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The bactericidal effect of shock waves

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Abstract. There are a variety of theories relating to the origins of life on our home planet, some of which discuss the possibility that life may have been spread via inter-planetary bodies. There have been a number of investigations into the ability of life to withstand the likely conditions generated by asteroid impact (both contained in the impactor and buried beneath the planet surface). Previously published data regarding the ability of bacteria to survive such applied shockwaves has produced conflicting conclusions. The work presented here used an established and published technique in combination with a single stage gas gun, to shock and subsequently recover *Escherichia coli* populations suspended in a phosphate buffered saline solution. Peak pressure across the sample region was calculated via numerical modelling. Survival data against peak sample pressure for recovered samples is presented alongside control tests. SEM micrographs of shocked samples are presented alongside control sets to highlight key differences between cells in each case.

1. Introduction

The extent to which simple life such as bacteria has the ability to survive high pressure conditions in a dynamic environment has relevance to a variety of fields including mass-extinction events, panspermia and shock-sterilisation. It is now widely believed that the Cretaceous-Paleogene mass-extinction event was caused by a large asteroid and resulted in planet-wide ecological change and damage [1, 2]. There is now a large body of evidence backing up the claim that the crater formed as a result of this event has been found at Chicxulub, Mexico [3]. Although it is thought by some that this planetary impact event was likely the trigger required to push an already imbalanced ecosystem over the edge [4].

Recent research has produced discoveries of life-forms capable of surviving some of the harshest conditions known. One such example is the discovery of microbial activity at $\sim$ 11 km below sea level. This study showed that levels of oxygen consumption at this extreme depth were high and subsequent testing of sediment samples also showed the presence of microbial cells [5]. Another investigation in a similar field has shown that *Escherichia coli* has the ability to survive hydrostatic compression of up to (and possibly beyond) 2 GPa [6]. There has also been interest in the bactericidal effects of shockwave application from other fields such as medicine. Traditionally, extracorporeal shockwave lithotripsy (ESWL) has mainly been used for the destruction of stones in the human body, however development in the research of shockwave effects on bacteria has sparked interest in its use for the sterilisation of blood samples [7].

One method of investigating the resilience of life to dynamic pressures, is to apply shockwave treatment to bacterial samples in a shock-recovery experiment and then test the bacterial colonies for

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growth and/or analyse the samples using microscopic techniques. Similar investigations include a previous paper looking at the effects of shock loading on *Escherichia coli*, *Enterococcus faecalis* and *Zygosaccharomyces bailii* [8]. In this particular study by Hazell *et al.*, the flyer-plate technique was employed to shock load samples of these bacterial strains and cavitation was minimised by careful target design in order to study the effects of shock alone. Results from this investigation showed that *E. coli*, *E. faecalis* and *Z. bailii* were largely unaffected by conditions experienced in this particular loading regime.

This paper has investigated the effects of quasi-one-dimensional shock loading on samples of one strain of *E. coli* in order to try and analyse the effects of increasing peak shock pressure (PSP) on bacterial cells' ability to grow and attempt to understand the damage mechanisms governing these changes. The initial hypothesis expected a trend of exponential decay, with a large number of cells being capable of surviving PSP's of < 1 GPa and increasingly smaller numbers surviving higher PSP's as pressures increased towards 2 GPa. This hypothesis was based on results seen previously, concerning the ability of plant seeds to survive shockwaves [9] and bacteria (and other forms of life) to survive high hydrostatic pressures [10].

