The effects of quasi-one-dimensional shock on *Escherichia coli* while controlling pressure and temperature

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Abstract

The response of microorganisms to high pressures is of growing interest in the literature, regarding areas of research including the sterilisation of foodstuffs, panspermia and, more generally, the study of extremophiles. When examining organisms under shock pressure, there are a number of caveats that need to be considered, including temperature and the nature of the shock wave front. Both of these caveats have been explored in this study through the application of the plate impact technique to create quasi-one-dimensional shock waves with controlled shock fronts through bacterial targets. This was achieved using typical planar flyer plates to study the dynamic pressure response of the bacterium, *Escherichia coli* NCTC 10538. Additionally, in order to create an adiabatic, off-Hugoniot loading path, a novel graded areal density flyer produced by the Surfi-Sculpt® approach was used to assess the effects of lowering temperature during shock on *E. coli* growth rates. The maximum temperature generated by a Surfi-Sculpt® flyer impact was 5 K less than that produced by the planar flyer analogue. Higher growth rates of bacterial colonies post-impact by the Surfi-Sculpt® flyer compared to those by the planar flyer were observed, with this behaviour determined to be a possible function of the nature, although temperature was also decreased with the use of this adiabatic ramp loading technique. In an effort to purposefully increase pressure and temperature for the *E. coli* samples, a modified form of a previously developed bacterial encapsulation system was also employed in this study, allowing pressures of up to 10 GPa and growth rates of up to 0.09% to be reached.

Keywords: Shock pressure; adiabatic loading; Surfi-Sculpt® method; *Escherichia coli*; growth rates; temperature response
1. Introduction

Pressurisation of microorganisms using a number of hydrostatic and hydrodynamic loading techniques has been documented in the literature (Ono et al., 2010; Vanlint et al., 2011; Price et al., 2013; Picard et al., 2014; Hazael et al., 2014). Interest in the pressurisation of microorganisms exists for a number of different fields, including; the food industry (Considine et al., 2008), the study of deep sea organisms (Picard et al., 2014; Picard and Daniel, 2013), panspermia and the origins of life on this planet, potentially via asteroid impact (Burchell et al., 2004). A number of simulated asteroid impacts have also been carried out to study the production of the building blocks to life. Such studies include the production of RNA nucleobases from formamide using high-power lasers (Ferus et al., 2015) and the formation of amino acids following the impact of cometary ice analogues (Martins et al., 2013). These findings, among others, have led to the consideration of asteroids and meteors as vehicles for the transport of life or its precursors to earth. However, exposure to different caveats including overpressure – 25-45 GPa for the impact of the Tenham meteorites (Langenhorst et al., 1995), for example – elevated temperature and UV ionising radiation must be overcome for any organism to survive asteroid impact and subsequent ejection into space (Fajar-Cavazos et al., 2009; Mileikowsky et al., 2000).

As part of the study of panspermia and lithopanspermia, various microorganisms have been examined under dynamic pressure loading. These experiments have included hypervelocity launches of *Bacillus subtilis* spores at 57.1 GPa with subsequent growth rates on the order of $10^{-5}$ (Fajardo-Cavazos et al., 2009) and, more recently, highly controlled 1D loading of *Shewanella oneidensis* (Hazael et al., 2017). The latter study indicated that this organism may be pressure adapted to survive higher dynamic pressures than wildtype strains.

