

**Disinfection of Gram – Negative and Gram – Positive Bacteria using DYNAJETS®  
Hydrodynamic Cavitating Jets**

Gregory Loraine, Georges Chahine, Chao-Tsung Hsiao, Patrick Aley

DYNAFLOW, INC. Jessup, MD, USA

**ABSTRACT**

Cavitating jet nozzles (DYNAJETS®) were investigated as a means of disinfection of gram-negative *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas syringae*, and *Pseudomonas aeruginosa*, and gram-positive *Bacillus subtilis*. The cavitating jets were found to be very effective in reducing the concentrations of all of these species. In general, the observed rates of disinfection of gram-negative species were higher than for gram-positive species. However, different gram-negative species also showed significant differences (*P. syringae* 6- log<sub>10</sub> reduction, *P. aeruginosa* 2-log<sub>10</sub> reduction) under the same conditions. Disinfection of *E. coli* repeatedly showed 5 orders of magnitude reduction in concentration within 45 to 60-minutes at low nozzle pressure (2.1 bar).

Optimization of nozzle design and operating pressures increased disinfection rates per MJ of power by several orders of magnitude. The power efficiencies of the hydrodynamic cavitating jets were found to be 10 to 100 times greater than comparable ultrasonic systems.

*Keywords:* hydrodynamic cavitation; disinfection; *E. coli*; *B. subtilis*; spores

**1. INTRODUCTION**

While ultrasonic cavitation processes have been shown to be an effective means of disinfection for bacteria in liquids [1-7], their use has been somewhat restricted to small volumes and specialized applications such as sterilization of medical and dental equipment and food processing [8-9]. A factor in limiting the application of cavitation for disinfection is the relatively high energy costs associated with ultrasonic cavitation. However, the energy efficiency of disinfection of water using hydrodynamic cavitating jets has been shown to be at least an order of magnitude higher than that of ultrasonic devices and the required energy inputs much lower [2,10,11]. Several recent studies have taken advantage of this and examined the application of hydrodynamic cavitation to the disinfection of bacteria in water and wastewater [2,10-15].

Hydrodynamic cavitation differs from ultrasonic cavitation in how cavitation is generated. In hydrodynamic cavitation, large fluctuating pressure forces in the shear layer causes preexisting microscopic bubble nuclei in the liquid to cavitate, that is to grow explosively and collapse violently. In ultrasonic cavitation high frequency sound generates cyclic pressure waves in the liquid that drive cavitation. As the cavitating bubble collapses its gas content is highly compressed creating very high pressures (~500 atm) and temperatures (~5,200 °K) inside the bubble [16,17]. These high pressures and temperatures result in chemical and physical processes that are significant in understanding disinfection of bacteria by liquid cavitation.

Gases including water vapor in the interior of the collapsing bubbles can undergo cleavage of molecular bonds. Volatile compounds in the bubble interior can be pyrolyzed. Water vapor can be split into OH<sup>•</sup> and H<sup>•</sup> radicals during bubble collapse. These free radicals can oxidize and reduce molecules in the vapor [16,17]. Compounds near the walls of the collapsing bubbles will also be exposed to OH<sup>•</sup> and other free radicals diffusing from the bubbles. These processes are most likely of limited importance in disinfection due to the large sizes of the organisms [12]. However, OH<sup>•</sup> are strong oxidizers and the direct oxidation of microorganisms during hydrodynamic cavitation has been reported [18].

In addition to the generation of oxidants, strong cavitation bubble collapse also results in shock waves, high shear, super critical water, and pressure and temperature spikes. This extremely adverse local environment can result in rupture of cell walls, interference with osmotic responsiveness, loss of intercellular material, and disruption of protein synthesis [2,3,12,18]. The question of which of these diverse mechanisms are the most critical in hydrodynamic cavitation is open. Elucidating the kill mechanism of hydrodynamic cavitation will increase its utility by allowing for the intelligent application of this technology.

Currently, studies of the disinfection mechanism of hydrodynamic cavitation are limited. Balasundaram and Harrison [13] studied the disruption of the cell walls of *E. coli* using hydrodynamic cavitation for the purpose of preferentially releasing engineered proteins from the organisms. The concentrations of proteins originating from the periplasm or cytoplasm of the *E. coli* were monitored as the bacteria were jetted through an orifice plate for up to 1000 cycles (passes through the orifices). They postulated a kill mechanism for multiple passes through an orifice. In the first stage the outer membrane is perforated allowing loss of periplasmic proteins. In the second stage the cytoplasm membrane is now exposed to the effects of cavitation and oxidation due to the loss of integrity of the outer membrane and cytoplasmic proteins are lost. Continued cavitation results in more complete breakdown of the outer membrane. Unfortunately, cell viability was not measured in this study. However after 400 cycles 40-50% of the soluble proteins and enzymes from the *E. coli* had been lysed.

High-pressure homogenization is another process in which bacteria in liquids are exposed to transient pressure changes analogous to the pressure spikes during cavitation [19,20]. In high pressure homogenization, liquid is pumped against a valve which requires a high pressure (100 -300 MPa) to open it. The high pressure conditions last for roughly one second [19]. Once the valve is open the liquid experiences a rapid pressure drop and high shear. It is often used as a disinfection process for food, such as milk. The susceptibility of bacteria to high pressures varies between species and even growth phases. Cells are more resistant during stationary growth phase than in exponential growth phase [21].

Wuytack et al. [19] and Kelemen and Sharpe [20] examined the effects of high-pressure homogenization on gram-positive and gram-negative bacteria species. Gram's staining is a method of classifying bacteria based on the structure of their cell walls [22]. Gram-positive bacteria have a cell wall composed of a thick layer of peptidoglycan and small amounts of teichoic acids. The peptidoglycan layer is much thicker than that of gram-negative bacteria. Gram-negative cell walls are made up of a composite of an outer membrane, a periplasmic space, a peptidoglycan membrane, a periplasmic space, and the

cytoplasmic membrane. The outer membrane is a bilayer of phospholipids and lipopolysaccharides. The periplasmic spaces are filled with liquid proteins. The peptidoglycan membrane is much thinner (5-10% of the cell wall) than that of gram-positive bacteria. The cytoplasmic membrane is a phospholipid bilayer. The resistance of a cell to mechanical stresses has been related to the level of cross-linking in the peptidoglycan layer [23]. Since the peptidoglycan layer in gram-negative cell walls is much thinner, even if it is extensively cross-linked, it will still be less resistant to rupture of the cell wall than would gram-positive species.

Kelemen and Sharpe [20] found that during high-pressure homogenization gram-negative *E. coli* were disrupted at lower pressures than were gram-positive *Bacillus subtilis*. This was attributed to the composition of their cell walls. Wuytack et al. [19] compared the disinfection of five species of gram-positive bacteria and six species of gram-negative bacteria using high-pressure homogenization and high hydrostatic pressure (200-400 MPa applied uniformly for several minutes). The kill mechanism of high hydrostatic pressure was reported to be interference with cellular functions inside the cytoplasm (interior) of the cells. There was no clear correlation between cell wall structure and susceptibility to hydrostatic pressures.

There was a clear correlation with cell wall structure with high-pressure homogenization [19]. The thicker walled gram-positive species were much more resistant than were the thin walled gram-negative species. They concluded that high-pressure homogenization kills bacteria through mechanical destruction of cell integrity.

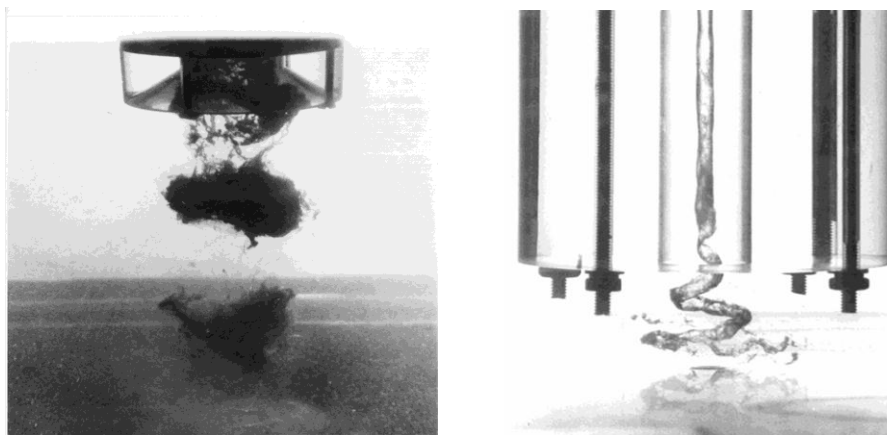
Thus, if the kill mechanism of bacteria undergoing hydrodynamic cavitation is mechanical disruption of the cell walls, then there would be large differences in the disinfection efficiencies of gram-positive and gram-negative bacteria. What is more, since the cavitation conditions experienced by bubble nuclei are dependent on the operating conditions and the design of the cavitation chamber, varying these parameters should also have a clear effect on the disinfection rates observed [12,24].

