-plate reactor; review.

# 1 1. Introduction

The lack of safe water in developing countries is affecting millions of people 2 causing major sanitation and economic issues. Prohibitive costs and difficult access to chemicals (as well as qualified staff) 1 2 prevent the implementation of water-disinfection technologies routinely adopted in developed countries. Such technologies also present shortcomings, the main one being associated with the fact that all chemicals used in the disinfection process may produce, under certain conditions, unhealthy and carcinogenic by-products (DBPs), such as trihalomethanes, haloacetic acids, bromate, and chlorite 3. As a result, in the ongoing review of drinking water quality guidelines, the World Health Organi-10 zation is updating risk assessments for dissolved chemicals, setting new stricter 11 limits for DBPs 4. From this picture it appears that there is a clear need to 12 implement chemical-free water disinfection techniques, which must also be sim-13 ple to use, robust and low-cost, especially to meet the demands of low-income 14 countries. 15

In this context, techniques based on cavitation seem to be promising. Cavita-16 tion exploits the phenomenon of formation, growth, and collapse of vapour/gas 17 bubbles triggered by pressure variations 5. When the fluid experiences a criti-18 cal pressure (i.e., lower than vapour pressure), the formation of cavities begins, 19 and the maximum size of the cavities is typically reached under isothermal 20 expansion. Subsequently, when higher pressure is recovered, bubbles undergo 21 adiabatic collapse. Such a collapse leads to the formation of pressure-waves and 22 micro-jets that instantly release a large amount of energy while generating in-23 tense normal and shear fluid stresses 6, 7, 8. In the scientific literature, these 24 severe conditions are considered as the main cause of cell membrane damage 25 and consequently of microorganism death or inactivation 9, 10, 11. Moreover, 26 high temperature peaks promotes chemical reactions, such as the dissociation of 27 water molecules into •OH radicals, which provide oxidizing power and increase 28 the efficiency of disinfection 12 29

Cavitation can be generated in two main ways: by ultrasonic waves travelling 30 through the liquid (i.e., acoustic cavitation, AC), or by forcing the fluid through 31 a constriction (i.e., hydrodynamic cavitation, HC) **13**. AC is energy demand-32 ing, works on batch and is effective only for fluid volumes in close proximity to 33 the acoustic source. Thus, AC is deemed unsuitable for the treatment of large 34 volumes of water 14, 15. In the case of HC (which has been investigated con-35 siderably less than AC 16), cavitation is tipically obtained by a pressure drop, 36 e.g. generated by an orifice plate or a Venturi tube. In contrast to AC, HC 37 is deemed as an energetically more efficient process 17 18 and allows for the 38 treatment of large volumes of moving water; so it is suitable for implementation 39 in drinking- and waste-water treatment plants 19 as well as in the food and 40 beverage processing 20 21, 22 and chemical synthesis 23 24, 25 26. 41

HC is induced by purely mechanical devices which can be used without 42 the presence of qualified staff and is therefore suitable for use in developing 43 countries. On the down side, HC is a more complex process than AC from 44 the fluid-dynamics prospective. AC involves bubbles growing and collapsing 45 in quiescent water, whereas HC commonly occurs in fast moving fluids whose 46 dynamics responds to complex (and currently poorly-understood) non-linear 47 interactions between bubbles and turbulence. As a consequence, the study of 48 fluid dynamics within HC reactors for water treatment is still in its infancy and 49 much more work is needed to identify governing parameters and quantifying 50 51 their role in the game of disinfection.

Recently, many research-works have focused on demonstrating the effective-52 ness of HC as well as exploring the effects of different HC-reactor-geometries 53 on disinfection efficiencies. Orifice plates 27, 14, Venturi tubes 28, 29, and 54 rotor-stator reactors (e.g., high speed homogenizers) 30 31 were the most in-55 vestigated devices. Other studies have focused on hybrid disinfection techniques 56 (i.e., the combination of cavitation with chemical disinfectants) in order to re-57 duce the amount of chemicals in the water treatment processes 32 33 34 35. 58 This interest in the topic witnesses the great potential of HC for water disin-59 fection [36]. However, the scientific literature on HC currently lacks of a sound 60 methodological approach as well as sound theoretical grounds 37, 29. In par-61 ticular, due to its complexity, the study of HC for disinfection purposes has been 62 commonly addressed using an empirical approach, although numerical studies 63 have also been proposed (see, e.g., 38, 39, 40, 41). However, to the best of 64 our knowledge, none of the existing studies in the literature has based the ex-65 perimental work on dimensional analysis. This clearly makes it difficult to: (i) 66 identify all the relevant non-dimensional groups controlling the problem; (ii) 67 isolate their effects on the observed disinfection efficiencies; and ultimately (iii) 68 scale up from laboratory to full-scale HC reactors. 69

The objectives of the present paper are: (a) to identify, by means of dimen-70 sional analysis, the non-dimensional parameters controlling disinfection efficien-71 cies in classical HC reactors such as orifice plates; (b) in light of this dimen-72 sional analysis, to provide a critical appraisal of the relevant literature (section 73 3) highlighting main results and knowledge gaps; (c) to present results from a 74 systematic set of experiments where the effects of the so-called cavitation num-75 ber (defined in the next section), were isolated and assessed. This parameter 76 was chosen as the target of the present paper as it quantifies the intensity of 77 cavitation and is therefore considered key for the design of HC reactors. 78

#### 79 2. Dimensional analysis

When a problem is as complex as HC, it is convenient to first attempt to tackle it by adopting an empirical approach whose very first step should be dimensional analysis. Towards this end, let us consider the simple case of a HC reactor where cavitation is induced by orifice-plates only. This is convenient because: (i) the geometry of Venturi-tubes (i.e., the other commonly-employed HC reactor) is much more complex than orifices as it is associated with many <sup>86</sup> more influencing variables, which make the analysis significantly more convoluted; (ii) as Venturi tubes, orifice plates have been largely investigated in the literature and therefore the results of the present paper can be easily put into context; (iii) we present novel experiments involving orifice plates only.

Since most experimental studies deal with the case of HC reactors implemented in closed loop systems, we consider the case of a fixed volume of water V which goes through a HC reactor multiple times  $n_p$ . At these conditions it can be argued that the bacterial concentration C of a specific pathogen (measured in Colony Forming Units, CFU, per unit volume of water) depends on the following set of parameters:

$$C = f(C_0, \mu, \rho, \gamma_s, v_h, P_2 - P_v, n_p, L_i),$$
(1)

where  $C_0$ , is the initial pathogen concentration;  $\mu$ ,  $\rho$  and  $\gamma_s$  are the kinematic 96 viscosity, the density and the surface tension of water, respectively;  $v_h$  is the 97 mean fluid velocity at the downstream end of the constriction,  $P_2$  is the abso-98 lute pressure recovered downstream of the orifice plate (see Figure 1),  $P_v$  is the 99 absolute water-vapor pressure,  $L_i$ , in general terms, defines the set of variables 100 characterizing the geometry of the reactor. In the simplest case of a circular 101 orifice plate, which is the subject of the present paper,  $L_i$  includes: the charac-102 teristic diameter of the orifice (i.e., the constriction) d, the diameter of the pipe 103 upstream and downstream of the plate D, the orifice-plate thickness b and the 104 number of orifices n. 105

As far as equation (1) is concerned, a few comments are in order: (i) as 106 in many other Fluid Dynamics problems, Equation (1) does not include simple 107 pressures but pressure-differences with respect to a reference value, which, due to 108 the importance of bubble formation and collapse, is here identified as the water-109 vapor pressure; (ii) the effects of temperature are indirectly taken into account 110 through parameters  $\mu, \rho, \gamma_s$  and  $P_v$ ; (iii) we did not consider the absolute water 111 pressure upstream of the orifice plate  $(P_1)$  as this is a direct function of  $v_h$  and 112  $P_2$  and is therefore redundant. 113

Relevant non-dimensional parameters can now be identified by application of the well-known Buckingham  $\pi$  theorem [42]. Towards this end  $\rho$ ,  $v_h$  and d are chosen as the three repeating variables, which contain all the primary dimensions appearing in Equation [1], namely length [L], mass [M] and time [T] (CFU appearing in the definition of concentrations are dimensionless numbers and therefore cannot be accounted for as a primary dimension). Simple dimensional arguments lead to the following set of non-dimensional parameters:

$$Cd^{3} = f_{1}\left(C_{0}d^{3}, \frac{\rho v_{h}d}{\mu}, \frac{\rho v_{h}^{2}d}{\gamma_{s}}, \frac{P_{2} - P_{v}}{\rho v_{h}^{2}}, n_{p}, \underbrace{\frac{D}{d}, \frac{b}{d}}_{k}, n\right).$$
 (2)

The dependent parameter on the left hand of Equation (2), can be combined with the first independent parameter to form a dimensionless bacterial <sup>123</sup> concentration  $\frac{C}{C_0}$ , so that Equation (2) becomes:

$$\frac{C}{C_0} = f_2 \left( C_0 d^3, \frac{\rho v_h d}{\mu}, \frac{\rho v_h^2 d}{\gamma_s}, \frac{P_2 - P_v}{\rho v_h^2}, n_p, \frac{D}{d}, \frac{b}{d}, n \right), \tag{3}$$

where,  $C/C_0$  is herein defined as a non-dimensional disinfection efficiency;  $(\rho v_h d)/\mu$ 124 is the Reynolds number of the jet forming at the downstream end of the ori-125 fice, which regulates turbulence and flow development within the HC reactors; 126  $\rho v_b^2 d/\gamma_s$  is the so-called Weber number, which takes into account surface ten-127 sion forces with respect to inertial forces and, presumably, strongly influences 128 the behaviour of bubbles [43]; D/d and b/d are geometrical parameters that, 129 together with the Reynolds number affect the flow characteristics of the orifice 130 and hence the fluid stresses bacteria may be subjected to (bacteria are strongly 131 sensitive to turbulence and fluid stresses, see e.g. <u>44</u>);  $(P_2 - P_v)/(\rho v_h^2)$  is the 132 so-called cavitation number, which quantifies the intensity of cavitation so that, 133 for values above the one corresponding to the onset of supercavitation, the lower 134 is its value the more intense is the formation and collapse of bubbles. It is worth 135 mentioning that, in the current literature, the cavitation number  $\sigma_v$  is usually 136 formulated adding a scaling factor 2, irrelevant for dimensional analysis, see 137 Equation (4);  $C_0 d^3$  is a dimensionless initial concentration, which, although ar-138 bitrarily defined, indicates that the effectiveness of a HC reactor might depend 139 on initial conditions. In Equations (1), (2) and (3),  $f, f_1$  and  $f_2$  are functional 140 relations between dependent and independent variables. 141

The next section provides an appraisal of the existing literature contextually to the dimensional analysis carried out above.