The research presented in this paper, following on from initial 1D dynamic loading experiments (Fitzmaurice et al., 2017), focuses on *Escherichia coli* NCTC 10538, a bacterium typically less well adapted to extreme environments, unlike other more robust organisms such as *S. oneidensis* and *B. subtilis*. However, certain strains of *E. coli* have been found to reproduce even at temperatures up to 49 °C (Fotadar et al., 2005), likely due to mutations of proteins occurring during heating allowing greater survival. *E. coli* has also been reported to grow at temperatures between 23 and 40 °C, but population growth slows with increasing pressure (Kumar and Libchaber, 2013). In a study by Russell and Fukunaga, lipid composition was determined to be altered when cell membranes are exposed to high temperatures (Russell and Fukunaga, 1990). Hydrostatic assessments of *E. coli* in both the MPa and GPa ranges (Black et al., 2013; Vanlint et al., 2011) have led to more in depth proteomic and genomic studies to be carried out. In an experiment carried out by Aertsen *et al*., promoter genes involved in the heat shock response of *E. coli* were found to also be induced by static high pressure loading (Aertsen et al., 2004b). This was followed by further high pressure experiments which identified the activation of proteins that have previously been found to be induced as a response to DNA damage (Aertsen et al., 2004a).

In light of both hydrostatic and hydrodynamic pressure studies on organisms, temperature is a vital component to sustaining life and one that may be difficult to control in the case of dynamic pressure loading, particularly. Hence, the study here focuses on a novel method of controlling temperature to avoid this problem during dynamic compression. A number of techniques, including magnetic flux, lasers and gas guns, as in this particular study, have been used to apply dynamic adiabatic loading, or ramp waves, across various media (Orlikowski et al., 2007; Taylor et al., 2014; Winter et al., 2015, 2014). Ramp wave loading may be carried out using a number of different techniques, from layered impactors with shock impedance gradients to
graded areal density flyer plates (impactors with a gradient density across the structure). Ramp waves can provide a greater understanding of the quasi-isentropic nature of such loading and there has been a keen interest to quantify the dissipation of energy in these quasi-isentropic processes in recent years (Orlikowski et al., 2007; Ray and Menon, 2011). Furthermore, the low temperature regime may prove useful for other types of temperature-sensitive targets, such as biological materials.

Graded areal density flyer plates are often produced by additive manufacturing techniques such as Toll Ceramic Stereolithography (CSL) and Selective Laser Melting (SLM) (Taylor et al., 2014; Winter et al., 2015). In the current investigation, ramp waves were yielded by a Surfi-Sculpt® flyer plate. This novel Surfi-Sculpt® (TWI Ltd) method involves the displacement of material to create texture across a planar surface. Previous studies using the Surfi-Sculpt® technique have been carried out to examine their effects on flat plates of stainless steel 316 (Painter et al., 2017). These flyers have allowed two of the main caveats to shock loading biological samples to be examined; the nature, or shape, of the wave front and the loading path experienced by the target and subsequent temperature generated. These have been addressed throughout this study using the plate-impact technique with Surfi-Sculpt® flyers to pressurise *E. coli* NCTC 10538.

2. Methods and materials

2.1 Plate-impact experiments

The plate-impact technique was used to generate a quasi-one-dimensional shock loading path on the bacterial samples. Stainless steel 316 Surfi-Sculpt® (Thomas et al., 2008) flyers (Fig. 1 a) with a 10-mm thick base and surface spikes of 1.5 mm (± 0.2 mm) were used for comparison with 10-mm thick (standard) planar flyers at each different pressure. The experiments were carried out on the 50-mm bore single stage gas gun at Cranfield University (Bourne, 2003).

![Figure 1. Surfi-Sculpt flyer plate (10 mm) with 1.5 mm spikes (a). Inner Al capsule (10 mm) with Teflon liner (6 mm) (b).](image)