2. Experimental Design and Method

A dual-capsule system and the flyer-plate technique were employed to shock-load samples of *E. coli*. A schematic diagram of the target setup used in the flyer-plate experiments is shown in figure 1. In addition, a close-up half-section of the sample-containing region within the inner capsule is shown in figure 2, with the sample dimensions and locations of the nodal monitoring points employed in numerical modelling, labelled. For reference, further information on this method has been published previously by Leighs *et al.* [11]. Shockwave experiments were performed using a 50 mm bore, 5 m length single stage gas gun. Projectiles used were 5 mm thick Al flyer-plates, accelerated to 150 - 300 ms^{-1}, corresponding to PSPs of 0.8 - 1.9 GPa. Surfaces perpendicular to the impact axis were machined flat with a tolerance of $\pm$ 10 μm ensuring inertial confinement on impact. In target assembly, a bacterial sample volume of 7 μl was loaded into the inner capsule, which was sealed via an o-ring and attached inside the resin-filled sacrificial outer capsule. Flyer-plate targets were attached to an Al target ring, which was then attached to a sacrificial barrel extension to aid alignment. Flyer-plate velocities were controlled by varying the driving gas pressure prior to firing. Projectile velocities were measured using a sequential infrared light-gate system connected to an oscilloscope. The nature of this particular shock-recovery technique was such that it was not possible to measure loading histories directly so these were interrogated via ANSYS Autodyn^{TM} hydrocode modelling. The numerical model employed has been previously validated with practical experiments employing Het-v as a diagnostic and a slightly modified capsule design; this was published previously by Leighs *et al*. [11].

Bacterial samples used in all shock experiments in this investigation were *E. coli* NCTC 10538, sourced from Technopath (Riverbridge House, Leatherhead, Surrey UK). *E. coli* cultures used in these experiments were incubated in lysogeny broth (Better Equipped Educational Supplies Ltd, Ivy House, Wrenbury Heath Rd, Nantwich, Cheshire UK) for 18 hours at 37°C and 250 RPM.

Shocked samples were recovered following shock-loading experiments and (alongside samples of the original solution) serial dilutions were made and cultured on agar plates in order to calculate the concentration of viable cells remaining, in Colony Forming Units per millilitre (CFU/ml). In addition a further two experiments were performed in which bacterial samples were shocked to a PSP of 1.9 GPa. Recovered samples from these experiments were pipetted on to postlip blotting paper, fixed in a solution of 2.5% glutaraldehyde in 0.1M HEPES buffer (pH controlled to 7.2) and coated with a thin layer of Gold-Paladium, to enable subsequent scanning electron microscopy (SEM) analysis.

3. Results

A series of flyer-plate experiments were conducted on *E. coli* samples, which were subsequently recovered for post-shock analysis. The target setup employed was such that loading histories needed to be calculated via hydrocode modelling. An example pressure vs. time trace is shown in figure 3,

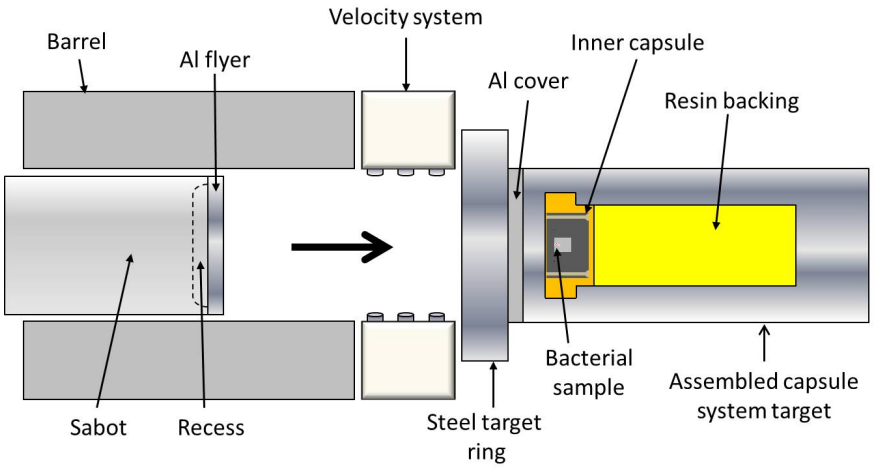


Figure 1. A basic diagram showing the target setup for shockwave experiments [9].