## <sup>144</sup> 3. A critical appraisal of the literature

As hydrodynamic cavitation has attracted considerable research interest, the 145 number of experiments available in the scientific literature is large and growing 146 fast. In Table 1 we selected 12 works on the basis of the following criteria: (i) 147 they all deal with HC induced by orifice plates or similar reactors such as noz-148 zles or partially closed valves; (ii) they all provide sufficient experimental details; 149 (iii) they all deal with disinfection of bacteria, except the work of Badve et al. 150 45 that used zooplankton, included for the sake of completeness. It is worth 151 noting that *Escherichia Coli* is the most commonly adopted bacterium in these 152 experiments as it is often present in naturally-contaminated water. Moreover, 153 the microbiological quality of drinking water relies largely on examination of 154 indicator bacteria such as coliforms, in particular E. Coli. For this reason, the 155 procedures to measure its concentration is internationally regulated. In addi-156 tion, E. Coli is simply cultivable in laboratory and is not particularly dangerous 157 to handle during the experiments. For the sake of completeness and to provide 158 an overall overview of the relevant literature, Table 1 provides information and 159 parameters that were reported by the authors of each referenced paper and 160 not only those already mentioned in the previous section. In order to interpret 161 Table 1 the following definitions apply: 162

• reactor type indicates the type of cavitating reactor used. OP refers to orifice plates; DynaJets<sup>®</sup>, DynaSwirl<sup>®</sup> and StratoJets<sup>®</sup> are patented reactors with a configuration comparable to an orifice plate; "valve" refers to as partially closed valve in which cavitation occurs; "pump" refers to experiments where the bacterial reduction solely due to the action of the pump was assessed;

• configuration is the geometry of the orifice plate used, e.g.  $25 \times 2$  mm indicates a plate with 25 holes of 2 mm of diameter. Additional information indicate the shape of the holes: squared (S), rectangular (R), if not specified otherwise, circular holes were adopted. Orifice plates put in series are indicated with the "+" sign;

• holes area is the total area of the holes in the plate;

•  $\alpha$  is the ratio of perimeter of the holes to their total area;

•  $\beta$  is the ratio of holes-area to cross-sectional area of pipe;

• cavitation number. This is considered one of the most important parameters to describe the intensity of cavitation. The literature, rather arbitrarily, introduced two types of cavitation numbers:

$$\sigma_v = \frac{P_2 - P_v}{1/2\,\rho \, v_b^2},\tag{4}$$

and,

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$$\sigma_{v,\Delta P} = \frac{P_2 - P_v}{P_1 - P_2},\tag{5}$$

• **t** is the total duration of the treatment;

• **initial/final CFU** are the initial and final concentration of bacteria used in the disinfection experiments;

• disinfection efficiency is the bacteria concentration reduction  $\delta C$  on percentage or in logarithmic unit, e.g.  $3 \log$  corresponds to a reduction in the bacterial concentration of three orders of magnitude.

Empty cells (-) in Table 1 indicate data not provided by the authors. Data with an asterisk were non directly provided by the referenced papers, but were derived by the authors of the present paper. Appendix A provides details about experimental methods and results provided by papers referenced in Table 1.

Table 1 witnesses the remarkable experimental efforts made by researchers to investigate the influence of the main variables involved in orifice-shaped reactors, e.g. the pressure drop, the velocity of the constricted flow, etc.. However, the dimensional analysis developed in the previous section highlights that the single dimensional variables are not the key information, but it is instead their suitable combination in dimensionless groups that is informative. The values of



Figure 1: Upper panel reports the qualitative behaviours of the pressure along the centerline. Lower panel shows the formation and successive implosion of cavities.

those numbers therefore play the crucial role in determining reactor behavior 193 and its effectiveness in inactivating bacteria. Aware of this fact, in Table 2 we 194 report the dimensionless numbers used in the works reported in Table  $\overline{1}$ . In 195 many of these studies, the experimental data necessary to calculate the dimen-196 sionless parameters were often not explicitly provided. Therefore, in Table 2, a 197 qualitative comparison is made by simply reporting which non-dimensional pa-198 rameters, among those of Equation 3, were left to vary (" $\times$ " symbol) and those 199 that were kept constant (" $\checkmark$ " symbol) in a specific set of trials. Therefore, 200 this table allows to asses whether the effects of one (or some) non-dimensional 201 parameters were actually isolated. 202

Table 2 shows that past studies and experiments were designed to investi-203 gate/isolate the effects of dimensional, rather than non-dimensional parameters 204 on disinfection efficiencies. The only non-dimensional group, whose effects were 205 isolated (by three studies only 46, 12, 47) is the one related to the initial 206 concentration, which seems to be negatively correlated with the disinfection ef-207 ficiency of orifice-based HC reactors. Therefore, while the available literature 208 plays a very important role in identifying and quantifying the effectiveness of 209 HC and different HC reactors, it does not allow to understand and explore the 210 physical mechanisms underpinning the disinfection efficiencies observed in the 211 experiments as these could be the effect of multiple variables and associated 212 physical processes. The authors believe that, in order to progress in this re-213 search field, future experimental work should be designed and carried out using 214 the dimensional analysis framework herein proposed or, if required, different 215 versions of it. 216

<sup>217</sup> Consistently with this idea, the remaining part of the paper is dedicated to <sup>218</sup> the the presentation of a set of experiments that the authors have carried out in <sup>219</sup> an orifice plate HC reactor to investigate mainly the effects of one of the afore-<sup>220</sup> mentioned dimensionless parameter, namely, the cavitation number  $\sigma_v$ . This <sup>221</sup> parameter is widely used to quantify the intensity of cavitation and is therefore <sup>222</sup> commonly considered extremely important to characterize disinfection efficien-<sup>223</sup> cies. In fact, since bubbles implosion is often considered the key physical process responsible for bacterial inactivation (although this hypothesis has recently been challenged, see (37, 29)), it is expected that disinfection efficiencies will be higher for lower  $\sigma_v$ . Experiments were also designed to further investigate the effects of initial bacterial concentration  $C_0$  on disinfection efficiencies.

#### 228 4. Experimental methods

All the experiments were carried out in the Water Engineering Laboratory 229 "Giorgio Bidone" at the Polytechnic of Turin (Italy) while bacteria preparation 230 and sample analysis was performed at the Research Centre of SMAT, which is 231 the Water Utility serving the city of Turin. The pilot plant used to induce cav-232 itation is shown in the upper panel of Figure 2 and it consists in a closed loop 233 pipe (stainless steel, 32 mm internal diameter) including a cylindrical holding 234 thank of 35 l volume (300 x 500 mm). The water temperature was controlled by 235 two chiller-units connected to a cooling coil placed inside the thank. A centrifu-236 gal multistage pump (Lowara 3SV-11, 2900 rpm, 1 kW) was used to recirculate 237 the water and an electromagnetic flow meter (Endress Hauser PROline Promag 238 10) was employed to monitor the flowrate. Two manometers, named M1 and 239 M2 (lower-left panel of Figure 2) were used to monitor  $P_1$  and  $P_2$ , respectively. 240 A ball-valve was used to control  $P_2$  and a transparent control section made of 241 glass (lower-right panel of Figure 2) was used to observe the occurrence of cavi-242 tation. The cavitation unit was mounted between two flanges and was made of 243 a stainless steel-plate of 16 mm thickness (lower-left panel of Figure 2), where 244 4 holes of 2.5 mm diameter were drilled and arranged in a diamond pattern. 245 Each test consisted in the treatment of 21 l of Milli-Q<sup>®</sup> water contaminated by 246 E. Coli bacteria at different concentrations. 247

A reference sample was taken at the beginning of each test, after contam-248 inated water was mixed within the whole hydraulic circuit for 10 minutes at 249 very low flow-rates that induced no cavitation. Successive samples were taken 250 at different times during each test. Each sample (300 ml), was then stored in 251 sterile plastic bottles that were kept at a constant temperature of 4 °C for a 252 period of maximum 24 hours. The samples were then brought to SMAT labs 253 for microbiological analysis to reconstruct the variation of the bacterial con-254 centration C with time during each experiment. After each experiment, the 255 entire hydraulic circuit was sterilized by injecting 2 ml of sodium hypochlorite 256 and then rinsed three times. At the end of the procedure a sample was taken 257 to verify the absence of either chlorine- or bacteria-residuals to make sure that 258 following experiments were carried out at identical "circuit" conditions. 259