Disinfection experiments were conducted using hydrodynamic cavitation generated with the DYNAJETS<sup>®</sup> nozzles described below. Several species were used including gram-negative *E. coli* MC 4100, *Klebsiella pneumoniae*, *Pseudomonas syringae* and *Pseudomonas aeruginosa*. *E. coli* is a common potential waterborne human pathogen. Measurements of *E. coli* in wastewater are often used as an indicator of the level of overall bacterial content. *Klebsiella pneumoniae* (gram-negative) was used as a surrogate species for human pathogens. *Pseudomonas aeruginosa* is a good representative of opportunistic pathogens of humans and is a gram-negative, aerobic rod. *P. aeruginosa* has a greater degree of cross-linking of peptidoglycan than does *E. coli* [25]. *Pseudomonas syringae* is very similar to *P. aeruginosa* except that it is a plant pathogen and could be easily grown for laboratory testing. *Bacillus subtilis* EM 1600 was used as the gram-positive species.

The *E. coli* and *B. subtilis* were extensively tested using various nozzle types and flow conditions for optimization studies because both are good indicators of a wide range of human pathogenic bacteria. They are also both rod shaped and are of similar size [20]. The effects of species, nozzle type, nozzle pressure, and microorganism concentration, were determined.

The DYNAJETS<sup>®</sup> cavitating jet technology employs various nozzle designs to induce cavitation in submerged water jets. The STRATOJET<sup>®</sup> incorporates acoustic

resonance in the flow to result in very high amplitude, periodic, oscillatory discharge (Figure 1 left) [26-28]. Acoustic resonance is achieved in the nozzle feed-tube assembly when a standing wave forms in the “organ-pipe” feed tube to the STRATOJET<sup>®</sup> nozzle.

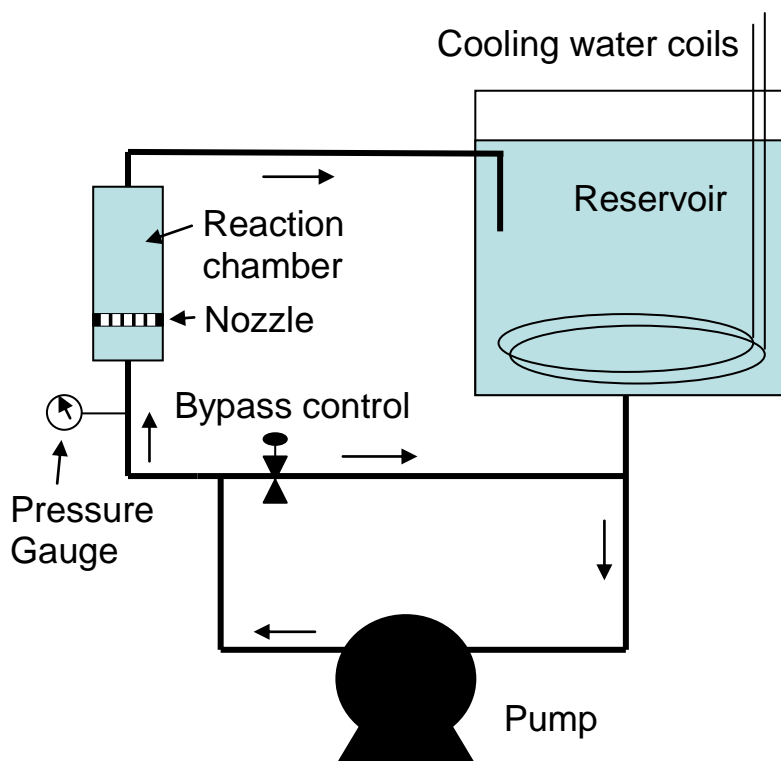


*Figure 1. Left hand side, Strobe photograph of cavitating vortex rings in a STRATOJET<sup>®</sup>. Right hand side, Flow visualization of DYNASWIRL<sup>®</sup> showing helicoidal cavity.*

The swirling cavitating jet, DYNASWIRL<sup>®</sup>, achieves cavitation at even higher cavitation numbers (i.e. lower jet velocities) than the STRATOJET<sup>®</sup>. Swirl is achieved by means of tangential injection slots, resulting in a central vortex, which appears as a helicoidal filament along the jet axis (Figure 1 right). The DYNASWIRL<sup>®</sup> jets have the advantage of generating very large cavity surface areas that start inside the nozzle assembly and extend in front of the nozzle orifice.

## **2. DISINFECTION EXPERIMENTAL PROCEDURES**

The disinfection experiments were conducted in batch reactors using either a DYNASWIRL<sup>®</sup> or STRATOJET<sup>®</sup> jet nozzle. A batch reactor consisted of a 3.7 KW positive displacement pump, a temperature controlled reservoir, and an in-line DYNAJETS<sup>®</sup> nozzle (Figure 2). The total sample volume was varied between 1.8 and 20 liters, depending on the test conditions.



**Figure 2. Schematic of Experimental set-up.**

*E. coli* MC 4100, *Klebsiella pneumoniae*, *Pseudomonas syringae* and *Pseudomonas aeruginosa* were obtained from the University of Maryland, and were cultured in sterile buffered salt solutions (M9 Minimal Salts, Sigma) fortified with glycerol (99%, Sigma), or LB Broth (LB Broth, Miller, US Biological). *B. subtilis* EM 1600 cultures were grown in M9 salts with 2% LB Broth. Flasks of media were inoculated from streak plates, and the cultures were grown overnight in a temperature controlled shaker water bath.

Incubation was continued until the midpoint of the exponential growth phase as determined by light absorbance (600 nm). This insured populations in a robust stage of growth – neither in saturation mode, such that no further growth would occur, nor at too low an initial concentration [21,29,30].

Bacteria concentrations in the experiments were adjusted by diluting the growth stock solution with sterile buffered salt solutions. The solution was added to the test loop and was circulated through the DYNAJETS<sup>®</sup> nozzles under controlled pressures and flow rates in a batch mode. The temperature was controlled by running chilled water through a cooling coil located inside the reservoir (Figure 2).

During the test, samples of approximately 5 ml were taken every 5 to 30 min, with the higher frequency occurring at the beginning of the tests. The samples were then serially diluted and plated on agar plates in duplicate. The plates were incubated at 37 °C for 48 hours after which viable colonies were counted [31]. The concentrations of the bacterial solutions were recorded as colony forming units per milliliter (CFU/ml). A portion of the bacteria laden liquid was maintained in a flask in a shaker water bath and monitored to serve as a control.

### 3. EXPERIMENTAL RESULTS

#### 3.1 Gram-Negative Species

Disinfection tests with pure cultures of gram-negative bacteria were run in batch tests using either a DYNASWIRL<sup>®</sup> nozzle or a STRATOJET<sup>®</sup> nozzle. Disinfection of *Klebsiella*, and *Pseudomonas* using hydrodynamic cavitation has, to our knowledge, rarely been reported in the open literature. Figure 3 shows the disinfection of *Klebsiella pneumoniae* and *E. coli* using a DYNASWIRL<sup>®</sup> cavitating jet with a single exit orifice with a diameter of 0.323 cm, and operated at 2.1 bar (30 psi). The initial concentrations of *Klebsiella pneumoniae* and *E. coli* were  $C_0 \sim 10^7$  CFU/ml. The test batch volume was 2 liters. Both cases achieved a reduction to a level of  $\sim 100$  CFU/ml within 60 minutes, a 5- $\log_{10}$  reduction, that is, 99.999% removal. The error bars in the figures are calculated from the standard deviations of the duplicates of the CFU/ml measurements.