A previously validated Al capsule design (Leighs et al., 2012) was used for the majority of the bacterial experiments, as outlined in Fig. 1 b and Fig. 2. This essentially comprised an outer Al capsule (designed to confine and capture samples post-shot) along with a smaller, Teflon lined, inner capsule (see Fig. 2). The use of a Teflon liner for the inner capsule provided both a biologically compatible environment for the bacteria under test, as well as a route to ‘wave shape’ incident shocks, ensuring a quasi-one-dimensional loading. Furthermore, in order to facilitate the production of higher pressure shock and ramp waves a modified version of the
capsule was also employed; this was comprised of Al inner and outer capsules along with a backing of 20% ballistic gelatine. A second set of Cu capsules were used for the higher pressure experiments. Two shots were carried out using this modified capsule design with an added layer of Mylar® to avoid contact with the bacterial broth. The bacterium used was *Escherichia coli* NCTC 10538. Lyophilised pellets of this strain were obtained from Microbiologics® and these were hydrated with phosphate buffered saline (PBS) before being added to Sigma-Aldrich® LB nutrient broth No. 2. This broth was prepared under aseptic conditions. After an 18 hour incubation period, 6 µl of the bacterial broth was added to a Teflon liner so that it was overfilled to prevent cavitation during plate-impact. The Teflon was then placed inside the aforementioned inner Al capsule (Fig. 1 b), before the entire assembly was secured inside the larger outer capsule, backed with ballistic gelatine in line with the schematic presented in Fig 2.

![Figure 2](image_url) Exploded diagram of the target set-up inside the target chamber of the single stage gas gun.

2.2 Hydrocode modelling

In order to determine the average peak pressures and estimate peak temperatures in the shocked samples between the Surfi-Sculpt® and planar flyers, Lagrangian hydrocode simulations via ANSYS® Autodyn were employed for each impact velocity. The material properties employed for the models are outlined in Table 1. Similar models have been previously validated with Heterodyne velocimetry experiments (Leighs et al., 2012), but in order to confirm the incoming pressures and temperatures to the modelled Teflon capsule in this study, a series of experiments employing manganin piezoresistive gauges and nickel temperature gauges were undertaken with the aim of replicating the work of Rosenberg *et al.* (Rosenberg et al., 1980; Rosenberg and Partom, 1981). Essentially, measured pressures and temperatures were compared to modelled data of Rosenberg’s study. It should be noted that the models for the bacterial experiments were not used to measure the length of time required by the sample to reach thermal equilibrium, as Autodyn is not reliable in this regard. The models were used to estimate the peak temperatures reached inside the Teflon capsule only. Temperature gauges estimated the peak temperature reached at a particular pressure. An example is shown in Fig. 3 where a peak temperature of 41.5 °C was found following an impact velocity of 205 m s⁻¹. This was compared to modelled data of the same impact velocity with a peak temperature of 44.85 °C (Fig. 4).
Table 1. Material properties for ANSYS® Autodyn simulations. All models were run using a 2D Lagrangian mesh. Five monitoring (gauge) points were located along the inside of the Teflon capsule and were subsequently used to obtain a mean pressure across the bacterial medium.

<table>
<thead>
<tr>
<th>Material</th>
<th>Strength model</th>
<th>Density (g cm(^{-3}))</th>
<th>Gruneisen coefficient</th>
<th>Thermal conductivity (J m(^{-1}) K(^{-1}) s(^{-1}))</th>
<th>Specific heat capacity (J kg(^{-1}) K(^{-1}))</th>
<th>Reference EOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>S/steel 316</td>
<td>Piecewise JC</td>
<td>7.86</td>
<td>1.67</td>
<td>N/A</td>
<td>4.23 x 10(^{2})</td>
<td>Matsuka, 1984</td>
</tr>
<tr>
<td>Copper</td>
<td>Piecewise JC</td>
<td>8.9</td>
<td>2.0</td>
<td>403</td>
<td>3.90 x 10(^{2})</td>
<td>Matsuka, 1984</td>
</tr>
<tr>
<td>Al 6061-T6</td>
<td>Steinberg-Guinan</td>
<td>2.703</td>
<td>1.97</td>
<td>247</td>
<td>8.85 x 10(^{2})</td>
<td>Steinberg, 1991</td>
</tr>
<tr>
<td>Teflon</td>
<td>von Mises</td>
<td>2.16</td>
<td>0.9</td>
<td>0.25</td>
<td>1.05 x 10(^{3})</td>
<td>Matsuka, 1984</td>
</tr>
<tr>
<td>Water</td>
<td>N/A</td>
<td>1.0</td>
<td>0.28</td>
<td>0.609</td>
<td>4.18 x 10(^{3})</td>
<td>Nagayama et al., 2002</td>
</tr>
</tbody>
</table>

Figure 3. Temperature profile an impact velocity of 205 m s\(^{-1}\). The front trace plateaus at 41.5 °C before gauge death.