E. Coli was chosen as the reference bacterium for this study since it al-260 lows a comparison with the works presented so far in the literature. E. Coli 261 (ATCC 8739, IELAB) was propagated on Chromogenic Coliform Agar (Oxoid) 262 overnight at 37°C. Colonies were resuspended in Maximum Recovery Diluent 263 (Oxoid) and live bacteria concentration was measured through absolute ATP 264 quantification by Dendridiag SW reagents (GLBiocontrol) following the man-265 ufacturer's instructions. The desired amount of bacteria was then transferred 266 into 1 l of Milli-Q<sup>®</sup> water and further diluted to a final volume of 21 liters of 267

Table 1: Experimental studies about water disinfection by orifice plate reactors or similar devices. Data marked with \* were not directly provided by the referenced papers, but derived by the authors of the present paper. (see text for the meaning of each column)

| Authors (man)                       | Reactor type                      | Bactoria               | Configuration  | Holes area             | 6            | 8            |              | 31.           | D.           | $D_{c}$    |            | :<br>-<br>-      | Λ        | +         | Initial CEII               | Final CEII              | Disinfaction afficiancy        |
|-------------------------------------|-----------------------------------|------------------------|--|------------------------|--------------|--------------|--------------|---------------|--------------|------------|------------|------------------|----------|-----------|----------------------------|-------------------------|--------------------------------|
| (mak) sinming                       | treactor to be                    | Datie                  | Coung a auon   | $[m^2]$                | $[mm^{-1}]$  | م <u>۲</u>   | [s/s]        | [m/s]         | [bar] [l     | bar]       | a_         | $v_{v,\Delta P}$ | [7]      | [min]     | [CFU/ml]                   | [CFU/ml]                |                                |
|                                     |                                   |                        |  |                        | •            |              |              |               | 1.72         | *0         |            | $0.57^{*}$       | 75       | 15        | 4580                       | $3180\pm10$             | $30\% \pm 0.22\%$              |
| Jyoti et al. (2001) 48              | Valve                             | Coliform               | Valve  | I                      |              | '            | ·            |               | 3.44         | *0 *       |            | $0.28^{*}$       | 13       | 15        | 4280                       | $3780 \pm 20$           | $11\% \pm 0.22\%$              |
|                                     |                                   |                        |  |                        |              |              |              |               | 5.17<br>5.17 | . *0       |            | 0.19*            | 6. 22    | c1<br>09  |                            |                         | $23\% \pm 0.22\%$ $44\%$       |
|                                     |                                   |                        |  |                        |              |              |              | - 4.1         | 3 - 5.17     | 0          | 0          | $24^* - 0.19^*$  | 1.5      | 40        | $10^8 - 10^9$              | $10^{5} - 10^{6}$       | 3 log                          |
| Kalumuck et al. (2003) 46           | $\mathrm{DynaJets}^{\circledast}$ | E. Coli                | $\mathrm{DynaJets}^{\otimes}$  |                        |              |              |              |               | 10.3         | 0 0        |            | 0.09*            | 1.5      | 120<br>30 | $10^8 - 10^9$              | $10^4 - 10^5$<br>$10^2$ | 5 log<br>5 log                 |
|                                     |                                   |                        | 95 v) mm   | 7 86F 06               | 00.6         | 11           | 00 6         | 44            | ц<br>Н       |            | 13         | 0000             | 06       | 8         | 0                          |                         | 907 D                          |
|                                     |                                   |                        | 1x12 mm  | 1.13E-04               | 0.33         | 0.20         | 3.86         | 34            | 13.8         | 0          | .17        |                  | 20       |           |                            |                         |                                |
| Balasundaran et al (2006) 40        | OP                                | E Coli                 | 1x15 mm  | 1.77E-04               | 0.27         | 0.32         | 5.28         | 30            | 10           | 0          | ).22       | '                | 20       |           |                            |                         |                                |
| Datasunation and the art (2000) The | 5                                 | . COM                  | 1x17 mm  | 2.27E-04               | 0.24         | 0.41         | 5.57         | 25            | 4            | 0          | ).32       | '                | 20       | ,         |                            | 1                       |                                |
|                                     |                                   |                        | 1x19 mm<br>1~99 mm   | 2.84E-04<br>3 80E-04   | 0.21         | 0.51         | 5.61         | 20<br>15      | . 31         | 0 0        | 0.49       |                  | 20<br>20 |           |                            |                         |                                |
|                                     |                                   |                        | 11111 77X1   | 0.00E-04               | 0T-0         | 0.00         | 10.0         | 61            | 0            |            | 787        |                  | 07       |           |                            |                         |                                |
|                                     |                                   |                        | 40x2  mm<br>32x(2x2)  mm (S)   | 1.26E-04<br>1 $28F-04$ | 2.00<br>2.00 |              | 4.58<br>4.16 | 36.4<br>32.5  |              | 0 0        | 0.14       |                  | 20<br>20 |           |                            |                         |                                |
| Deleaned anon of al (9011) 07       | đŎ                                | 1° 0 1                 | 16x(2x4) mm (R)  | 1.28E-04               | 1.50         | '            | 4.24         | 33.1          | ,            | 0          | 0.18       | '                | 20       |           | I                          |                         | I                              |
| Balasundaran et al. (2011) 24       | OF                                | E. C00                 | 25x2 mm  | 7.85E-05               | 2.00         | ,            | 2.68         | 34.1          | ı            | 0          | 0.17       | '                | 20       | ı         | I                          | ,                       | I                              |
|                                     |                                   |                        | 5x5 mm<br>1×14 mm  | 9.82E-05<br>1.54E-04   | 0.80         |              | 3.26<br>5.07 | 33.2<br>32.9  |              | 0 0        | 0.18       |                  | 20       |           |                            |                         |                                |
|                                     |                                   |                        | 1 0 1 0 1 0 1 0 1 0  |                        | 000          |              |              | 1 1           | 000          |            |            |                  | ì        |           | 1101 1                     |                         | 0 . Alaot                      |
|                                     |                                   | E. Coli<br>R. Sabbilie | $1 \times 0.10 + 1 \times 0.16$<br>$1 \times 0.10 \pm 1 \times 0.92$ |                        |              |              |              | 355.7         | 906<br>800   |            | 154<br>037 |                  |          |           | 1.4x10**                   |                         | 100% in 3 passes               |
| Azuma et al. (2007) 50              | OP                                | B. Halodurnas          | $1x0.10 \pm 1x0.23$<br>$1x0.10 \pm 1x0.23$                           |                        |              |              |              | 353.7         | 800          |            | 037        |                  |          |           |                            |                         | 100% in 6 passes               |
|                                     |                                   | P. Putida              | 1x0.10 + 1x0.23  | ı                      | '            | '            | ,            | 353.7         | 800          | -          | 037        | '                | '        | ı         |                            | I                       | 100% in 4 passes               |
|                                     |                                   | E. Coli                | 1x0.10 + 1x0.23  |                        | '            |              |              | 353.7         | 800          | - 0        | 037        | '                |          |           | ı                          |                         | 100% in 3 passes               |
|                                     | OP                                |                        | 25% open   | 7.85E-05               | 0.40         |              | 0.80         | 10            | 3.7          | 1          | 5.13       |                  | 50       |           |                            |                         | 79% in 1 pass                  |
|                                     | OP                                |                        | 50% open   | 1.56E-04               | 0.29         | ,            | 1.70         | 11            | 3.2          | ,          | 3.94       | '                | 50       | ï         | 1                          | I                       | 75% in 1 pass                  |
| Sawant et al. (2008) 45             | OP                                | Zoonlankton            | 75% open   | 2.35E-04               | 0.12         | ,            | 1.30         | 9             | 3.1          | -          | 1.68       | '                | 20       | ,         |                            | '                       | 82% in 1 pass                  |
|                                     | Valve                             |                        | 20% open   | 5.73E-06<br>9.73E-06   |              | •            | 1.00         | 16            | 3.4          |            | [.93       |                  | 20       | ı         |                            |                         | 57% in 1 pass                  |
|                                     | Pump                              |                        | 40 /0 орен<br>-  |                        |              |              | 2.80         | - 1           | 2.9<br>2.9   |            | - 10       |                  | 20       |           |                            |                         | 28% in 1 pass<br>28% in 1 pass |
|                                     | 4                                 |                        | 15   | 1 065 05               | 0.00         |              | =            | 12            |              |            |            |                  | 60       | 190       | 107                        |                         |                                |
|                                     |                                   |                        | fx2 mm   | 1.30E-05<br>1.88E-05   | 0.00<br>2.00 |              |              | 26<br>26      |              |            |            |                  | 20       | 120       | 107                        |                         |                                |
|                                     |                                   |                        | 25 xl  mm  | 1.96E-05               | 4            | ,            | 1.11         | 57            |              | 1          | $12^{*}$   | '                | 50       | 120       | 107                        | I                       | I                              |
| Arrojo et al. (2008) 12             | OP                                | E. Coli                | 25 xl  mm  | 1.96E-05               | 4            |              | 1.11         | 57            | ·            | 1.5 0      | $16^{*}$   | '                | 50       | 120       | 107                        | ·                       |                                |
|                                     |                                   |                        | 25x1 mm  | 1.96E-05               | 4 -          | •            | 1.11         | 57            |              | 0 0<br>0 0 | 19*        | '                | 202      | 120       | 102                        |                         | 100%                           |
|                                     |                                   |                        | 25x1 mm<br>25x1 mm   | 1.96E-05<br>1.96E-05   | 4            | • •          |              | 97<br>57      |              | 0 0<br>7 7 | . 19*      |                  | 20       | 120       | $10^{4}$                   |                         | %02<br>%02                     |
|                                     |                                   |                        |  | 0 10E 06               |              |              |              | ;             | - C          |            | ;          | *48.0            | 6        | 9         | 106                        | lot                     | 50.0<br>2012                   |
|                                     |                                   |                        | 8x1.14 mm  | 0.19E-00<br>8.17E-06   |              |              |              |               | 2.1<br>16.5  |            |            | 0.06*            | 7 8 1    | 120       | 100                        | 101                     | 5 log                          |
|                                     |                                   |                        | $72 \times 0.38 \text{ mm}$  | 8.17E-06               | '            |              | ,            | ,             | 5.2          |            | ,          | $0.19^{*}$       | 2        | 240       | 106                        | 10                      | 6 log                          |
|                                     | DynaSwirl <sup>®</sup>            | E. Coli                | 1x4.5 mm   | 1.59E-05               | '            | •            | ,            | ,             | 3.45         | 1          | ).33<br>   | $0.28^{*}$       | i.       | 270       | 107                        | 1                       | 4 log                          |
| Loraine et al. (2012) 47            |                                   |                        | 1x4.5 mm   | 1.59E-05<br>1 50F 05   |              |              |              |               | 1.2          |            | 0.5        | $0.47^{*}$       |          | 19.0      | 107                        |                         | / log<br>A log                 |
|                                     |                                   |                        | 1x3.2 mm   | 8.04E-06               |              |              |              |               | 2.1          |            | 0.5        | 0.47*            | 5        | 210 1     | $0^3 - 10^5 - 10^7 - 10^9$ | $10^{2}$                | 7 log                          |
|                                     | DynaSwirl <sup>®</sup>            | Klebsiella             | 1x3.23 mm  | 8.19E-06               | ,            |              |              | ,             | 2.1          |            |            | 0.47             | 5        | 09        | 107                        | $10^{2}$                | 5 log                          |
|                                     | StratoJet <sup>®</sup>            | P. Aeruginosa          | 8x1.14 mm  | 8.17E-06               | 1            |              |              | ,             | 16.5         |            |            | $0.06^{*}$       | 1.8      | 06        | $\sim 10^8$                | $\sim 10^5$             | 3 log                          |
|                                     | StratoJet <sup>®</sup>            | P. Syringae            | 8x1.14 mm  | 8.17E-06               |              | •            | •            |               | 16.5         |            |            | 0.06*            | 1.8      | 20        | $\sim 10'$                 | $\sim 10^{1}$           | 6 log                          |
|                                     |                                   |                        | 33x2 mm  | 1.04E-04               | 2.00         | 0.40         | ,            | ,             | 3.5          | ,          | ,          | $0.28^{*}$       | 25       | 09        | 103                        |                         | 57.30%                         |
|                                     |                                   |                        | 33x2 mm<br>33x2 mm   | 1.04E-04<br>1.04E-04   | 2.00         | 0.40<br>0.40 |              |               | 4.5          |            |            | $0.24^{\circ}$   | 25       | 8 8       | 103                        |                         | 00%<br>87.30%                  |
| Wang et al. (2015) <b>35</b>        | OP                                | ÷                      | 33 xl  mm  | 2.59E-05               | 4.00         | 0.02         | ,            | ,             | 4.5          |            |            | $0.22^{*}$       | 25       | 09        | 103                        | 1                       | 87%                            |
|                                     |                                   |                        | 33x2 mm  | 1.04E-04               | 2.00         | 0.40         | ,            | ,             | 4.5          | ,          | ,          | $0.22^{*}$       | 25       | 09        | 103                        |                         | 63%                            |
|                                     |                                   |                        | 20x2 mm<br>17x3 mm   | 6.28E-05<br>1.20E-04   | 2.00<br>1.33 | 0.05         |              |               | 4.5          |            |            | $0.22^{*}$       | 25<br>25 | <u>6</u>  | 103                        |                         | 58%<br>40%                     |
| D-1                                 | uQ                                | 4-10 B                 |  | 9 14E 06               | 5            | 2000         |              |               | •            |            | 000        |                  | -        | 8         | 107                        |                         | 14.07                          |
| Badve et al. (2015) 51              | 0P                                | E. Coli                | lx2 mm   | 3.14E-06               | 2.71         | •            | •            |               | ~            |            | 0.62       | '                | 4        | •         | , 10'                      | 1 0                     | 15%                            |
| Filho at al (2015) 52               | Norrho                            | E Coli                 | 1x2 mm<br>1~9 mm   | 3.14E-06<br>3.14E-06   | 2.71         | 900.0        | 0.48         | 87.8<br>100.8 | 80           |            |            |                  | 40       | 30        | 10 <sup>5</sup>            | $10^{3}$                | 98.30%<br>00 06%               |
| LIIIU GU GU. (2010)                 | argzout                           | - C0%                  | 1x2 mm   | 3.14E-06               | 2.71         | .006         | 0.48         | 131.8         | 120          |            |            |                  | 40       | 30<br>8   | 102                        | 101                     | 100%                           |
| Liu et al. (2016) 53                | OP                                | E. Coli                | 49x1 mm  | 3.85E-05               |              | .048         | 0.56         |               | 2.5          | 0 0        | 92*        | 0.40*            | 20       | 120       | $1.60 \times 10^{3}$       |                         | %66                            |