Disinfection of high concentrations of *Pseudomonas aeruginosa* ( $\sim 10^8$  CFU/ml) and *Pseudomonas syringae* ( $\sim 10^7$  CFU/ml) were conducted using an eight-orifice STRATOJET<sup>®</sup> at a pump pressure of 16.5 bar (240 psi), and a batch volume of 1.8 liters (Figure 4). A nearly 3- $\log_{10}$  decrease in *P. aeruginosa* concentration was observed in 90 minutes. *P. syringae* concentrations were reduced in excess of 6- $\log_{10}$  in 20 minutes. The differences in the disinfection rates of the gram-negative species may be related to the degree of cross-linking in the peptidoglycan layer of the cell walls. Young [25] determined that *P. aeruginosa* had a higher degree of cross-linking than did *E. coli*. These results are thus consistent with a disinfection mechanism based on rupture of cell walls. As can be seen in Figure 3 and Figure 4, the disinfection rate for *E. coli* using the DYNASWIRL<sup>®</sup> nozzle was roughly twice as great as was seen with the STRATOJET<sup>®</sup> nozzle. A more complete analysis of the effect of nozzle type on disinfection rate is discussed below.

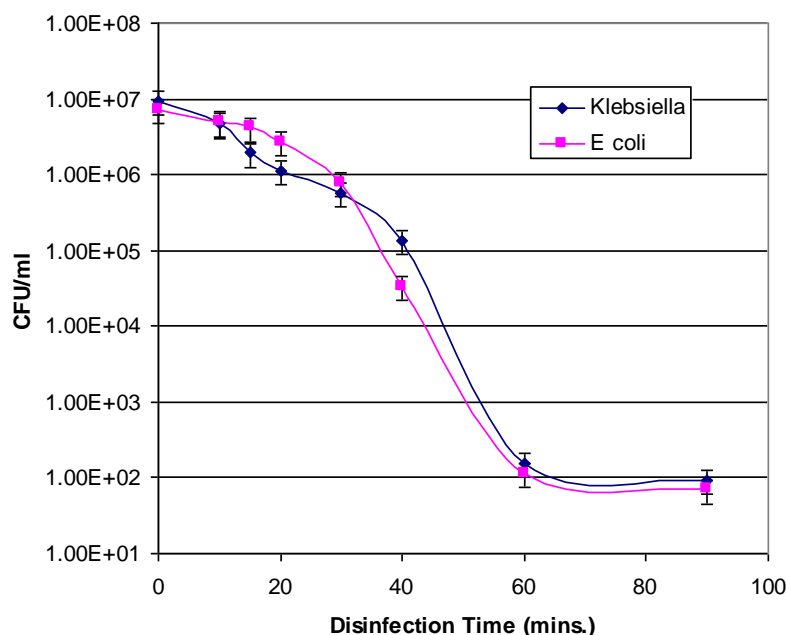


Figure 3. Comparison of reductions of *Klebsiella* and *E. coli* for a single 0.32 cm orifice DYNASWIRL® cavitating jet operating at 2.1 bar.

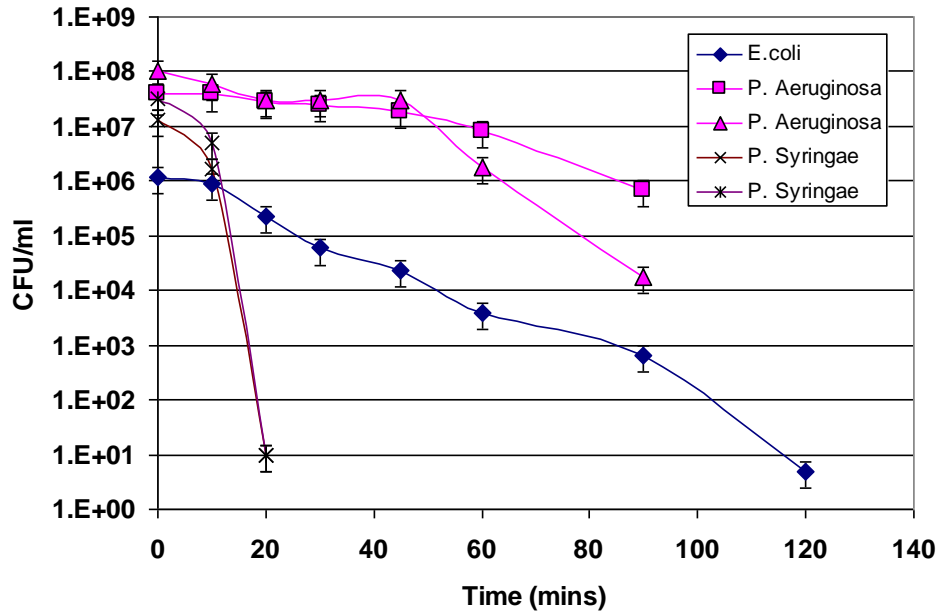


Figure 4. Disinfection of *Pseudomonas aeruginosa*, *Pseudomonas syringae*, and *E. coli* using an 8-orifice STRATOJET® cavitating jet nozzle operating at a pump pressure of 16.5 bar.

### 3.2 Influence of DYNAJETS® Types and Operating Conditions

One important concept in comparing the experimental results is the number of times a fluid particle has been subjected to cavitation, i.e. the number of cycles it has gone through the loop. To account for various volumes, pressures and flow rates, we normalize the disinfection time by the ratio of the system volume,  $\mathcal{V}$ , and the flow rate through the loop,  $Q$ , to get the number of times or cycles through the loop,  $N$ , that a fluid particle has been exposed to cavitation.

$$N = t / (\mathcal{V} / Q). \quad (1)$$

In order to compare the decreases in bacteria concentration of experiments with different initial concentrations  $C_0$ , we normalize the concentration by  $C_0$ .

The observed bacteria reduction was correlated to the hydraulic energy input to the liquid  $E_h$  given by the product of the input hydraulic power  $\mathcal{P}_h$  and disinfection time,  $t$ . For a volumetric flow rate,  $Q$ , and a pump pressure rise of  $\Delta P$ , this is given by:

$$E_h = \mathcal{P}_h t = Q \Delta P t. \quad (2)$$

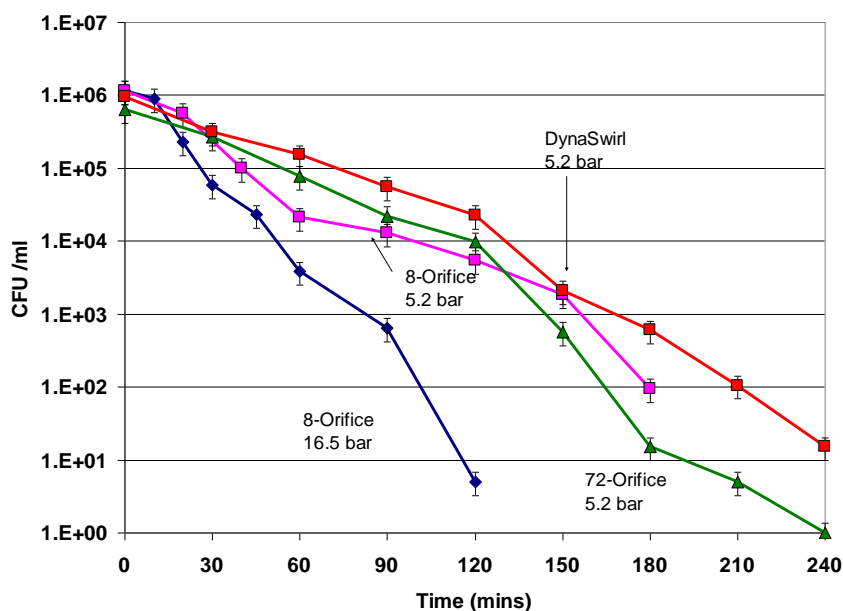
In order to generalize this, we can divide the energy by the liquid volume to obtain an input hydraulic energy per unit volume  $E_h^*$ :

$$E_h^* = E_h / \mathcal{V} = \mathcal{P}_h t / \mathcal{V} = Q \Delta P t / \mathcal{V}. \quad (3)$$

This can be utilized to compare different configurations operating at different pressures and flow rates. As will be seen below, although one configuration may produce a more rapid disinfection, it may be less energy efficient than another.