Figure 4. Modelled temperature profile for PMMA following an impact velocity of 205 m s\(^{-1}\). The peak temperature measured at the front gauge was 318 K.
3. Results

A series of experiments were conducted within the pressure range 0.7 - 10.0 GPa. From the data presented in Table 2, it was observed that the growth rate of *E. coli* colonies appeared to decay exponentially under increasing shock pressure; this was seen with both planar and Surfi-Sculpt® flyers. Of particular note, there was a noticeable difference between colony forming units at lower pressures than at higher pressures. This relationship is shown in more detail in Fig. 5, with the nominally linear relationship between the logarithm of the growth rate and impact pressure demonstrating the aforementioned exponential relationship. Growth rates at the lowest pressure (0.7 GPa) were 2% for the planar flyer and 3% for the Surfi-Sculpt®, each with a temperature difference of 4 K. At pressures reaching up to 10 GPa, a growth rate of 0.09% was seen with each flyer type. The higher pressures also led to less variation in temperature for both flyer plates.

<table>
<thead>
<tr>
<th>Flyer type</th>
<th>Capsule material</th>
<th>Flyer velocity (m s⁻¹)</th>
<th>Average pressure (GPa)</th>
<th>Average temperature (K)</th>
<th>Growth rate (%)</th>
<th>Colony Forming Units (CFU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P SS</td>
<td>Al</td>
<td>107 (±3%)</td>
<td>0.7 (±7%)</td>
<td>315 (+0.1/-0.2%)</td>
<td>2 (+25/-17.2%)</td>
<td>2.3x10⁷</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>311 (±0.2%)</td>
<td></td>
<td>3 (+24.5/-21.3%)</td>
<td>1.51x10⁸</td>
</tr>
<tr>
<td>P SS</td>
<td>Al</td>
<td>173 (±1.2%)</td>
<td>1.2 (+4/-5%)</td>
<td>320 (±0.2%)</td>
<td>1 (+19.2/-21.9%)</td>
<td>1.14x10⁷</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>315 (+0.4/-0.3%)</td>
<td></td>
<td>0.9 (+20.1/18.7%)</td>
<td>1.8x10⁷</td>
</tr>
<tr>
<td>P SS</td>
<td>Al</td>
<td>316 (±1%)</td>
<td>3.6 (±6%)</td>
<td>333 (±0.6%)</td>
<td>0.01 (+10.7/-12%)</td>
<td>8.4x10⁵</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>330 (+0.9/-0.5%)</td>
<td></td>
<td>0.1 (+15.2/-10.8%)</td>
<td>1.1x10⁶</td>
</tr>
<tr>
<td>P SS</td>
<td>Al*</td>
<td>385 (±4.6%)</td>
<td>4.5 (+4/-9%)</td>
<td>336 (±1%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>334 (+1.2/-1.1%)</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P SS</td>
<td>Cu</td>
<td>175 (±1.4%)</td>
<td>6.6 (+5/-12%)</td>
<td>335 (+0.3/-0.5%)</td>
<td>0.06 (+9.6/-6.8%)</td>
<td>3.3x10⁴</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>332 (±0.6%)</td>
<td></td>
<td>0.1 (+4)</td>
<td>2.22x10⁴</td>
</tr>
<tr>
<td>P SS</td>
<td>Cu</td>
<td>268 (±3.2%)</td>
<td>10.0 (+15/-16%)</td>
<td>341 (+1/-2%)</td>
<td>0.09 (+1.7/-0.5%)</td>
<td>6.0x10⁴</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>341 (+1.4/-1.2%)</td>
<td></td>
<td>0.09 (+2.6/-3.5%)</td>
<td>3.33x10⁴</td>
</tr>
</tbody>
</table>