Figure 2: Experimental set-up: upper image shows the schematic representation of the experimental set-up, lower left image shows the orifice plate and pressure measurements points, lower right image shows the transparent test section illuminated by red laser light during disinfection experiments.

| Authors (year)                  | $C_0 d^3$    | $rac{ ho v_h d}{\mu}$ | $\frac{\rho v_h^2 d}{\gamma_s}$ | $\frac{D}{d}$ | $\frac{b}{d}$ | $\frac{P_2-P_v}{\rho v_h^2}$ | n            | $n_p$        |
|---------------------------------|--------------|------------------------|---------------------------------|---------------|---------------|------------------------------|--------------|--------------|
| Jyoti et al. (2001) 48          | ×            | ×                      | ×                               | ×             | ×             | ×                            | ×            | ×            |
| Kalumuck et al. $(2003)$ (a) 46 | $\times$     | ×                      | ×                               | $\checkmark$  | $\checkmark$  | ×                            | $\times$     | $\checkmark$ |
| Kalumuck et al. $(2003)$ (b) 46 | $\times$     | $\checkmark$           | $\checkmark$                    | $\checkmark$  | $\checkmark$  | $\checkmark$                 | $\checkmark$ | $\checkmark$ |
| Balasundaran et al. $(2006)$ 49 | ×            | ×                      | ×                               | $\times$      | ×             | ×                            | $\times$     | ×            |
| Balasundaran et al. $(2011)$ 27 | $\times$     | ×                      | ×                               | $\times$      | ×             | ×                            | $\times$     | $\times$     |
| Azuma et al. (2007) 50          | $\times$     | ×                      | ×                               | $\times$      | ×             | ×                            | $\times$     | $\times$     |
| Sawant et al. (2008) 45         | $\times$     | ×                      | ×                               | $\times$      | ×             | ×                            | $\times$     | $\times$     |
| Arrojo et al. (2008) (c) 12     | $\times$     | ×                      | ×                               | $\times$      | ×             | ×                            | $\times$     | $\checkmark$ |
| Arrojo et al. (2008) (d) 12     | $\times$     | $\checkmark$           | $\checkmark$                    | $\checkmark$  | $\checkmark$  | $\checkmark$                 | $\checkmark$ | $\checkmark$ |
| Loraine et al. $(2012)$ (e) 47  | $\times$     | ×                      | ×                               | $\checkmark$  | $\checkmark$  | ×                            | $\times$     | $\checkmark$ |
| Loraine et al. $(2012)$ (f) 47  | $\times$     | $\checkmark$           | $\checkmark$                    | $\checkmark$  | $\checkmark$  | $\checkmark$                 | $\checkmark$ | $\checkmark$ |
| Wang et al. (2015) (g) 35       | $\times$     | ×                      | ×                               | $\checkmark$  | $\checkmark$  | ×                            | $\times$     | $\times$     |
| Wang et al. (2015) (h) 35       | $\times$     | ×                      | ×                               | $\times$      | ×             | ×                            | $\times$     | $\checkmark$ |
| Badve et al. (2015) 51          | $\checkmark$ | $\checkmark$           | $\checkmark$                    | $\checkmark$  | $\checkmark$  | $\checkmark$                 | $\checkmark$ | $\checkmark$ |
| Filho et al (2015) <b>52</b>    | $\times$     | ×                      | ×                               | $\checkmark$  | $\checkmark$  | ×                            | $\times$     | $\times$     |
| Liu et al. (2016) <b>53</b>     | $\checkmark$ | $\checkmark$           | $\checkmark$                    | $\checkmark$  | $\checkmark$  | $\checkmark$                 | $\checkmark$ | $\checkmark$ |
| Our results                     | $\checkmark$ | $\checkmark$           | $\checkmark$                    | $\checkmark$  | $\checkmark$  | ×                            | $\checkmark$ | $\checkmark$ |
| (numerical value)               | $\checkmark$ | 154900                 | 65900                           | 12.8          | 6.4           | ×                            | 4            | 410          |

Table 2: Dimensional analysis of the works presented in Table 1  $\checkmark$ : parameters kept constant in all the tests.  $\times$ : parameters varied between the tests.

