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On the response of *Escherichia coli* to high rates of deformation

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Abstract. While a large body of work exists on the low strain-rate loading of biological systems such as bacteria, there is a paucity of information on the response of such organisms at high rates of deformation. Here, the response of a readily accessible strain of bacteria, *Escherichia coli* (*E. coli*), has been examined under shock loading conditions. Although previous studies have shown greatly reduced growth in shock conditions up to several GPa, relationships between loading conditions and bacterial response have yet to be fully elucidated. Initial results of a more rigorous investigation into the 1D shock loading response of *E. coli* are presented here, expectantly leading to a more comprehensive view of its behaviour when exposed to high pressures. Comparison has been drawn to provide insight into the importance of the nature of the loading regime to the survival of these biological systems.

INTRODUCTION

There are a number of reasons for studying the effects of high pressures on organisms and biological materials and, in line with the focus of this study, especially shock pressures. From the sterilisation of foods by pressure loading bacteria to gaining a better understanding of the types of micro-organisms that survive in extreme environments, there has been a surge in research on the high pressures on a variety of organisms. More specifically, and within the scope of this paper, there have been a number of investigations into how micro-organisms might fare in the face of panspermia (the possible transfer of life and its building blocks through space) and equally, the extinction of life that can be caused by such an occurrence. In order to survive transfer through space, an organism must be capable of surviving the pressures and temperatures involved in their ejection into space and exposure to other risks such as UV radiation [1, 2].

There is evidence to support the resilience of at least small percentages of some microbial life under extremely high pressures and temperatures. In fact, recent evidence of amino acid formation upon impact of an ice mixture found on comets [3] has led to more questions about not only whole cells surviving asteroid impact pressures, but also individual cellular components. Pressures that are associated with asteroid impact are in the range of 1-100 GPa. It was also shown by Melosh (1984) [4] that upon asteroid impact onto a planetary body, small ejecta (between 1 and 5% of the mass of the original impactor) can result and manage to escape actual shock pressures. This could potentially mean even greater rates of survival for micro-organisms exposed to these impact events.

More evidence has been gathered in support of the concept of panspermia and lithopanspermia (the transfer of life through space via rocks) with examinations of the survival rates of bacteria, including *Escherichia coli* and spores of *Bacillus subtilis*, on the surface of rocks undergoing dynamic impact [5, 6]. Dynamic pressure loading of *B. subtilis* cells by Burchell *et al*. [7] even showed survival rates of 10^{-7} at pressures of close to 100 GPa; within the region of pressures faced by rock ejection into space and asteroid impact. A more complex eukaryotic organism,

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Saccharomyces cerevisiae (baker's yeast), was also investigated in a similar manner by Price *et al*. [8] and found to have a survival rate of ~10^{-4} at a peak pressure of ~43 GPa.

While shock loading of micro-organisms has become more extensive, the area of interest here is in quasi-one dimensional loading of these biomaterials. Loading regimes likely play a part in micro-organism survival rates, evidence for which has been seen from contrasting *E. coli* survival rates between particular past studies [5, 9]. However, this paper aims to provide additional data to previous work on the one-dimensional shock loading of *E. coli* by Leighs *et al*. [10] and may contribute to further understanding of what the nature of the loading regime does to bacterial survival before eventually examining what mechanisms may be affected within the cell.

EXPERIMENTAL TECHNIQUE

The shock loading experimental set-up, outlined in Fig. 1, included the plate-impact technique carried out on a 50 mm bore single stage gas gun for quasi-one-dimensionally loading the bacteria samples. *E. coli* NCTC 10538, a genetically modified lab-safe strain of this bacterium, was used in this investigation. Lysophilised (freeze-dried) pellets of the bacteria were rehydrated and incubated overnight at 37°C for 18 hours (based on previous measurements of their growth curve to encourage maximum production of colonies) [10]. The incubated *E. coli* broth was then introduced to the aluminium capsule system [11] shown in Fig. 1, within a Teflon® (PTFE) liner which held 6 μl of broth.