Table 2. Summary of results from each dynamic loading experiment and resulting growth rates of bacterial colonies. There is a general trend of increasing temperature with pressure with a greater temperature difference between both planar (P) and Surfi-Sculpt® (SS) flyer types at lower pressures. *The Al capsule failed at this pressure, preventing safe retrieval of the bacterial solution.
In order to validate the peak temperatures measured by the hydrocode models, nickel temperature gauges were employed as described by Rosenberg & Partom (1981). Temperature duration was not compared since this factor could not be reliably recorded by the hydrocode. The models were therefore employed to measure “worst case scenario” peak temperatures. Errors in temperature, based on four gauge points within the modelled Teflon capsule were found to be ≤ 2 % in each case (Table 2).

The prototype Surfi-Sculpt® flyers created ramped waves through the target, which were more pronounced at lower impact velocities. As expected, the waves produced by Surfi-Sculpt® flyers at higher pressures gradually produced more 1D traces than at lower pressures; this is illustrated in Fig. 6 which compares modelled impact pressures and peak temperatures (based on the data presented in Table 2). As a result, the highest-pressure experiment at 10 GPa yielded the same temperature for both flyer types (341 K), but there were noticeable changes in temperature at lower pressures, e.g. at 0.7 GPa there was a temperature difference of 4 K (Fig. 4 and Fig. 5). Peak pressures were found to be the same with a maximum error of 7% at the lowest pressure (0.7 GPa) and a maximum of 16% for the highest pressure reached (10 GPa).

In each case in Fig. 6, even at the lowest impact velocity of 107 m s⁻¹, ramped traces are visible for the Surfi-Sculpt® flyers. Although a modest ramp is also seen for the planar flyer at lower velocity impacts, which will be discussed in the next section, there is a distinct difference between the overall rise times for both flyer types (Fig. 6). At the lowest impact velocity, the planar pulse had a duration of ~ 4 µs, while the Surfi-Sculpt® flyer produced a pulse lasting ~ 5.5 µs. The differences in temperatures recorded for each experiment became smaller as the pressure increased.
Figure 6. Sample pressure and temperature traces from within the Teflon liner using planar and Surfi-Sculpt® flyers at two different impact velocities, (a) Pressure profile from Al capsule at 107 m s⁻¹; (b) temperature profile from Al capsule at 107 m s⁻¹; (c) pressure profile from Cu capsule at 268 m s⁻¹; (d) temperature profile from Cu capsule at 268 m s⁻¹.

In order to produce higher shock pressures, Al capsules were replaced with Cu capsules for use during higher velocity impacts. The outer Al capsule failed at 4.5 GPa with no retrievable sample from the Teflon liner; Cu, with a greater density and shear modulus was used for obtaining pressures above this (up to 10 GPa). Although Cu is generally bio-incompatible, this issue was avoided by adding a layer of 50 μm Mylar® to the underside of the inner capsule lid, acting as a barrier between the lid and the surface of the Teflon liner.
4. Discussion