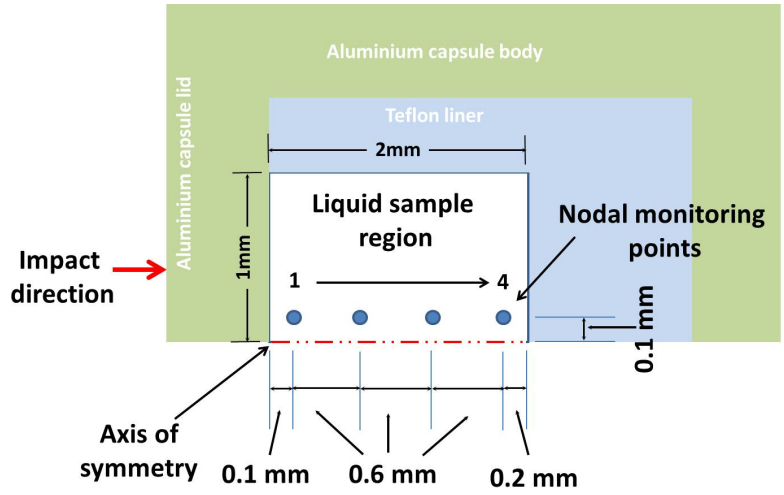


Figure 2. A schematic diagram outlining the setup of the inner capsule, highlighting the dimensions of the sample region and the location of the nodal monitoring points employed in numerical simulations. Note: the image shown is a half-section through the center of the target, with the axis of symmetry labelled.

with nodal monitoring points 1-4 being equi-spaced along the 2 mm deep sample region. This particular example is for a projectile velocity of 300 ms^{-1}, which corresponds to a PSP of 1.9 GPa. Percentage survival results are presented in figure 4, which were calculated via plate-counts of serial dilutions (using a pH-balanced, phosphate-buffered saline solution) comparing shocked and unshocked (control) results. To put the presented results into context, it is important to note that a percentage survival of 0.52% (the lowest PSP presented) corresponds to a drop in cell count from 2.03×10^{11} to 1.07×10^{9} colony forming units per millilitre (CFU/ml) following shock loading and subsequent sample recovery.

In addition, a series of two further shots were performed at the highest pressure, 1.9 GPa, following which samples were recovered for SEM imaging analysis in order to interrogate the damage mechanisms which may influence the cells' ability to grow and divide. From each of these two plate-impact experiments, the original sample was also fixed for imaging and these formed the control samples against which shocked results are contrasted. A series of representative micrographs obtained from the two

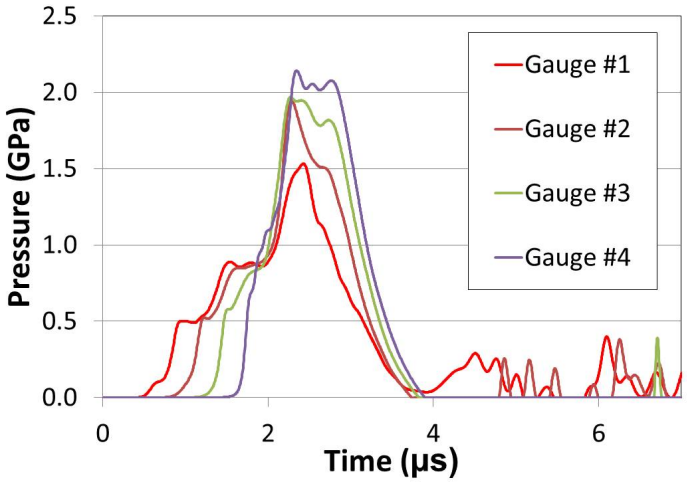


Figure 3. Pressure vs. time histories taken from ANSYS Autodyn^{TM} numerical simulations of the experimental setup for four nodal monitoring points equi-spaced along the 2 mm length bacterial sample holder. The example shown above is for a projectile velocity of 300 ms^{-1} corresponding to a PSP of 1.9 GPa (+0.24/-0.37) - this shows the level of variation of pressure across the sample region.