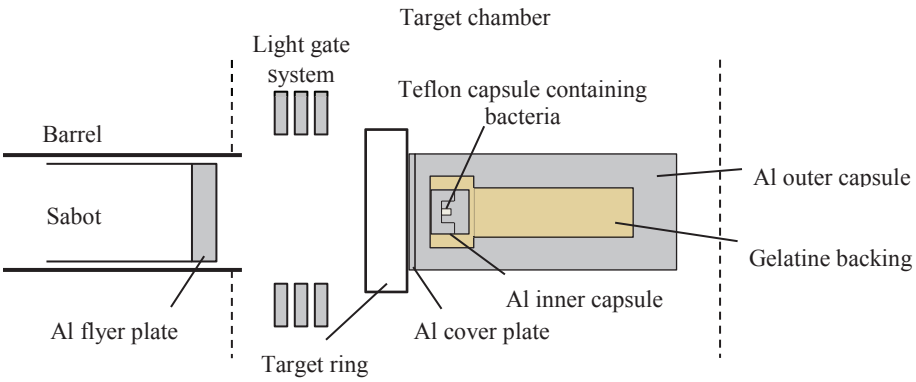

FIGURE 1. Experimental set-up with the Al capsule and Teflon system in the target chamber of the 50 mm bore gas gun.

The purpose of the Teflon liner was to ensure a quasi-one-dimensional shock wave for as long as possible through the sample and to attenuate the shock to prevent any excess ringing and reduce the effects of rarefaction. The liner was overfilled to avoid cavitation in the bacterial broth during the shock. The cavity in the larger Al capsule was filled with 20% ballistic gelatin to also attenuate the shock and reduce rarefaction. In place of pressure gauges to measure pressure during the shock loading event, peak shock pressures reached for each bacterial sample were measured using a Lagrangian model employed via ANSYS Autodyn®.

After shock loading, the bacterial broth was plated on an agar nutrient medium and incubated for 18 hours. The process was repeated for the control samples which consisted of un-shocked *E. coli* from the original broth. After incubation, the *E. coli* colonies were counted and survival rates calculated according to population measured in colony forming units (CFU) per millilitre.

RESULTS AND DISCUSSION

Shock profiles from the hydrocode models provided the mean peak pressures reached for the three shock loading experiments carried out in this investigation, which are listed in Table 1. Error in the pressure measurements was considerably reduced by validation of the models by previous Heterodyne velocimetry experiments to calculate real shock pressures [10]. A representative depiction of the modeled Teflon liner and the points at which pressures were

measured during simulation is shown in Fig. 2. Four peak pressures were obtained for each experiment in order to obtain the mean peak pressure experienced by the bacteria within the capsule. An example of the modelled shock profiles for one experiment is displayed in Fig. 3.

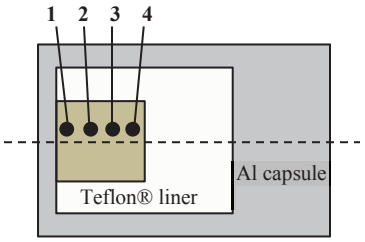

FIGURE 2. Teflon® liner (4 mm x 4 mm) filled with bacterial broth. Dashed line depicts the axial symmetry used in the hydrocode models. Gauges in the models are labelled 1-4.

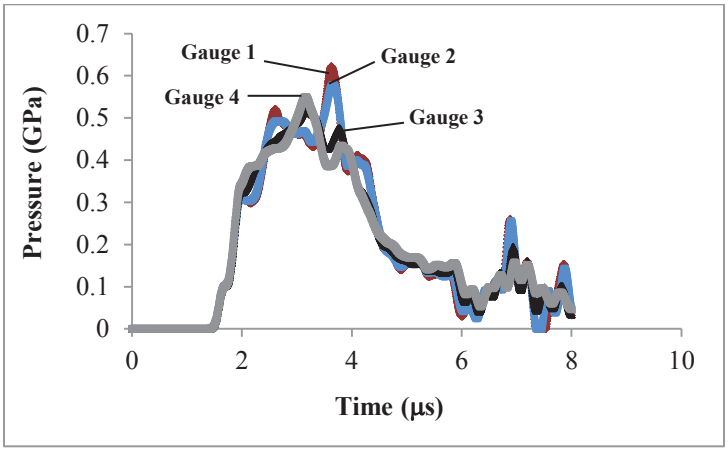

FIGURE 3. Shock profile from ANSYS Autodyn® model showing the peak pressures from four different gauges within the bacterial broth. The mean of these pressures was taken as the final peak pressure (in this case 0.55 GPa).

TABLE 1. Impact velocity, peak pressure and percentage survival for each shot on E. coli NCTC 10538; comparison between data from this study with previous data (Leighs *et al*. 2014).