The ability of *E. coli* survivors to form colony forming units up to pressure at least 10 GPa in these experiments suggests that the quasi-one-dimensional shock waves produced during these experiments are likely to invoke a protective response in the bacterium. Survival of these longitudinal waves may depend on the viscoelasticity of the cell wall and the ability for the cell to maintain its integrity under shock compression. Organisms have even been shown to survive high pressure conditions under static compression (Sharma, 2002) and more recently under 1D dynamic compression (Hazael et al., 2017), despite a high probability of ice phases occurring at these pressures. The fact that specific proteins, such as RecA and LexA in the *E. coli* SOS mechanism, are known to be involved in the static high pressure response of *E. coli* (Aertsen et al., 2004a) suggests another possible mechanism for their survival during a much shorter loading period induced here; a pulse length of ~ 4 µs at the lowest pressures and 2 µs at the higher pressures. The production of controlled shock wave fronts as part of this study may also promote greater numbers of survivors than would be seen as the result of a radially expanding shock wave. The relatively simplified loading path that was followed in this particular study by impact using the planar flyers frequently resulted in lower growth rates for the *E. coli* than their Surfi-Sculpt® counterparts. Given the relative decrease in growth rates for the planar flyers, it may be inferred that elevated temperatures reached as a result of the planar shock wave at lower pressures may affect the mechanisms governing growth rates.

The dynamic loading profiles from the Surfi-Sculpt® and planar flyers in each experiment described here resulted in different loading paths but similar pressure plateaus in every case. While ramped waves traversed the entire target after Surfi-Sculpt® impact, there was a greater time delay for the peak pressure to be achieved and this facilitated temperature control throughout the loading process. The lower velocity impacts for the Surfi-Sculpt® flyers have
resulted in more exaggerated ramped traces as expected, although this would likely be pronounced with longer spikes on the flyer surface. In addition, at lower velocities the traces for the planar flyers indicated a slight ramped wave occurring (due to the complexity of the target capsule construction), although in these cases the associated temperatures inside the capsule were consistently higher. Since changes in temperature were not statistically significant overall between the two flyer types, but there are differences in the temperatures reached at different impact pressures, it is likely that the differences in growth rates seen were due to the nature of the dynamic loading path.

As stated previously, the adiabatic pathway is followed by the use of these graded areal density flyers in dynamic compression. The Hugoniot and isentropic curves for water from Nagayama et al. (2002) suggest the pathway followed by the bacterial broth in these experiments lies between the Hugoniot and the isentrope and therefore does not follow a traditional shock loading path. This is also likely due to the overall complexity of the target; the thickness of the capsule and the transition from high impedance materials (stainless steel and aluminium) to lower impedance materials (PTFE and bacterial broth) which may dampen the wave once it reaches the Teflon liner.

Additionally, the use of a Cu capsule, with a greater shock impedance than Al, facilitated higher pressures being induced inside the Teflon capsule that could not be achieved with the original capsule design. Since Cu has a greater shear modulus than Al, the integrity of the Cu capsule was sustainable under higher impact velocities, which allowed the bacteria to be reliably and safely retrieved from the capsule. The survivability of the E. coli could then be explored under a greater pressure range (up to 10 GPa).

5. Conclusions

A series of plate-impact experiments have been conducted using both planar and complex (Surfi Sculpt®) impactors with the dual aims of establishing a novel technique to allow for enhanced temperature control of biological systems during shock loading, as well as the investigation of the effects of temperature on such systems under shock. In terms of the response of the E. coli, a general exponential decay in growth rate with impact pressure was apparent in all cases. However, interestingly, at a given pressure, use of a Surfi Sculpt® flyer – and the associated decrease in shock temperature (at lower pressures) – led to a consistent higher growth rate of survivors (bar a single data point where sample recovery was compromised). This implies that there may be a particular mechanism or mechanisms governing growth rate, which respond to these changes in temperature/load path at the pressures investigated. The resultant number of survivors may also be a result of the nature of the ramp loading vs a more 1D shock wave front. Equally, the ability to reach these shock pressures well into the GPa range offers new opportunities to examine the effects of controlled dynamic pressure on a variety of microorganisms. While the particular intercellular mechanisms governing the growth rates of E. coli under these conditions are yet to be determined, ongoing studies in protein responses to pressure loading may offer some insight into the subcellular effects of dynamic compression.
6. Acknowledgements

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References