Figure 5 to Figure 7 present results of experiments conducted with *E. coli* in buffered salt media for different types of DYNAJETS<sup>®</sup> and for different pump pressures. In all cases, the initial bacteria concentration was about 10<sup>6</sup> CFU/ml. Figure 5 presents the bacteria concentration, *C*, versus time, while Figure 6 presents the normalized concentration, *C/C*<sub>0</sub>, versus the number of cycles, *N*. Figure 7 presents the normalized data, *C/C*<sub>0</sub>, versus the applied hydraulic energy.

In all four cases 4 to 6 log<sub>10</sub> reductions in *E. coli* concentrations were observed. Disinfection using the 8-orifice STRATOJET<sup>®</sup> was faster at 16.5 bar than at 5.2 bar (Figure 5) but was less efficient (Figure 7). The faster disinfection rate at the higher pressure was due to the faster flow rate (more cycles per unit time). When the disinfection rates are normalized by *N* (Figure 6) this difference was seen to be minimal.

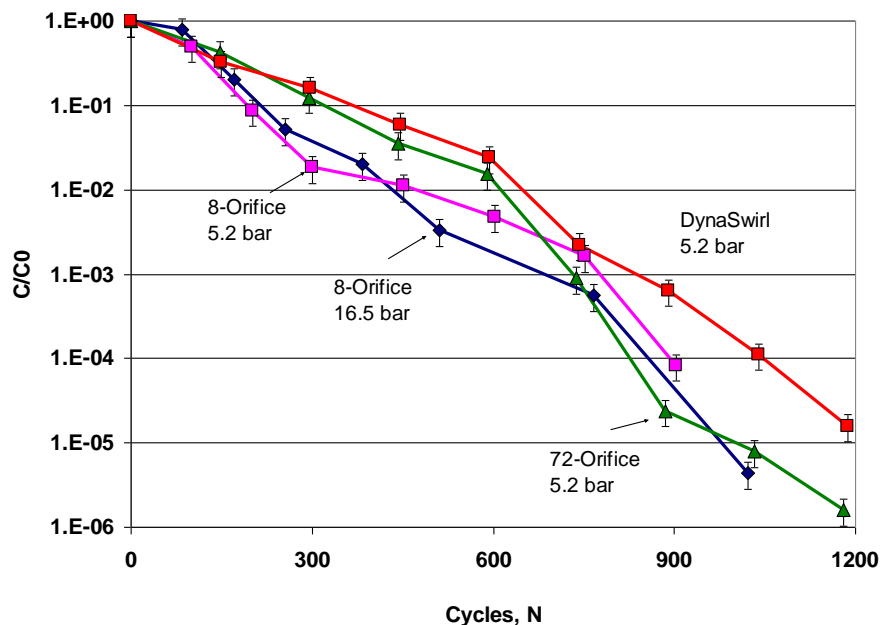


**Figure 5. Comparison of the reductions of *E. coli* achieved for the 8-orifice plate STRATOJET<sup>®</sup> operating at 16.5 bar (240 psi) and 5.2 bar (75 psi), the 72-orifice manifold DYNAJETS<sup>®</sup> operating at 5.2 bar (75 psi), and the single orifice swirling DYNAJETS<sup>®</sup> operating at 5.2 bar (75 psi).**

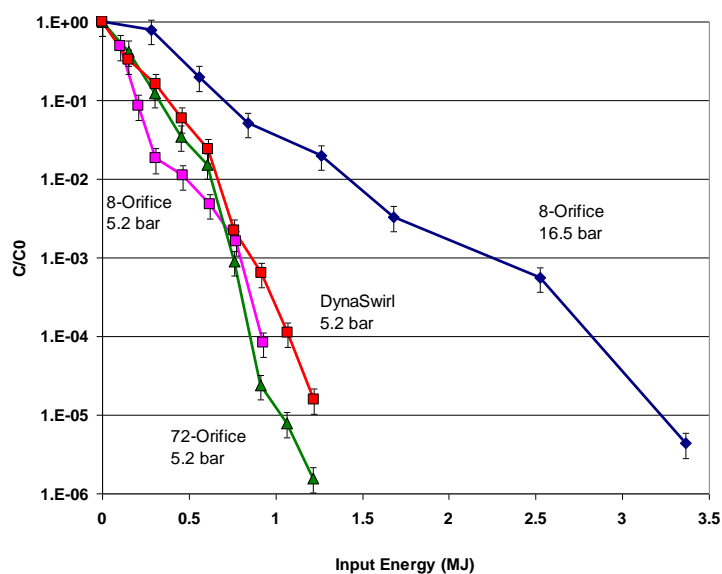
Figure 5 through Figure 7 also compare the performance at the same operating pressures for three different types of DYNAJETS<sup>®</sup>: an 8-orifice plate STRATOJET<sup>®</sup> with 0.114 cm diameter orifices, a 72-orifice manifold configuration with 0.038 cm diameter orifices, and a slotted DYNASWIRL<sup>®</sup> with a single exit orifice of 0.323 cm diameter. All three nozzles had the same flow rates and total opening area (0.082 cm<sup>2</sup>) so the results were due to hydrodynamic effects of the different nozzle geometries. However, for *E. coli* the differences in removal efficiencies were relatively small. This was not the case



for *B. subtilis* which is discussed below. Figure 7 shows that the lower pressure tests were much more efficient than the 8 orifice 240 psi test.



*Figure 6. Data of Figure 5 expressed in non-dimensional form.*



*Figure 7. Reduction in bacteria count ratio versus expended hydraulic energy for data of Figure 5.*

Arrojo et al. [12] also reported the rate of *E. coli* disinfection using hydrodynamic cavitation was dependent on nozzle configuration. They found that venturi type nozzles outperformed orifice plate type nozzles. They concluded that *E. coli* were more susceptible to extended lower intensity cavitation than to more intense localized cavitation.

### 3.3 INFLUENCE OF PUMP PRESSURE ON THE DYNASWIRL<sup>®</sup> JET

From Equation (2) it can be seen that reducing the pump pressure reduces the hydraulic energy required. As can be seen from Figure 7, there was an inverse relationship between pump pressure and disinfection efficiency for *E. coli*. Unlike ultraviolet disinfection for instance, increasing power input does not necessarily increase disinfection efficiency.

One of the objectives of this study was to determine the lowest operating pump pressure that could achieve significant disinfection using the DYNASWIRL<sup>®</sup> cavitating jet. Figure 8 and Figure 9 present results of experiments conducted with *E. coli* using a DYNASWIRL<sup>®</sup> with a single orifice (0.45 cm diameter) at three pressures. In all cases, the initial bacteria concentration was 10<sup>7</sup> CFU/ml. Figure 8 shows the relative reduction in bacteria concentrations for nozzle pressure drops of 3.45, 2.1, and 1.0 bar. Figure 9 shows the normalized data versus hydraulic energy input.

As can be seen in these figures, significant reductions in bacteria count were achieved in both the 3.45 bar and the 2.1 bar cases, while the microorganism removal for the 1.0 bar case stagnated after 2-log<sub>10</sub> removal. This can be examined with respect to the cavitation number,  $\sigma$ , is the dimensionless parameter characterizing cavitation,

$$\sigma = \frac{P_{amb} - p_v}{\rho V^2 / 2} = \frac{P_{amb} - p_v}{\Delta P}, \quad (4)$$

where  $P_{amb}$  is the ambient pressure,  $p_v$  is the liquid vapor pressure,  $\rho$  is the liquid density,  $V$  is the characteristic flow velocity, and  $\Delta P$  is the pressure drop across the nozzle. For applications where the jet discharges at ambient atmospheric pressure,  $\Delta P$  is the pump pressure relative to ambient.

The particular value at which cavitation is incipient is defined as

$$\sigma_i = \left[ \frac{P_{amb} - p_v}{\Delta P} \right]_{\text{at inception}}. \quad (5)$$

Thus if the operating conditions for a submerged jet are such that  $\sigma/\sigma_i < 1$ , cavitation will occur, and as  $\sigma/\sigma_i$  continues to decrease below unity the amount of cavitation will increase.