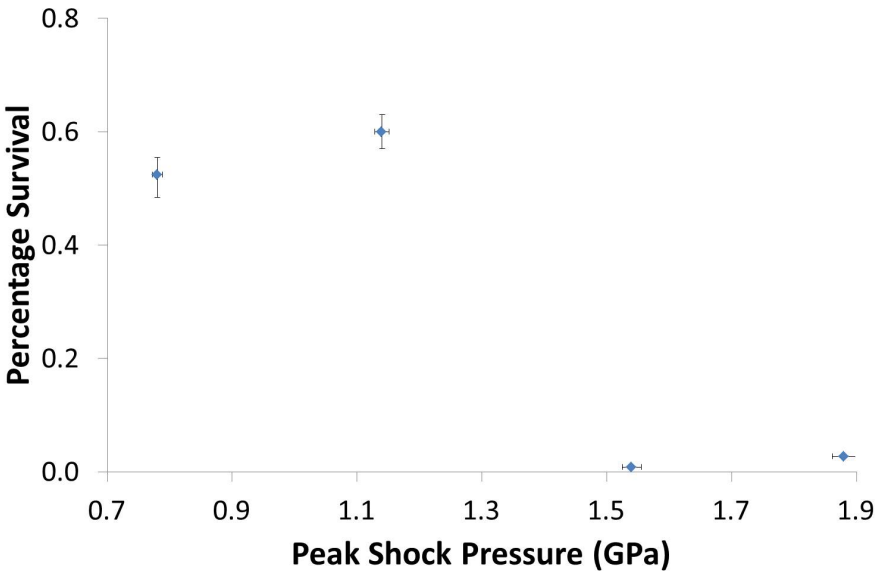


Figure 4. Percentage survival of *E. coli* populations as a function of loading pressure. Bacterial survivors were calculated from plate counts of serially diluted recovered samples.

control sets are shown in figure 5. SEM analysis of recovered samples from plate-impact experiments produced a large amount of data, however a select series of images is shown in figure 6 which highlight some of the key results found.

4. Discussion

It was initially anticipated that the rate of change of percentage survivability with increasing PSP would follow a trend of exponential decay. Although further investigation is required to confirm an exponential fit, figure 4 shows that there is a definite trend of decreasing survivability with increasing PSP. In more

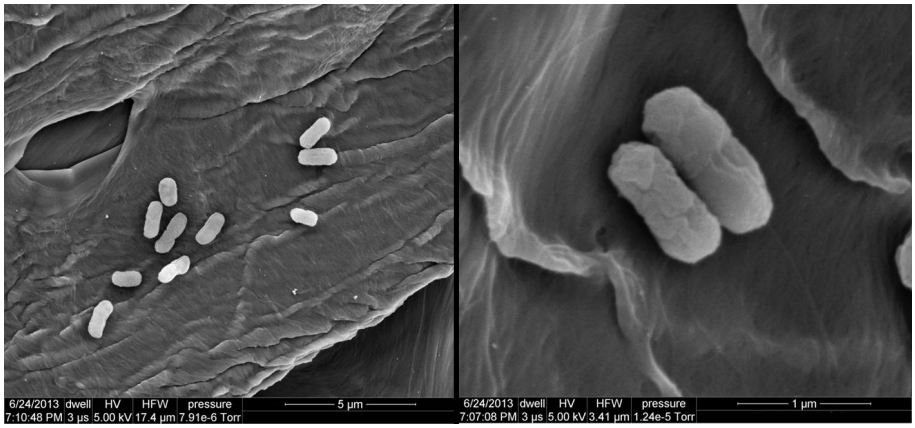


Figure 5. Scanning Electron Microscopy micrographs of *E. coli* control samples, which have not been subject to dynamic pressures.