Milli-Q<sup>®</sup> water while filling the tank at the inlet of the circuit to reach the desired concentration. The starting bacteria concentration of each experiment was confirmed by Colilert Quanti-Tray 2000 assay (IDEXX). *E. Coli* concentration at the different time points was determined by Colilert Quanti-Tray 2000 assay (IDEXX) according to standard procedures **54**.

Three groups of experiments were performed to analyze the effect of different cavitation numbers  $\sigma_v$  on the disinfection efficiency. As expressed in Equation (4), assuming constant temperature conditions (and hence constant values of fluid properties such as  $P_v$ ,  $\gamma_s$ ,  $\rho$  and  $\mu$ ), the variables involved in the computation of  $\sigma_v$  are the recovery pressure  $P_2$  and the orifice fluid velocity  $v_h$ . The former was directly measured, whereas the latter was estimated simply

| $\sigma_v$ [-] | Configuration | Holes area $[m^2]$ | Q $[l/s]$ | $v_h$ [m/s] | $P_1$ [bar] | $P_2$ [bar] | V<br>[1] | t[min]   |
|----------------|---------------|--------------------|-----------|-------------|-------------|-------------|----------|----------|
| 0.20           | 4x2.5  mm     | 1.96E-05           | 0.6       | 30.5        | 7.5         | 0           | 21       | 30 - 360 |
| 0.40           | 4x2.5  mm     | 1.96E-05           | 0.6       | 30.5        | 7.5         | 1           | 21       | 30 - 120 |
| 0.65           | 4x2.5 mm      | 1.96E-05           | 0.6       | 30.5        | 7.5         | 2           | 21       | 30 - 240 |

Table 3: Hydraulic and geometric characteristics of the orifice plate reactor.

as the ratio between the flow rate and the holes area (see also the discussion section for more details on the definition of  $v_h$  and its shortcomings).

The downstream recovery pressure (or back-pressure)  $P_2$  was varied by means of the ball-valve (see Figure 2) in order to vary  $\sigma_v$ . As shown in Table 3, the other parameters (orifice velocity and flow rate) were kept constant and so were all the non-dimensional parameters identified in Equation (3).

In the first group of experiments the configuration characterized by  $\sigma_v = 0.20$ was studied. Seven tests with initial concentration  $C_0$  between  $10^2 \ CFU/100 \ ml$ and  $10^5 \ CFU/100 \ ml$  were carried out. The duration of the experiments varied between 120 and 360 minutes, which correspond to a number of passages  $n_p \sim$ 205 and 620, respectively. Samples were taken every 30 minutes.

The second group of experiments was performed at  $\sigma_v = 0.40$ . Six experiments with initial concentration between  $10^2 \ CFU/100 \ ml$  and  $10^4 \ CFU/100 \ ml$ were performed. The total duration of the tests was 120 minutes ( $n_p \sim 205$ ) and samples were taken every 30 minutes.

In the last group of experiments, the configuration with  $\sigma_v = 0.65$  was studied. Three tests of 240 minutes  $(n_p \sim 410)$  with initial concentrations between  $10^3 \ CFU/100$  ml and  $10^6 \ CFU/100$  ml were performed. Samples were taken at 60, 120, 180 and 240 minutes.

Two control experiments were performed by removing the orifice plate to investigate the effects of the pump on disinfection efficiencies. In those scenarios the flow rate was higher due to the absence of the orifice plate. The initial concentration was  $10^2 \ CFU/100$  ml and the tests lasted for 120 minutes, corresponding to ~ 360 passes (the number of passes in this case is higher due to the higher flow rate). Samples were taken every 30 minutes. The bacterial concentration remained constant for the entire duration of the experiment.

During all the orifice-plate experiments, and the control experiments without the orifice-plate reactor, the water-temperature was controlled by means of two chiller units. It is finally pointed out that for all hydrodynamic configurations, the ball-valve was always working in a non cavitating regime and, therefore, it never played any role in the game of disinfection.

## 310 5. Results

Figure 3 shows  $C/C_0$  vs  $n_p$  curves for each individual trial. In order to avoid 311 overcrowding of the figure, the 95% confidence intervals (as estimated from the 312 Quanti-Tray/2000 method 54) associated with each experimental data-point, 313 are reported in Table 5 in Appendix B. Figure 3 indicates that the orifice plate 314 employed in the experiments caused a reduction in bacterial concentration in 315 all the experimental configurations investigated. Confidence intervals associ-316 ated with each measurement (see Table 5) are quite large and make it difficult 317 to identify statistically-significant trends. However, it seems that, contrary to 318 what reported in the previous literature 12, 46, the initial concentration value 319  $C_0$  of bacteria (or its dimensionless counterpart  $C_0 d^3$ ) have no clear effect on 320 the non-dimensional disinfection efficiencies at all the cavitation numbers in-321 vestigated. Moreover, contrary to what reported in the literature [12] [47], the 322

 $C/C_0$  vs  $n_p$  curves do not show any obvious initial plateau (or quasi-stationary phase), which is commonly interpreted as a colony fragmentation, rather than an effective disinfection phase. However, it should be noted that the concentrations of bacteria used herein (much lower than those used by [46] [47]) are unlikely to generate colonies and therefore this could be the reason underpinning the observed results.

Since no clear effects of the initial concentration were observed, average  $C/C_0$ 320 vs  $n_p$  curves were computed from each group of experiments corresponding to 330 each cavitation number (i.e., each curve is the average of the curves shown 331 in panels 3a - 3c) and are reported in Figure 4a. In this Figure the shaded 332 error bars represent the standard deviations of concentration obtained from each 333 experiment group. As previously predicted, Figure 4a shows that the average 334  $C/C_0$  vs  $n_p$  curves drop faster for lower values of the cavitation numbers  $\sigma_v$ . 335 This is in agreement with the idea that a more intense cavitation (i.e., a lower 336  $\sigma_v$ ) promotes a more efficient disinfection. 337

The series of mean disinfection values were then fitted by the exponential law  $C/C_0 = \exp(-r \cdot n_p)$  as shown in Figure 4b, in order to obtain the bacterial reduction rate, r, typical of each cavitation number. Aiming to a fair comparison, the same number of sampling values were considered for all cavitation numbers. The rates obtained are reported in Table 4 and confirm that at lower cavitation numbers correspond higher disinfection rates. The R-square values shown in Table 4 witnesses goodness of data fitting.

Table 4: Bacterial reduction rates r and coefficients of determination  $R^2$  corresponding to the exponential fitting of the average disinfection curves shown in Figure 4b

| Cavitation number | $r(\cdot 10^3)$ | $\mathbb{R}^2$ |
|-------------------|-----------------|----------------|
| $\sigma_v = 0.2$  | 10.5            | 0.980          |
| $\sigma_v = 0.4$  | 9.56            | 0.993          |
| $\sigma_v = 0.6$  | 7.10            | 0.997          |

#### 345 6. Discussion

It is now important to point out that dimensional analysis represents a valid 346 starting point for the design of experiments and for the development of empirical 347 formulae, but it is certainly not free from drawbacks, which are now discussed 348 to clarify the significance of the results presented herein and frame the scope of 349 future research-works. A key problem of dimensional analysis is associated with 350 the fact that it is not always straightforward to rigorously take into consideration 351 all the factors influencing a problem, often because it is difficult to associate such 352 factors with well-defined and measurable variables. For example, in the case of 353 orifice-plates, the onset of cavitation (i.e., the critical number of  $\sigma_v$  below which 354 cavitation occurs), can be very sensitive to fine experimental-conditions. This 355 means that if no-control on these details is possible, the cavitation number may 356



Figure 3: Disinfection efficiency of the orifice-plate reactor at different cavitation numbers. Each color represents a different order of magnitude of E. Coli initial concentration  $(C_0)$ .



Figure 4: Average behavior of the disinfection curves at different cavitation number. In the panel (a), the shaded regions correspond to the standard deviation. In the panel (b), the exponential fitting are shown.

not represent an objective parameter to quantify consistently the intensity of 357 cavitation among different experiments. In particular, the onset of cavitation 358 may depend on fine geometrical details of the orifice (e.g. small manufacturing 359 defects such as irregular edges of the inlet or artificial roughness due to milling), 360 upstream flow conditions (i.e. velocity statistics, turbulence length-scales and 361 the flow-structure in general) and the chemical properties of water (including the 362 concentration of nuclei) 43. These are all factors that are difficult to identify 363 with a parameter (or a set of parameters), yet, they can have a measurable effect 364 on disinfection efficiency. In order to circumvent this issue, the experiments 365 presented herein were carried out using always the same hydraulic circuit (which 366 presumably maintained similar flow conditions upstream of the HC reactor), the 367 same orifice-plate (i.e., no changes in the slightest details of the orifice-geometry) 368 and ultra-pure water (which, from the point of view of water-chemistry, should 369 guarantee similar initial conditions). However, it is not always straightforward. 370 especially in applications, to have such controlled conditions, therefore caution 371 should be used when either comparing results from experiments carried out in 372 different facilities or when extending laboratory results to field applications. 373