The data in Figure 8 and Figure 9 show that the 2.1 bar case resulted in a more rapid reduction than the 3.45 bar case. This may be due to a change in the cavitation characteristics as the jet exits the orifice, i.e. at 3.45 bar the DYNASWIRL<sup>®</sup> was cavitating too much. This indicates better operation at a cavitation number closer to inception,  $\sigma \approx 0.5$  for the 2.1 bar case than at  $\sigma \approx 0.33$  for the 3.45 bar case. When the pressure drop across the nozzle was further lowered to 1.0 bar ( $\sigma \approx 1$ ), the limit value for cavitation to occur was approached and cavitation benefits were limited. The disinfection results in this case are comparable to those of the higher pressure cases, and a 2-log

reduction was achieved in the first 30 min. However, limited additional reduction was achieved from there on, during the subsequent 90 minutes (400 cycles). In contrast, continued reductions were measured for the 2.1 and 3.45 bar pressure cases. The lower limit of operating pump pressure to obtain good microorganism reduction with the DYNASWIRL<sup>®</sup> swirling jet appears to be in the 1.4 to 2.0 bar range.

The efficiency of disinfection per MJ of power is 10 times greater at 2.1 bar than at 3.45 bar due to a higher disinfection rate and lower hydraulic power input (Figure 9). Based on this, the 2.1 bar condition was selected to conduct a number of subsequent experiments to study the influence of other parameters on the performance of the DYNASWIRL<sup>®</sup> cavitating jet.

Balasundaram and Harrison [13] found that the amount of proteins released by *E. coli* when the cell membranes were disrupted using hydrodynamic cavitation was dependent on the cavitation number of the system. They found that the lower the cavitation number the more efficient was the extraction of proteins and enzymes. As discussed above, they did not measure the viability of the cells however. It is possible that cell death occurs at a lower cavitation number than does release of periplasmic proteins.

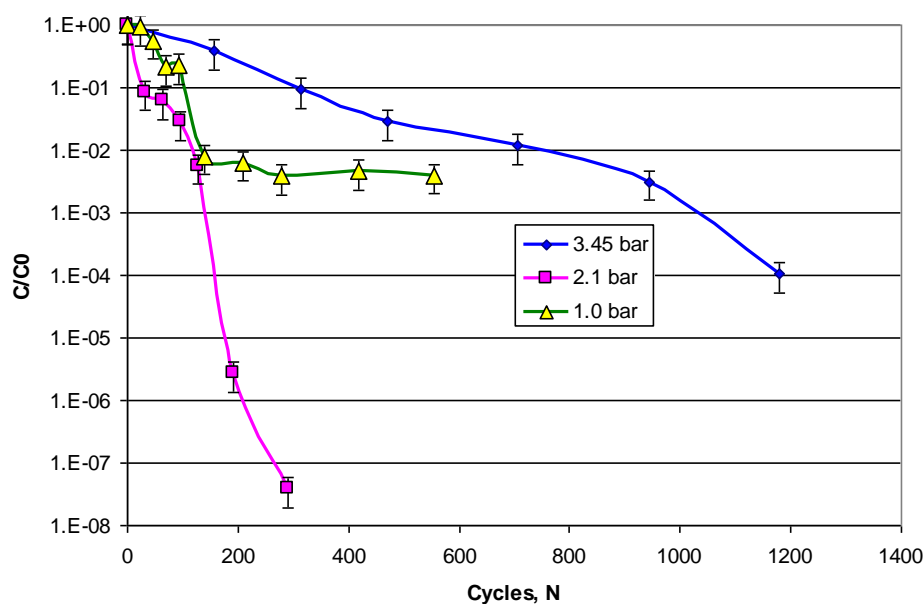
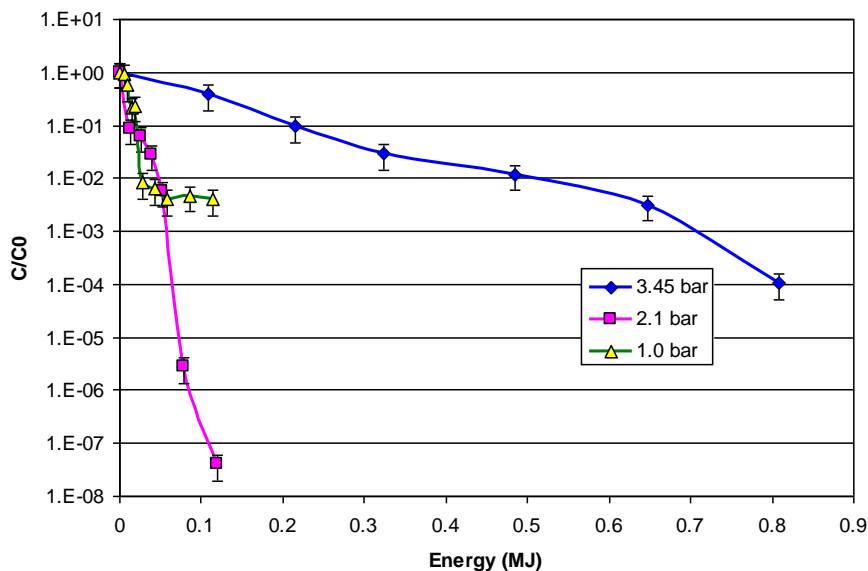


Figure 8. Comparison of the reductions of *E. coli* in M9 achieved for a single 0.45 cm orifice DYNASWIRL<sup>®</sup> cavitating jet operating at 1.0, 2.1, and 3.45 bar (15, 30 and 50 psi). Initial concentrations were 0.9 to  $2 \times 10^7$  CFU/ml.



**Figure 9. Reduction in relative bacteria count versus expended hydraulic energy for data of Figure 8.**

### 3.4 INFLUENCE OF THE INITIAL CONCENTRATION OF MICROORGANISMS

A set of experiments was conducted to study disinfection performance of the DYNAJETS<sup>®</sup> for different initial *E. coli* concentrations. Four initial bacteria counts, spanning a six order of magnitude range, were tested:  $C_0 \sim 10^3, 10^5, 10^7, 10^9$  CFU/ml, all for the same DYNAJETS<sup>®</sup> nozzle configuration and under the same set of hydrodynamic operating conditions: a 2-liter batch volume and a single 0.32 cm orifice DYNASWIRL<sup>®</sup> cavitating jet at 2.1 bar. Results of these experiments (Figure 10) demonstrate the same general trend: the reduction starts relatively slowly then increases rapidly till the concentrations fell below about 100 CFU/ml.

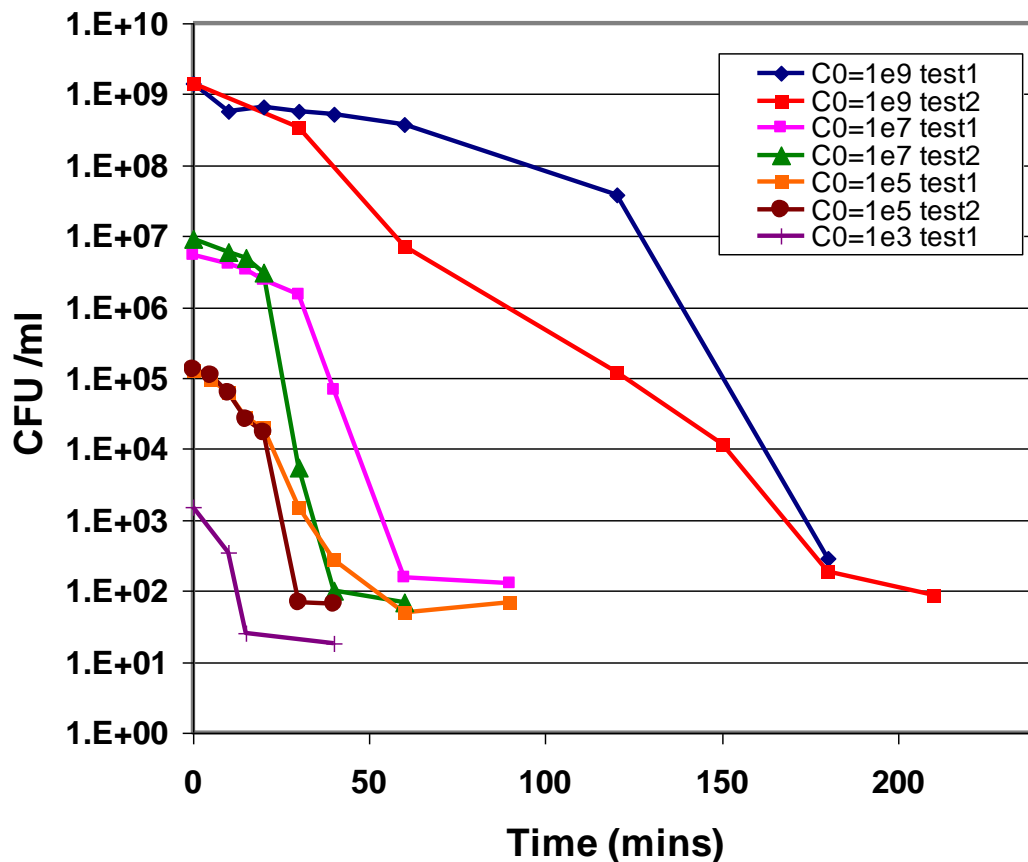


Figure 10. Effect of initial concentration on reduction of *E. coli* in buffered salt solution for the slotted DYNASWIRL<sup>®</sup> cavitating jet operating at 2.1 bar.