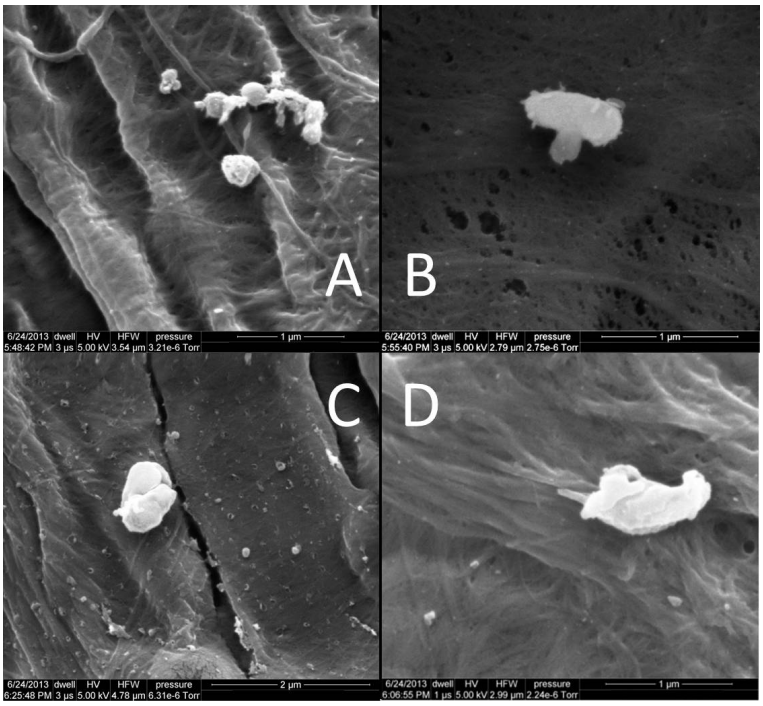


Figure 6. Scanning Electron Microscopy micrographs of *E. coli* samples subjected to shock loading with a PSP of 1.9 GPa.

detail, a PSP of $\sim$ 1 GPa has shown a 2-Log reduction, compared to a 3-Log reduction which was seen in experiments with a PSP $>$ 1.5 GPa (Note: a 2-Log reduction in a bacterial population refers to a drop of two orders of magnitude in living bacterial cells). As a result, the particular strain of *E. coli* investigated here appears to be quite susceptible to damage caused by shock-loading, with PSPs in the region between 0.8 and 1.9 GPa. This contrasts with results seen previously by Hazell *et al.* [8] where bacterial populations were largely unaffected by pressures within a similar range (ca. 1.2 GPa). It is thought the differences arising here are probably due to the difference in sample sizes and loading regimes. However, results seen previously by Willis *et al.* [12], did show a 4-Log reduction in *E. coli* populations experiencing shock loading with a PSP of 2.23 GPa.

Results from SEM analysis show clear differences between shocked and control sample sets. The control bacterial cells do appear to show some minor surface abnormalities, however this is thought to be an artefact of the sample preparation process, during which all of the water is removed from the cells. In addition, the control sample images appear to be relatively clean aside from the *E. coli* cells and the larger fibres from the postlip blotting paper. In contrast, the shocked images appear to show a large amount of 'debris' in addition to the cells and paper fibres. It is currently unknown what the debris consists of, however it is thought to be a combination of parts of broken bacterial cells as well as particulates remaining from the drilling of the teflon bacterial sample holder insert. Aside from this, the cells themselves appear to show a variety of deformities; it is impossible to definitively identify the very deformed shapes as *E. coli* cells. However, given the approximate length scales are very similar to the control cells, A-D (figure 6) are thought to be remains of previously viable cells. Image A appears to be a severely broken up cell, while images B and C seem to have suffered cell wall deformation or lysis (cell disintegration via cell membrane and/or wall rupture) and finally image D looks as though it has been substantially deformed.

5. Conclusion

E. coli samples have been subjected to quasi-one-dimensional shock-loading using a capsule-within-a-capsule system [9] to PSP's of 0.8 - 1.9 GPa. Recovered bacteria have been tested for survivability using plate-counts of serially diluted samples. Results from serial dilutions suggest some validity of the initial hypothesis of expecting exponential decay of survival with increasing PSP. However, further work is required in order to confirm the exact form of this relationship and to test the survivability beyond the pressures reached here. SEM analysis of recovered samples from two separate experiments which saw samples of *E. coli* being shocked to 1.9 GPa, has shown clear differences between examples of shocked and control cells, although further work is underway in order to analyse exactly what is being seen in the SEM micrographs.

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