Another key issue is that it is not easy to perfectly isolate the effect of indi-374 vidual non-dimensional parameters, often because technical limitations prevent 375 to control or monitor the actual value of some dimensional parameters. For ex-376 ample, the experiments presented herein were designed to isolate the effects of 377 the cavitation number  $\sigma_v$  as, for each series of trials, the other non-dimensional 378 parameters listed in Equation (3) were assumed to be constant. A key hypoth-379 esis underpinning this argument is that  $v_h$ , could be estimated from continuity 380 principles, as the ratio between the flow rate and the holes area. This is rep-381 resentative of the velocity at the downstream end of the holes in the case of 382 non-cavitating flows. When cavitation occurs, it is well known that, due to the 383 pressure drop caused by flow separation at the orifice inlet, a cloud of water-384 vapor forms, meaning that the flow exiting from the orifice is multiphase with 385 an average density and velocity, which are very difficult to measure/control and 386 are clearly dependent on the cavitation number 55, 56. Therefore, strictly 387 speaking, besides  $\sigma_v$ , the non-dimensional parameters containing  $v_h$  (i.e. the 388 Reynolds and the Weber number) probably varied a little among different tests 389 pertaining to the same group (i.e. the same value of  $\sigma_v$ ). Whether such vari-390 ations can have significant effects on the disinfection efficacy remains an open 301 question. One of the difficulties in providing an answer to this question and, 392 more generally, in the use of empirical approaches, is that dimensional analysis 393 is only a tool to find links between dimensional variables but hardly gives any 394 hint to understand the processes controlling the problem of interest, which is a 395 key prerequisite for the interpretation of experimental data. Moreover, this lack 396 of understanding makes it difficult to quantify the effects of non-dimensional pa-397 rameters other than through blind data-fitting, whose validity is often limited 398 to the dataset it is applied to. 399

Within this context, the authors claim that, one of the tightest bottlenecks for the development of efficient HC reactors is the complete lack of understanding of what, from a purely mechanical point of view, kills bacteria. This is

because, in HC reactors, besides imploding bubbles, many other processes are 403 triggered, which could be harmful to microorganisms. For example, Dular and 40 co-workers 57 29, argue (and provide good evidence) that fast and abrupt 405 pressure differences are much more effective than imploding bubbles in killing 406 pathogens in water. Moreover, there is quite a substantial literature demonstrat-407 ing that turbulence can induce fluid stresses that can be lethal to microorganisms 408 44. Until it will not be possible to quantify the sensitivity of microorganisms 409 to fluid shear and normal stresses (and to the non-dimensional parameters that 410 411 control the magnitude of such stresses), it will be extremely difficult to design and optimize HC reactors or other mechanically-based means of water disinfec-412 tion. 413

414

#### 415 **7.** Conclusions

The interest in the use of HC as a water-disinfection technique has grown 416 fast in the recent years, both from an academic and an industrial point of 417 view. The studies available from the literature have proved that HC is a very 418 promising and flexible technique which can be used alone or in series with other 419 methods (e.g., chlorination). However, robust and reliable design tools that 420 allow to go from the laboratory to full scale applications are, to the best of 421 the authors' knowledge, not available yet. This is clearly caused by the fact 422 that cavitating flows are poorly understood, and hence difficult to model, as 423 they involve turbulent multiphase flows occurring in complex geometries, which 424 leave little hope to theoretical or computational modeling approaches. 425

As a result of this complexity, the vast majority of the literature approaches 426 the problem from an empirical point of view. Empirically-derived design-relations 427 can be very effective but must be determined from a large number of experi-428 ments, which must be designed and carried out on the basis of a rigorous dimen-429 sional analysis. While dimensional analysis is customarily adopted to tackle an 430 enormous amount of engineering problems within the remit of Fluid Mechanics, 431 it has surprisingly never been adopted within the field of HC and this represents 432 a major shortcoming the present paper attempts to address. In particular, by 433 application of dimensional analysis and the Buckingham- $\pi$  theorem, we have 434 derived Equation (3), which provides a set of non-dimensional parameters gov-435 erning the simple problem of disinfection via HC triggered by circular orifice plates. 437

On the basis of this set of parameters, a number of experiments were de-438 signed and carried out to investigate the effects of the cavitation number and 439 the dimensionless initial concentration on disinfection efficiencies. Results from 440 these experiments indicate that  $C/C_0$  vs  $n_p$  curves are not influenced by the 441 initial concentration whereas, although heavily masked by experimental uncer-442 tainty, the effects of  $\sigma_v$  seem to be present. This points towards confirming the 443 significant role played by the formation and implosion of bubbles in the game 444 of disinfection and provides a first step towards the development of effective 445 empirical formulae for the design of HC reactors. 446

However, as discussed in the previous section, the development of effective empirical formulae cannot be left to an arid coupling between experiments and dimensional analysis but must be supported by a sound understanding of the physical processes controlling disinfection in HC reactors. In particular, the authors recommend that future research efforts should be directed towards fundamental studies aiming at understanding the effects of fluid stresses on microorganisms.

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#### 634 8. Appendix A

Jyoti and Pandit 48 explored the microbicidal effectiveness of various cav-635 itating reactors for naturally-contaminated bore well water. They made a com-636 parative analysis of different disinfection techniques, including ultrasonication 637 (AC), high-speed homogenisation (HC), high-pressure homogenisation (HC) and 638 a cavitating valve (HC). In ultrasonication and high-speed/pressure homogeni-639 sation they treated a small water volume  $(1 \ l)$ . For the case of the cavitating 640 valve, they treated 75 l of bore well water at three different pump discharge pres-641 642 sures  $(P_1)$  of 1.72, 3.44 and 5.17 bar, obtaining an increase in the disinfection efficiency when the pump discharge pressure increased. They observed that HC 643 was, energetically, the most efficient technique, resulting in maximum bacteria 644 concentration drops of 44% at  $P_1 = 5.17$  bar. The authors provided confidence 645 intervals of the results estimated via repeated trials but failed to provide details 646 about the geometry of the valve and the cavitation numbers reached during the 647 experiments.

Kalumuck et al. 46 used the DynaJets® cavitating device to investigate the 649 effects of cavitation on a small volume of 1.5 liters of high concentrated solution 650 of E.Coli  $(5 \times 10^8 - 2 \times 10^9 \text{ CFU/ml})$ . Four experiments were conducted in 651 a pressure ranges of  $P_1$  between 4.13 and 5.17 bar and a single experiment at 652 10.3 bar, but no information on the associated cavitation number were provided. 653 In the run performed at 10.3 bar, they achieved up to  $5 \log_{10}$  reduction in the 654 concentration of E. Coli in 30 minutes, while the experiments executed in the 655 pressure range between 4.13 and 5.17 bar shown a  $3 \log_{10}$  reduction in the first 656 20 - 40 minutes. Three more experiments were performed at moderate initial 657 concentration of E. Coli ( $10^7$  CFU/ml). In this case, they obtained a  $3 \log_{10}$  and 658  $5 \log_{10}$  reduction in bacteria concentration at 20 and 30 minutes, respectively. 659 They also reported a bacterial reduction of  $0.6 \log_{10}$  attributed exclusively to 660 the pump. No data are provided about the reactors' geometry. 661

Balasundaram and Harrison. 49 investigated the E. Coli cell damage due 662 to hydrodinamic cavitation, by analysing the periplasmic and cytoplasmic pro-663 teins released from the cell wall destruction. A wide range of cavitation numbers 664  $\sigma_v$  between 0.13 and 0.92 was investigated and the maximum extent of proteins 665 release was found at  $\sigma_v = 0.17$ . They also investigated the influence of cell 666 growth rate, finding a lower resistance to cavitation of cells grown at a higher 667 growth rate. In a later work 27 they presented the influence of the geometry 668 and the number of orifices on selective release of periplasmic proteins. Config-669 urations with circular, squared and rectangular orifices were studied. For the 670 same holes-area, the release of total soluble proteins was similar, however the 671 plate with circular holes allowed for a greater release of acid phosphatase. They 672 also studied the influence of the flow rate on the release of acid phosphatase 673 after 1000 passes, finding higher percentage of release for higher flow rates. The 674 best configuration was the one with the higher number of circular holes, were 675 the flow rate was maximum. Unfortunately, in this study no information about 676 initial concentration and bacterial survival rate was provided. 677

Azuma et al. 50 proposed a high pressure cavitating device with two cav-

itating orifices in series and a plunger pump capable of discharging pressures 679 up to 1050 bar. The cavitation numbers  $(\sigma_v)$  used in the study varied between 680 0.037 and 0.487, while the upstream nozzle velocity varied between 176 m/s 681 and 384 m/s. No information about the downstream nozzle velocity and cav-682 itation number were provided. In the second phase of the experiments they 683 compared sterilization rate among Gram-positive (Bacillus Subtilis, Bacillus 68/ Halodurans) and Gram-negative (Escherichia Coli, Pseudomonas Putida) bac-685 teria. The disinfection mechanisms suggested in this work are the high shear stresses reached in the orifice and the shock waves generated by bubbles' col-687 lapses. They achieved a complete disinfection of a mixture of water and E. 688 Coli in three successive treatments at  $\sigma_n = 0.154$ . The experiments compar-689 ing Gram-positive and Gram-negative bacteria resistance to cavitation showed 690 that Gram-positive bacteria are stronger than Gram-negative bacteria under 691 the two conditions studied, namely  $\sigma_v = 0.104$  and  $\sigma_v = 0.037$ . This behavior 602 was ascribed to the more resistant cell-wall of Gram-positive bacteria. 693

Sawant et al. 45 studied the effect of a single orifice plate on the disinfection 694 of the zooplankton in sea water. In all the experiments just once pass through 695 the cavitation device was made. The test loop was composed of a centrifugal 696 pump, a valve and a single orifice-plate positioned in sequence. During the 697 experiments, they isolated the effects of the cavitating valve, the orifice plate 608 and the pump, individually. The maximum percentage of disinfection due to the 699 pump and the valve was 57% while almost 28% of the zooplankton was killed by 700 the pump alone. The maximum percentage of killing achieved with the orifice 701 plate (and the valve fully open) was 82%, related to a cavitation number ( $\sigma_v$ ) 702 equal to 14.68. Similar values of disinfection efficiencies were obtained in spite 703 of wide differences in cavitation numbers tested. This behavior was explained 704 as an effect of the weak cell wall of zooplankton. 705