The duration of the slower disinfection rate phase at the beginning of the tests was dependent on the initial concentration. The higher the initial concentration was, the longer the duration of the slow reduction phase. During the rapid reduction phase, the disinfection rates are comparable for all cases until the concentration reached 100 CFU/ml.

Similar concentration effects have been reported for ultrasonic disinfection. Feril and Kondo [32] reported that cell lysis of human myelomonocytic lymphoma cells was inhibited at concentrations greater than 5 million/ml. Majumdar et al. proposed that the increased viscosity of solutions with high cell densities inhibits cavitation [33]. This also appears to be the case with hydrodynamic cavitation. Although Arrojo et al. [12] reported no initial lag period using hydrodynamic cavitation; they used lower initial concentrations of  $10^3$  to  $10^5$  CFU/ml. Our experiments did not show significant lag periods at these initial concentrations.

### 3.5 OPTIMIZATION STUDY FOR *B. SUBTILIS*

Disinfection of *Bacillus subtilis*, a gram-positive bacteria, was investigated using the DYNASWIRL<sup>®</sup> nozzle at pump pressures ranging from 2.1 to 8.3 bar. In addition, a complete matrix of tests for *B. subtilis* was performed in the same pressure range using an 8-orifice STRATOJET<sup>®</sup>.

Figure 11 through Figure 13 present results of experiments conducted with *B. subtilis* in buffered salt solution and 2% LB media with the DYNASWIRL<sup>®</sup> jet at pump pressures of 2.1, 4.1, 6.2, and 8.3 bar. Figure 11 presents the raw data, while Figure 12 and Figure 13 present normalized concentrations,  $C/C_0$ , versus number of cycles,  $N$ , and hydraulic energy input,  $E_h$  (MJ), respectively. All cases, except the 4.1 bar case show reductions in bacteria counts of four orders or more within 2 hours.

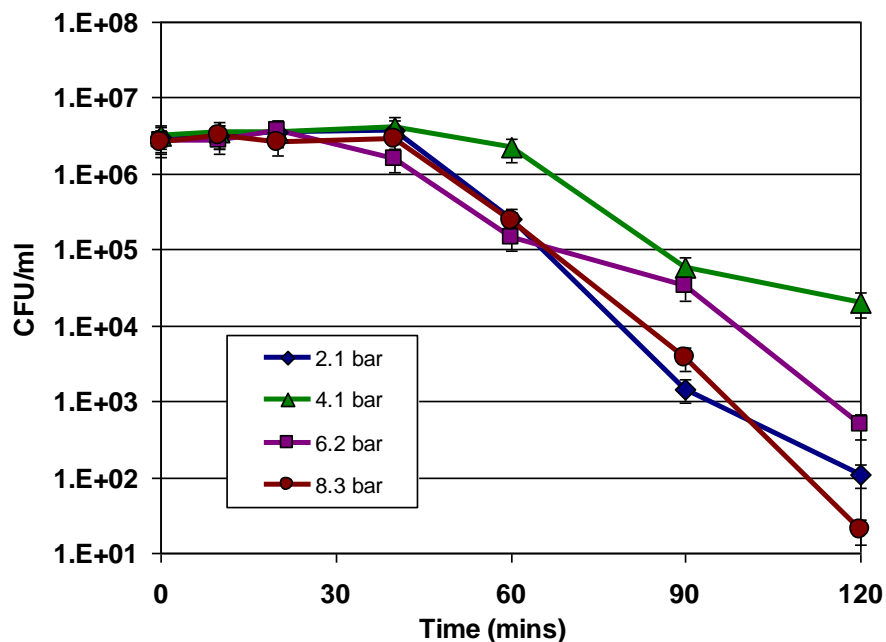


Figure 11. Comparison of the reductions of *Bacillus subtilis* achieved for a single 0.32cm orifice DYNASWIRL<sup>®</sup> cavitating jet operating at 2.1, 4.1, 6.2, and 8.3 bar (30, 60, 90 and 120 psi).

It is interesting to note that there was little reduction of *B. subtilis* concentrations in any of the experiments until after 150 to 250 cycles. After this point the disinfection rates were relatively large. This was not observed with any of the gram-negative species, even at  $C_0 > 10^7$  CFU/ml. There appears to be an additive affect at work. Each pass through the nozzle may weaken the cell walls of *B. subtilis* until a point is reached where the integrity of the membrane is compromised.

Jyoti and Pandit [2,10] studied the synergistic effects of cavitation followed by chemical oxidants such as hydrogen peroxide and ozone, for disinfection. One of the mechanisms they proposed for the enhancement of chemical disinfectants is that cavitation increases the permeability of the cell walls allowing greater transfer of the oxidants into the cell. To our knowledge, gram-positive species such as *B. subtilis* have not been used in this type of study. It would be interesting to see if the enhancement of chemical disinfectants also required multiple cavitation episodes. This could provide a useful probe for this system.

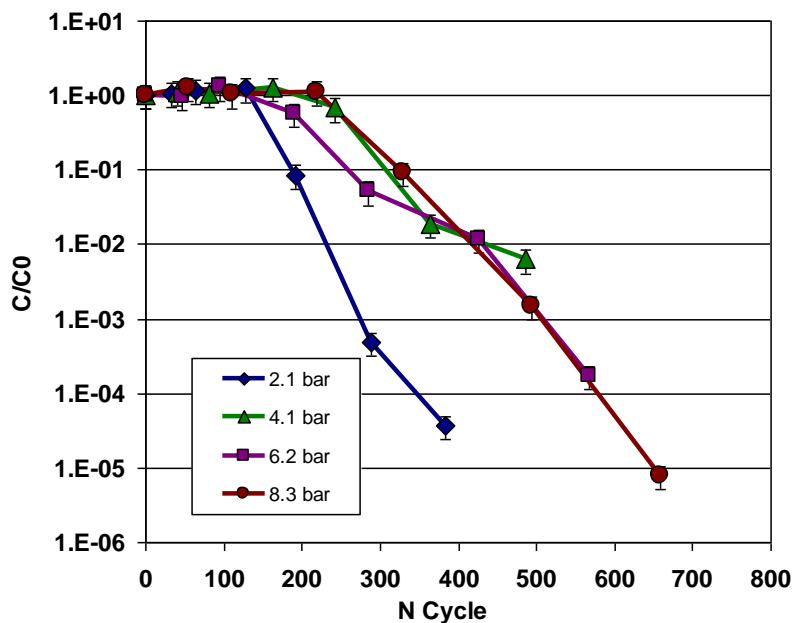


Figure 12. Reduction in relative bacteria count versus cycle number for data of Figure 11.