Study	Velocity (ms^{-1})	Pressure (GPa)	% Survival
Present data	152	0.55	6
Present data	233	1.2	1
Present data	247	1.3	0.08
Leighs et al., 2014	181	0.78	0.52
Leighs et al., 2014	223	1.1	0.6
Leighs et al., 2014	265	1.5	0.01
Leighs et al., 2014	298	1.88	0.03

Upon calculating the population of *E. coli* colonies, survival rates were determined and plotted alongside the preceding data (Table 1, Fig. 4). Results by Leighs *et al*. [10] revealed relatively low rates of survival and showed slightly more sensitivity to shock pressures in the 1 GPa range compared to those found in this study. The two data points from the present investigation that were plotted above 1 GPa compare relatively closely with the previous data, although the rates of survival show some variance between the two studies, despite being carried out within the same pressure regime. The most significant variance is the 6% survival rate observed at 0.55 GPa, although this rate of survival is debatable since the next highest pressure obtained by Leighs *et al*. [10] was 0.78 GPa with a survival rate of 0.52%. Further work into quasi-one-dimensionally shock loading *E. coli* at these pressures would help to verify these data and possibly reduce scatter while confirming where survival increases at the lower end of this scale. However, the apparent exponential decrease in survival with pressure increase in the present data does match up with previous work on *E. coli* and other types of bacteria [5, 7], while demonstrating that the nature of the loading regime likely effects survival. It is also clear from both investigations that there is a drop in magnitude of survival within the 1–1.5 GPa range.

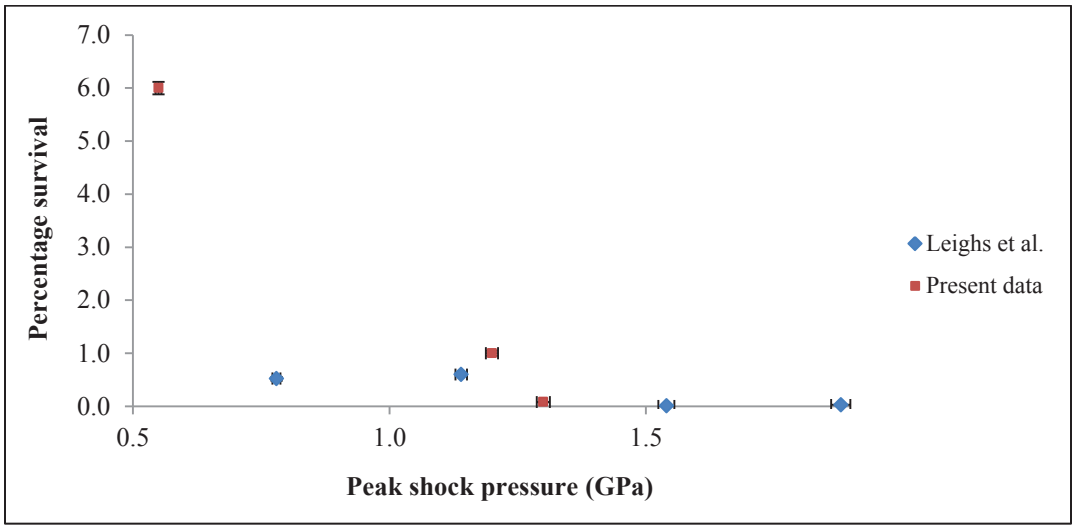

FIGURE 4. Comparison of percentage survival rates of *E. coli* found during the present study and previously by Leighs *et al*. (2014) within the 1 GPa range.

CONCLUSIONS

In an attempt to provide a more detailed view of the behavior of *E. coli* NCTC 10538 under shock loading conditions this study has provided new data to be considered with the previous work carried out on this bacterium. This was achieved by varying pressures to observe where the *E. coli* fit on the survival-pressure curve. Pressures in this investigation ranged from 0.55 GPa to 1.3 GPa with a possible exponential decline in survival rates from 6% to 0.08%. The discrepancies found between the current and previous set of experiments may be noteworthy, although with the current focus on a relatively small range of pressures it remains to be seen whether scatter in the data would be as significant on a larger scale. Ultimately, it would be of interest to continue shock loading at both higher and lower velocities to get a better sense of *E. coli* survival rates over a wider range of pressures. This would also be in the interest of panspermia which sees a pressure range of 1-100 GPa. While attempting to reach quasi-one-dimensional shock pressures at low MPa would be arguably more challenging, to observe differences in survival rates on a broader scale would be of importance for future in-depth studies of cellular mechanisms governing these responses. Further investigation would aim to see how particular cellular components and the biochemistry of *the E. coli* cell are affected in order to understand the effects of shock loading at a more fundamental level.

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