Arrojo et al. 12 compared the disinfection efficiency of different orifice 706 plates and Venturi tubes, varying the numbers of holes, the discharge pres-707 sure and the initial concentration of E. Coli. For an initial concentration of 708  $10^7$  CFU/ml, they found a higher disinfection efficiency for the configuration 709 with the highest number of holes with the smallest diameter. The experiments 710 performed with orifice plates showed a first stage where the CFU number in-711 creased. This lag-phase lasted for about 30 minutes and the authors explain this 712 behavior as an effect of bacteria-agglomerates fragmentation. from the compar-713 ison between the orifice plate and the Venturi-tube they found that, in order 714 to develop the same number of cavitating events, orifices plates need a higher 715 discharge pressure  $(P_1)$  than Venturi tubes. They also point out that cavitation 716 achieved with orifice plates is resulting in more violent cavity collapses due to 717 the sudden pressure recovery. Acting on in initial concentration in the interval 718  $10^3$  -  $10^5$  CFU/ml, they found that, for orifice plates, the higher is the ini-719 tial concentrantion the lower is the disinfection efficiency while Venturi-tubes 720 showed no correlation between disinfection efficiency  $(C/C_0)$  and initial E. Coli 721 concentration. In this study the cavitation number for the various trials is not 722 specified. 723

Loraine et al. 47 compared different types of cavitating devices, including

the so-called DynaJets<sup>®</sup>, orifice plates, the so-called StratoJet<sup>®</sup> and a single 725 orifice DynaSwirl<sup>®</sup>, all with the same total holes' area. The first group of dis-726 infection experiments aimed at comparing the disinfection efficiency associated 727 with different types of gram-negative bacteria. The first test was performed 728 with a single orifice DynaSwirl<sup>®</sup> cavitating jet operating at 2.1 bar. The initial 729 concentration was  $10^7$  CFU/ml with a test batch volume of 2 litres. Both Kleb-730 siella Pneumoniae and E. Coli underwent a  $5 \log_{10}$  reduction in 60 minutes, 731 corresponding to a 99.99% removal. A similar experiment with an 8-orifice 732 StratoJet<sup>®</sup> operating at 16.5 bar and a batch volume of 1.8 l was used to com-733 pare disinfection efficiency for E. Coli, Pseudomonas Syringae and Pseudomonas 734 Aeruginosa. This test showed approximately half efficiency in E. Coli disinfec-735 tion  $(5 \log_{10} \text{ reduction in } 120 \text{ minuts})$ . Nearly  $3 \log_{10} \text{ decrease in } P.$  Aeruginosa 736 concentration was observed in 90 min, while P. Syringae concentrations showed 737 a  $6 \log_{10}$  reduction in 20 min. These differences in disinfection efficiencies were 738 ascribed to the degree of cross-linking in the peptidoglycan layer of the cell walls. 739 However, when the results are presented as a function of the number of passes 740 through each reactor, the differences in removal efficiency of E. Coli between 741 the single orifice DynaSwirl<sup>®</sup> and the 8-orifice StratoJet<sup>®</sup> were relatively small. 742 These authors investigated the DynaSwirl<sup>®</sup> at operating pressure drops 743  $(P_1 - P_2)$  of 3.45, 2.1 and 1 bar, corresponding to cavitation numbers  $(\sigma_v)$ 744 of 0.33, 0.5 and 1, respectively. The best disinfection efficiency was found for 745  $P_1 - P_2 = 2.1$ . At this pressure drop the authors investigated disinfection 746 efficiencies for E. Coli (gram negative) and B. Subtilis (gram positive). B. 747 Subtilis concentrations were reduced by  $4.5 \log_{10}$ , while E. Coli concentrations 748 were reduced by more than  $7 \log_{10}$ . This experiment confirms that the thick 749 cell wall of gram-positive bacteria is more resistant to cavitation then the thin 750 cell wall of gram-negative species. A sensitivity analysis was carried out by 751 varying the initial E. Coli concentration between  $10^3$  and  $10^9$  CFU/ml. Gen-752 eral trends showed a slow initial reduction in the concentration followed by a 753 higher reduction rate until the concentration fell below 100 CFU/ml. The initial 754 lag period, where the bacterial concentration remained approximately constant, 755 lasted longer for higher concentrations, while during the rapid reduction phase 756 the disinfection efficiencies were comparable for all cases. Standard deviation of 757 the bacteria concentrations were calculated from the duplicates of the CFU/ml 758 measurements, but no information about the number of trials were provided. 759

Wang et al. 35 evaluated the effectiveness of hydrodynamic cavitation on 760 bore well water disinfection. They compared the effect of HC alone with a hybrid 761 system whereby HC was combined with the use of sodium hypochlorite and 762 chlorine dioxide. All the hybrid experiments showed an increase in disinfection 763 efficiency. This study also investigates the effects of the reactor geometry (i.e. 764 by varying the number and diameter of holes) and of the inlet pressure  $(P_1)$ , 765 but no information on the investigated cavitation numbers were provided. All 766 the experiments were carried out using relatively low concentration of E. Coli 767 (2500 - 3000 CFU/ml). It was observed that the higher the inlet pressure (i.e. 768  $P_1$ ) the higher the disinfection efficiency. Furthermore it was observed that 769 for a given constriction area, more holes of smaller diameter lead to improved 770

disinfection efficiencies. In this study, confidence intervals on the measuredconcentration are not provided.

<sup>773</sup>Badve et al. <sup>51</sup> investigated HC within the context of microbial disinfection <sup>774</sup>of ships ballast water. The initial concentration of microbes for all the exper-<sup>775</sup>iments was around 10<sup>7</sup> CFU/ml. They compared orifice plates and Venturi <sup>776</sup>tubes limiting the number of passes through the devices to 50. Results show <sup>777</sup>that Venturi tubes work better than single orifice plates. No precise information <sup>778</sup>about the cavitation numbers of the various configurations were provided.

Filho et al. 52 used a high pressure cavitating jet apparatus to inactivate 779 E. Coli in artificially - and natural - contaminated water. For the former, they 780 achieved a disinfection efficiency up to 90% in 15 minutes at 100 bar. After 781 30 minutes, the inactivation rate reached 98.30, 99.96 and 100% at pressure of, 782 80, 100 and 120 bar, respectively. No information about the cavitation number 783 characterizing the system was found. For naturally-contaminated water (i.e., for 784 concentrations of E. Coli around 10 - 100 CFU/ml) the disinfection efficiency 785 was independent of the jet pressure. After 30 minutes, inactivation rates of 98.89 786 and 97.31% were reached for discharge pressures of 100 and 50 bar, respectively. 787 Also in this work, confidence intervals on the measured concentration are not 788 provided. 789

Liu et al. 53] used a multi-orifice plate made of 49 holes of 1 mm diameter for the disinfection of *E.Coli*. A single reactor geometry was studied with an initial concentration of bacteria equal to  $1.6 \times 10^5 CFU/100$  ml. This device reached a disinfection efficiency of 98% in 60 minutes. The authors did not provide information regarding the cavitation number characterizing the system studied as well as they did not indicated the number of trials and the confidence intervals on the measured concentration.

#### 797 9. Appendix B

Table 5: Most Probable Number (MPN) of the CFU values in the single disinfection experiment (run) plotted in Figure 3 with upper and lower limit of the 95% confidence interval 54.

| $\sigma_v$ | run | t (min) | $n_p$ | MPN/100 ml | Lower limit | Upper limit |
|------------|-----|---------|-------|------------|-------------|-------------|
| 0.2        | 1   | 0       | 0     | 579400     | 379100      | 847200      |
| 0.2        | 1   | 30      | 51    | 101900     | 72700       | 140400      |
| 0.2        | 1   | 60      | 103   | 88200      | 62900       | 120200      |
| 0.2        | 1   | 90      | 154   | 49500      | 34400       | 69300       |
| 0.2        | 1   | 120     | 206   | 26200      | 16600       | 39700       |
| 0.2        | 1   | 150     | 257   | 18900      | 11300       | 30400       |
| 0.2        | 1   | 180     | 309   | 21300      | 12700       | 32600       |
| 0.2        | 1   | 210     | 360   | 14600      | 8200        | 24600       |
| 0.2        | 1   | 240     | 411   | 18500      | 11000       | 29200       |
| 0.2        | 1   | 270     | 463   | 12200      | 6800        | 21400       |
| 0.2        | 1   | 300     | 514   | 6300       | 2900        | 13700       |