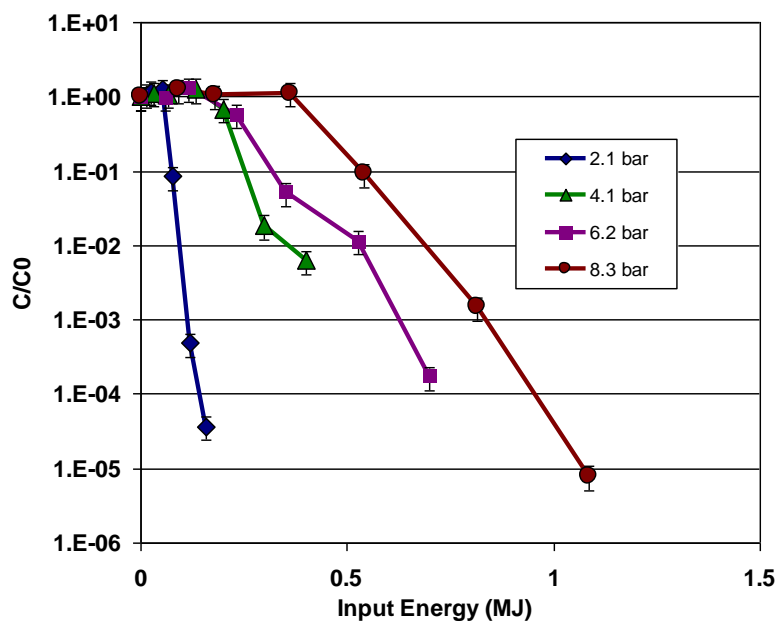


Figure 13. Reduction in relative bacteria count versus hydraulic energy input for data of Figure 11.

The disinfection efficiencies of both *E. coli* and *B. subtilis* using the DYNASWIRL<sup>®</sup> nozzle at a nozzle pressure of 2.1 bar are compared in Figure 14. *Bacillus subtilis*

concentrations were reduced by 4.5-log at 0.16 MJ, while *E. coli* concentrations were reduced by more than 7-log<sub>10</sub> after 0.12 MJ of hydraulic energy input. Thus hydrodynamic cavitation was more than twice as efficient in disinfecting *E. coli* than for *B. subtilis*. In addition, the disinfection efficiency of the DYNASWIRL<sup>®</sup> was seen to be greater than for the STRATOJET<sup>®</sup> for both species.

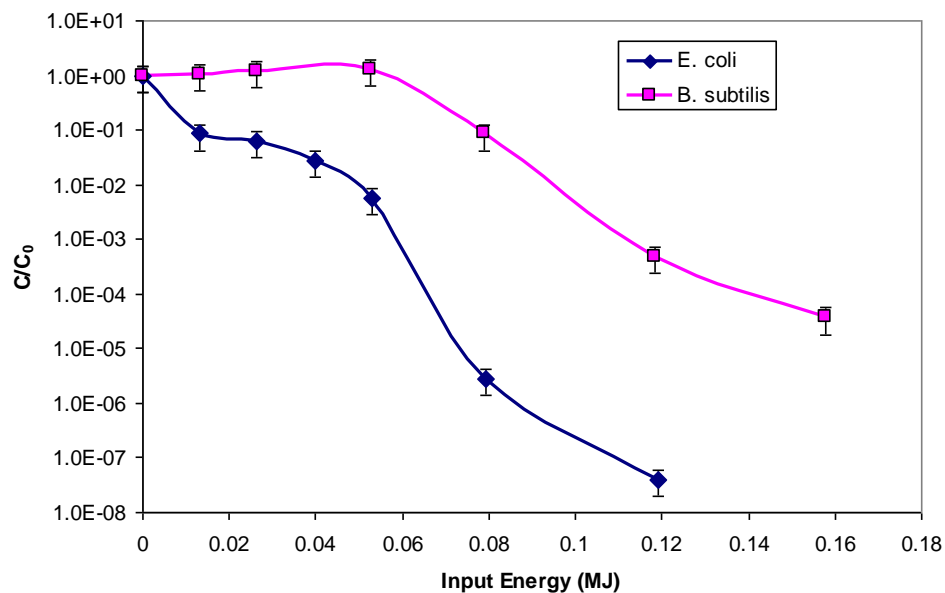


Figure 14. Disinfection efficiency of DYNASWIRL<sup>®</sup> nozzle at 2.1 bar for *E. coli* and *B. subtilis*.

Figure 15 present the *B. subtilis* concentration data from tests conducted at pump pressures of 2.1, 4.1, 6.2, and 8.3 bar using the multi-orifice STRATOJET<sup>®</sup>. The multi-orifice STRATOJET<sup>®</sup> results (Figure 15) did not show good reduction for the pressure range studied.



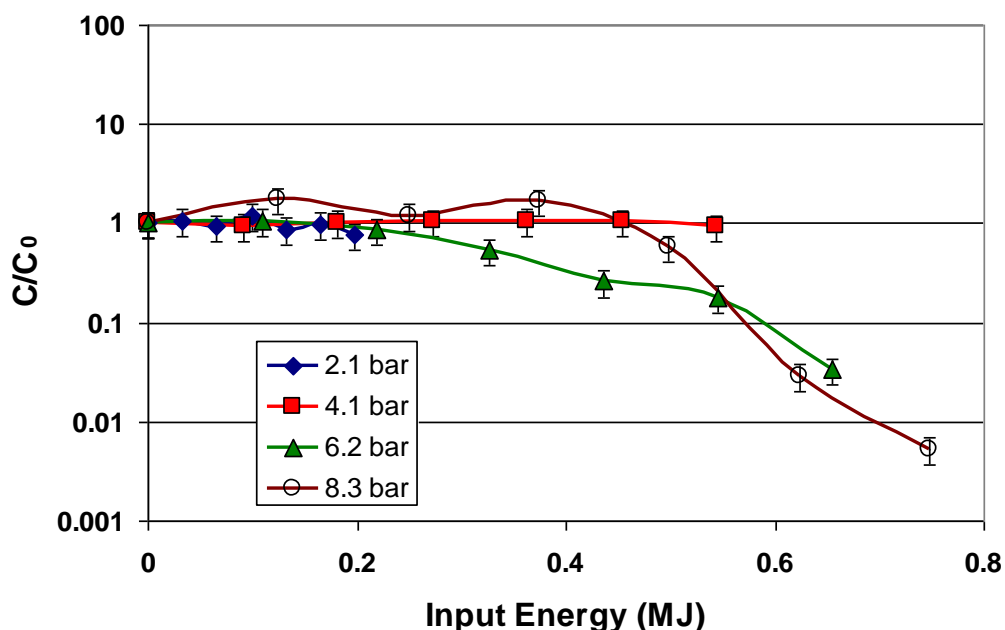


Figure 15. Comparison of the reductions of *Bacillus subtilis* achieved for multi-orifice StratoJet® operating at 2.1, 4.1, 6.2, and 8.3 bar (30, 60, 90 and 120 psi).

The disinfection of *B. subtilis* and *E. coli* using the STRATOJET® nozzle are compared in Figure 16. The nozzle pressure in the *E. coli* experiment was 5.2 bar, the pressure in the *B. subtilis* experiment was 6.2 bar. There was roughly 4-log removal of *E. coli* after 0.9 MJ of hydraulic energy were used, while after 0.85 MJ were input only 1.4-log removal of *B. subtilis* was observed (Figure 15).

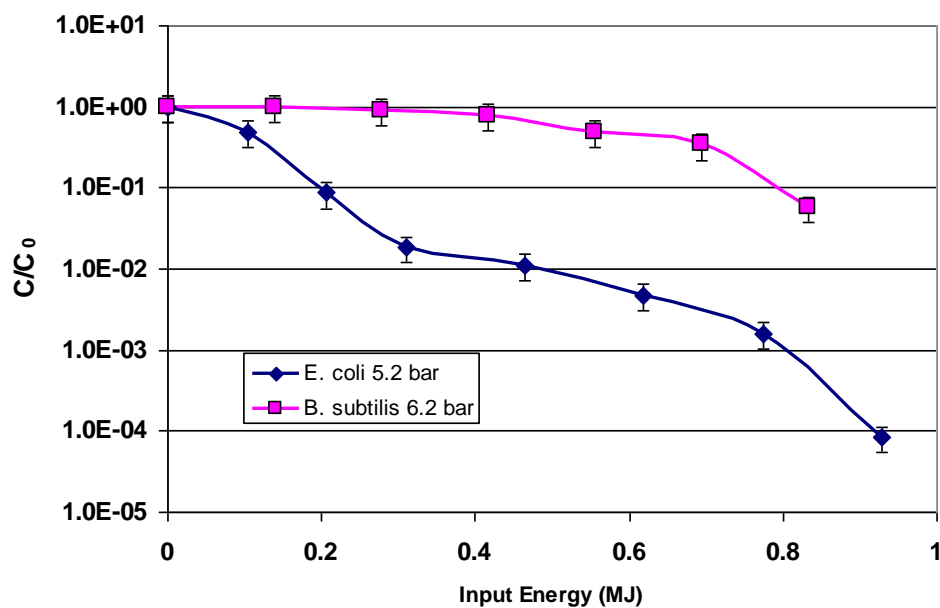
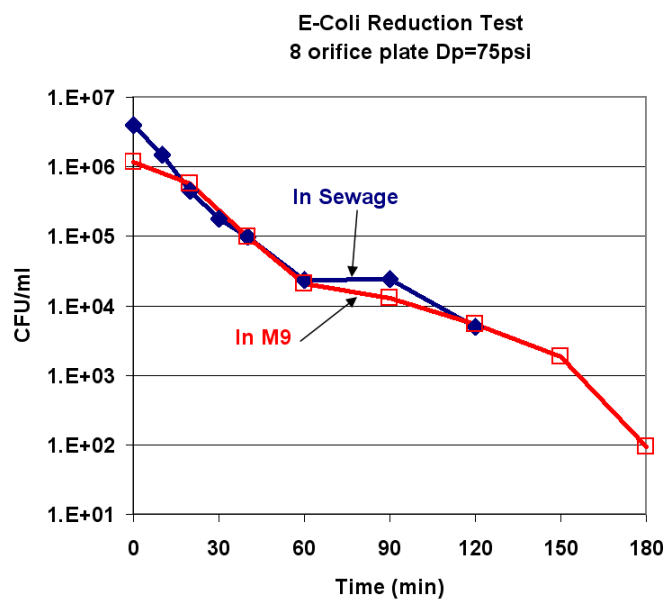


Figure 16. Comparison of energy required to disinfect solutions of *E. coli* and *B. subtilis* using a STRATOJET® nozzle.

### 3.6 DISINFECTION IN WASTEWATER

In order to assess the ability of the DYNAJETS<sup>®</sup> technology to work efficiently in complex matrices, we utilized sewage obtained from a nearby municipal wastewater treatment plant (Little Patuxent Water Reclamation Plant, Howard County, MD). The samples were obtained post-primary settling and the media was sterilized in an autoclave prior to use. The sterile sewage media was inoculated with *E. coli* (MC4100) and incubated until the measured *E. coli* concentration was  $4 \times 10^6$  CFU/ml. It was then placed in the test loop and exposed to cavitation.



**Figure 17. Reduction of *E. coli* in sewage media compared to that in growth media using an 8-orifice STRATOJET<sup>®</sup> operating at 5.2 bar.**

Results of the matrix testing (Figure 17) were compared with data from an experiment conducted with *E. coli* in culture media under otherwise identical conditions (1.8 L volume, 8-orifice STRATOJET<sup>®</sup> at 5.2 bar). The results for a given exposure time are virtually identical for the two cases. This finding strongly indicates that the matrix, such as sewage, does not affect disinfection by hydrodynamic cavitation.

### 3.7 ENERGY EFFICIENCY COMPARISON WITH ULTRASOUND

The energy efficiency of the DYNAJETS<sup>®</sup> was compared to similar ultrasonic cavitation studies using the method of Jyoti and Pandit [34]. In this method the energy efficiency is calculated based on the electrical energy consumption. The disinfection efficiency of the DYNAJETS<sup>®</sup> with *E. coli* was calculated by dividing the rate of disinfection per minute by the power density,  $E_h^*$  from Equation (5).

$$Eff. = \frac{-\log_{10}\left(\frac{C}{C_0}\right) \text{min}^{-1}}{E_h^*} \quad (6)$$

Apparatus	$-\log(C/C_0)$ $\text{min}^{-1}$	Power Density (W/L)	Efficiency ( $\log_{10}$ -L/W)	Species	Ref.
STRATOJET <sup>®</sup> 8- orifice, 5.2 bar	0.0087	8.84	$9.84 \times 10^{-4}$	<i>E. coli</i> MC 4100	Present study
DYNASWIRL <sup>®</sup> 0.127 orifice, 2.1 bar	0.0148	1.12	$1.3 \times 10^{-2}$	<i>E. coli</i> MC 4100	Present study
US 20 KHz	0.044	260	$1.68 \times 10^{-4}$	<i>E. coli</i> ATTC 11229	[35]
US 20 KHz	0.031	280	$1.11 \times 10^{-4}$	<i>E. coli</i> ATTC 11229	[35]
US 20 KHz	0.046	470	$9.79 \times 10^{-5}$	<i>E. coli</i> ATTC 11229	[35]
US 20 KHz	0.07	126	$5.55 \times 10^{-4}$	<i>E. coli</i> 0157:H7	[1]
US 20 KHz	0.10	290	$3.44 \times 10^{-4}$	<i>E. coli</i> 0157:H7	[1]
US 20 KHz	0.29	654	$4.43 \times 10^{-4}$	<i>E. coli</i> 0157:H7	[1]
US 20 KHz	42	12600	$3.33 \times 10^{-4}$	<i>E. coli</i> XL1-Blue	[36]

**Table 1. Rates of disinfection of *E. coli* normalized by power density applied for DYNAJETS<sup>®</sup> and Ultrasonic treatment.**

The disinfection rates and applied power densities from several citations from literature in which *E. coli* was removed using ultrasound are compared with those found in this study in Table 1 [1,35,36]. The literature references are from similar bench scale studies. The power densities of the ultrasound studies were calculated as the electrical energy applied to the ultrasound probe divided by treated volume.

$$Power\ density = \frac{Watt \times Surface\ Area\ of\ Tip}{Volume} \quad (7)$$

The power densities used in the DYNAJETS<sup>®</sup> studies were 2 to 4 orders of magnitude lower than in the ultrasound studies, and showed removal efficiencies that were 1 to 2 orders of magnitude higher than conventional ultrasonic devices. This finding is in

agreement with similar studies of efficiencies on hydrodynamic cavitation and ultrasonic cavitation [2,10, 34].

#### **4. CONCLUSIONS**

Comparison of the disinfection rates of gram-positive and gram-negative species under identical disinfection conditions showed that the gram-negative species with thinner cell walls were more enable to hydrodynamic cavitation than were the thick walled *B. subtilis*. Amongst the gram-negative species, the most resistant species was *P. aeruginosa*. The greater degree of cross-linking of the peptidoglycan in *P. aeruginosa* makes it more resistant to cell wall rupture. These findings support the conclusion that the kill mechanism of hydrodynamic cavitation is rupture of the cell wall.

*E. coli* and *B. subtilis* were further studied to determine the effects of nozzle geometry, operating pressure, temperature, and initial concentration. Nozzle pressure greatly affected disinfection efficiency of both *E. coli* and *B. subtilis*. The most efficient configuration for both species was the DYNASWIRL<sup>®</sup> nozzle operated at 2.0 bar nozzle pressure. This corresponds to a cavitation number of about 0.5.

The two species showed differing reactions to nozzle type. The *E. coli* reduction rates were independent of nozzle geometry at constant pressure and flow rates. However, *B. subtilis* disinfection was dependent on nozzle type. *B. subtilis* can be more effectively disinfected using the DYNASWIRL<sup>®</sup> nozzle at 2.0 bar, than the STRATOJET<sup>®</sup> nozzle at 6.2 bar. Thus nozzle design was more important than operating pressure in the disinfection of resistant gram-positive species.

Investigation of the importance of the initial microorganism concentration on the disinfection rate with the DYNASWIRL<sup>®</sup> shows that the microorganism disinfection is more efficient when the initial concentration is lower. High initial bacteria concentrations were found to delay the onset of disinfection. This may be due to higher viscosity or clumping of the biomass at concentrations above  $\sim 10^6$  CFU/ml.

The DYNASWIRL<sup>®</sup> system was also shown to reduce microorganisms and total coliforms in wastewater. Disinfection rates of *E. coli* were found to be independent of solution matrix. There was no significant difference found between disinfection in municipal wastewater and growth media. In terms of energy efficiency, the DYNAJETS<sup>®</sup> were 10 to 100 times more efficient than ultrasonic disinfection as compared to several studies published in the literature.

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