| $\sigma_v$ | run      | t (min) | $n_p$ | MPN/100 ml | Lower limit | Upper limit |
|------------|----------|---------|-------|------------|-------------|-------------|
| 0.2        | 1        | 330     | 566   | 3100       | 700         | 8900        |
| 0.2        | 1        | 360     | 617   | 5200       | 1800        | 10800       |
| 0.2        | 2        | 0       | 0     | 365400     | 231900      | 555500      |
| 0.2        | 2        | 30      | 51    | 209800     | 145500      | 301100      |
| 0.2        | 2        | 60      | 103   | 77600      | 55300       | 104500      |
| 0.2        | 2        | 90      | 154   | 69700      | 49700       | 95300       |
| 0.2        | 2        | 120     | 206   | 58300      | 40500       | 80600       |
| 0.2        | 2        | 150     | 257   | 25600      | 15700       | 38400       |
| 0.2        | 2        | 180     | 309   | 34500      | 23300       | 50100       |
| 0.2        | 2        | 210     | 360   | 26900      | 17100       | 39800       |
| 0.2        | 2        | 240     | 411   | 16100      | 12400       | 32300       |
| 0.2        | 2        | 270     | 463   | 14800      | 8500        | 25100       |
| 0.2        | 2        | 300     | 514   | 5100       | 1700        | 10600       |
| 0.2        | 2        | 330     | 566   | 6300       | 2900        | 13700       |
| 0.2        | 2        | 360     | 617   | 3000       | 700         | 7400        |
| 0.2        | 3        | 0       | 0     | 32550      | 20660       | 49810       |
| 0.2        | <b>3</b> | 30      | 51    | 18720      | 12610       | 28100       |
| 0.2        | <b>3</b> | 60      | 103   | 14210      | 10130       | 19680       |
| 0.2        | <b>3</b> | 90      | 154   | 8570       | 6110        | 11720       |
| 0.2        | <b>3</b> | 120     | 206   | 8130       | 5790        | 11140       |
| 0.2        | <b>3</b> | 150     | 257   | 4320       | 2910        | 6140        |
| 0.2        | <b>3</b> | 180     | 309   | 3180       | 2080        | 4640        |
| 0.2        | <b>3</b> | 210     | 360   | 2180       | 1340        | 3390        |
| 0.2        | <b>3</b> | 240     | 411   | 630        | 290         | 1370        |
| 0.2        | <b>3</b> | 270     | 463   | 200        | 30          | 710         |
| 0.2        | 3        | 300     | 514   | 100        | 10          | 550         |
| 0.2        | 4        | 0       | 0     | 4884       | 3100        | 7215        |
| 0.2        | 4        | 30      | 51    | 2481       | 1623        | 3719        |
| 0.2        | 4        | 60      | 103   | 2143       | 1402        | 3209        |
| 0.2        | 4        | 90      | 154   | 1658       | 1149        | 2380        |
| 0.2        | 4        | 120     | 206   | 767        | 546         | 1062        |
| 0.2        | 5        | 0       | 0     | 3076       | 1953        | 4712        |
| 0.2        | 5        | 30      | 51    | 2098       | 1455        | 3011        |
| 0.2        | 5        | 60      | 103   | 1081       | 770         | 1472        |
| 0.2        | 5        | 90      | 154   | 657        | 468         | 892         |
| 0.2        | 5        | 120     | 206   | 537        | 383         | 740         |
| 0.2        | 6        | 0       | 0     | 1664       | 1154        | 2340        |
| 0.2        | 6        | 30      | 51    | 404        | 273         | 574         |

Table 5: Most Probable Number (MPN) of the CFU values in the single disinfection experiment (run) plotted in Figure 3 with upper and lower limit of the 95% confidence interval 54.

| $\sigma_v$ | run | t (min) | $n_p$ | MPN/100 ml | Lower limit | Upper limit |
|------------|-----|---------|-------|------------|-------------|-------------|
| 0.2        | 6   | 60      | 103   | 218        | 134         | 339         |
| 0.2        | 6   | 90      | 154   | 109        | 56          | 195         |
| 0.2        | 6   | 120     | 206   | 52         | 23          | 119         |
| 0.2        | 7   | 0       | 0     | 727        | 476         | 1049        |
| 0.2        | 7   | 30      | 51    | 501.2      | 357         | 688         |
| 0.2        | 7   | 60      | 103   | 261.3      | 171         | 399         |
| 0.2        | 7   | 90      | 154   | 172        | 116         | 261         |
| 0.2        | 7   | 120     | 206   | 73.8       | 53          | 100         |
| 0.4        | 8   | 0       | 0     | 17220      | 11940       | 24500       |
| 0.4        | 8   | 30      | 51    | 13540      | 9650        | 18400       |
| 0.4        | 8   | 60      | 103   | 10860      | 7740        | 15000       |
| 0.4        | 8   | 90      | 154   | 9060       | 6460        | 12410       |
| 0.4        | 8   | 120     | 206   | 8160       | 5820        | 11030       |
| 0.4        | 9   | 0       | 0     | 5810       | 4140        | 7950        |
| 0.4        | 9   | 30      | 51    | 4410       | 3060        | 6250        |
| 0.4        | 9   | 60      | 103   | 3170       | 2070        | 4660        |
| 0.4        | 9   | 90      | 154   | 1610       | 930         | 2680        |
| 0.4        | 9   | 120     | 206   | 1460       | 820         | 2460        |
| 0.4        | 10  | 0       | 0     | 2142       | 1527        | 2944        |
| 0.4        | 10  | 30      | 51    | 987        | 723         | 1337        |
| 0.4        | 10  | 60      | 103   | 441        | 306         | 625         |
| 0.4        | 10  | 90      | 154   | 189        | 113         | 304         |
| 0.4        | 10  | 120     | 206   | 75         | 36          | 149         |
| 0.4        | 11  | 0       | 0     | 410.6      | 260.6       | 618.9       |
| 0.4        | 11  | 30      | 51    | 148.3      | 123.1       | 177         |
| 0.4        | 11  | 60      | 103   | 21.8       | 13.4        | 33.1        |
| 0.4        | 11  | 90      | 154   | 6.3        | 2.5         | 12.7        |
| 0.4        | 11  | 120     | 206   | 0          | 0           | 0           |
| 0.4        | 12  | 0       | 0     | 295        | 188         | 440         |
| 0.4        | 12  | 30      | 51    | 301        | 197         | 442         |
| 0.4        | 12  | 60      | 103   | 135        | 78          | 234         |
| 0.4        | 12  | 90      | 154   | 120        | 60          | 203         |
| 0.4        | 12  | 120     | 206   | 20         | 3           | 71          |
| 0.4        | 13  | 0       | 0     | 166.4      | 115.4       | 234         |
| 0.4        | 13  | 30      | 51    | 90.8       | 66.5        | 123.1       |
| 0.4        | 13  | 60      | 103   | 28.8       | 18.3        | 42.7        |
| 0.4        | 13  | 90      | 154   | 11         | 5.7         | 20.1        |
| 0.4        | 13  | 120     | 206   | 9.8        | 4.7         | 18.4        |

Table 5: Most Probable Number (MPN) of the CFU values in the single disinfection experiment (run) plotted in Figure 3 with upper and lower limit of the 95% confidence interval 54.

| $\frac{\sigma_v}{}$ | run | t (min) | $n_p$ | MPN/100 ml | Lower limit | Upper limit |
|---------------------|-----|---------|-------|------------|-------------|-------------|
| 0.65                | 14  | 0       | 0     | 1732900    | 1167700     | 2709500     |
| 0.65                | 14  | 60      | 103   | 1046200    | 705000      | 1509000     |
| 0.65                | 14  | 120     | 206   | 727000     | 475700      | 1048900     |
| 0.65                | 14  | 180     | 309   | 290900     | 190400      | 446100      |
| 0.65                | 14  | 240     | 411   | 151500     | 108000      | 207800      |
| 0.65                | 15  | 0       | 0     | 32700      | 19000       | 44400       |
| 0.65                | 15  | 60      | 103   | 21800      | 13400       | 33900       |
| 0.65                | 15  | 120     | 206   | 7500       | 3600        | 14900       |
| 0.65                | 15  | 180     | 309   | 2000       | 300         | 7100        |
| 0.65                | 15  | 240     | 411   | 0          | 0           | 370         |
| 0.65                | 16  | 0       | 0     | 2755       | 1857        | 4168        |
| 0.65                | 16  | 60      | 103   | 860        | 613         | 1155        |
| 0.65                | 16  | 120     | 206   | 201        | 124         | 318         |
| 0.65                | 16  | 180     | 309   | 10         | 1           | 55          |
| 0.65                | 16  | 240     | 411   | 10         | 1           | 55          |
| No Plate            | 17  | 0       | 0     | 307.6      | 195.3       | 471.2       |
| No Plate            | 17  | 30      | 60    | 344.8      | 218.9       | 520.7       |
| No Plate            | 17  | 60      | 120   | 461.1      | 292.7       | 687.9       |
| No Plate            | 17  | 90      | 180   | 344.8      | 218.9       | 520.7       |
| No Plate            | 17  | 120     | 240   | 344.8      | 218.9       | 520.7       |
| No Plate            | 18  | 0       | 0     | 209.8      | 145.5       | 301.1       |
| No Plate            | 18  | 30      | 60    | 204.6      | 137.9       | 306.9       |
| No Plate            | 18  | 60      | 120   | 185        | 131.9       | 256.3       |
| No Plate            | 18  | 90      | 180   | 204.6      | 137.9       | 306.9       |
| No Plate            | 18  | 120     | 240   | 185        | 131.9       | 256.3       |
| No Plate            | 19  | 0       | 0     | 3448       | 2189        | 5207        |
| No Plate            | 19  | 10      | 20    | 3654       | 2319        | 5555        |
| No Plate            | 19  | 20      | 40    | 4884       | 3100        | 7215        |
| No Plate            | 19  | 30      | 60    | 3255       | 2066        | 4981        |
| No Plate            | 19  | 60      | 120   | 5172       | 3384        | 7636        |
| No Plate            | 19  | 90      | 180   | 3076       | 1953        | 4712        |
| No Plate            | 19  | 120     | 240   | 4352       | 2762        | 6500        |

Table 5: Most Probable Number (MPN) of the CFU values in the single disinfection experiment (run) plotted in Figure 3 with upper and lower limit of the 95% confidence interval 54.