Food Microbiology, 1996, **13**, 109–114

ORIGINAL ARTICLE

An apparatus for studying the effect of transient temperature on growth of bacteria in a gel matrix

T. F. Brocklehurst, R. B. Piggott, A. C. Smith* and D. C. Steer

Apparatus was constructed to subject colonies of bacteria, formed from single cells immobilized in solidified culture media contained between sheets of polymer, film to time-varying temperatures in the range 2–80°C with rates of change of 1°C s^{-1}. Results for the growth of Salmonella typhimurium *LT2 in medium solidified with gelatin under sinusoidally-varying temperatures between 12 and 22°C and of period 12 or 22 min are presented and were predictable by time–temperature integration using isothermal growth rates.*

Introduction

Mathematical models are increasingly used to predict the growth of food-poisoning bacteria (e.g. Anon. 1992, Skinner et al. 1994). Models are frequently based on data derived from growth in liquid cultures at constant temperature. This approach is relevant to growth in many foods, however it does not necessarily allow for either effects of immobilization and subsequent growth as colonies or for rapid fluctuations in growth temperature. Surface colonies of *Salmonella typhimurium*, as a function of gradients of temperature have been investigated by Thomas et al. (1991). The effects of fluctuating temperatures on microbial growth in liquid media are reviewed by Mitchell et al. (1994, 1995).

In this work an apparatus for studying the effect of controlled transient temperature on bacteria in the bulk of a gel is described. The inoculated gel is held in a cassette (Robins et al. 1994, Brocklehurst et al. 1995a) that is in contact with a heating and cooling block. Temperatures from 2–80°C may be controlled with rates of change up to 1°C s^{-1}. The apparatus can be used to study the thermal inactivation of bacteria. Here the growth of *S. typhimurium* LT2 immobilized in gelatin subject to sinusoidally-varying temperatures within the growth-temperature range is described.

Materials and Methods

Preparation of gel cassette

S. typhimurium, strain LT2 (NCIMB 10248) was obtained from the UK National Collection of Industrial and Marine Bacteria (Aberdeen, UK).

Stock cultures were maintained on heart infusion agar (Difco) slopes stored at 1°C and sub-cultured monthly. Liquid-growth medium of trypticase soy broth (TSB,

Received: 7 July 1995

Institute of Food Research, Norwich Laboratory, Norwich Research Park, Colney, Norwich NR4 7UA, UK.

*Corresponding author.

0740-0020/96/020109+06 $18.00/0

Baltimore Biological Laboratory, Baltimore, MD), plus yeast extract (Difco), 0·3% (w/v) and glucose, 1% (w/v) (TSBYG) was used. The pH of this medium was adjusted to 7·0 with HCl, 1 mol l^{-1}. The TSBYG contained NaCl at a concentration of 0·5% (w/v) and was initially sterilized through filtration with a 0·22 μm membrane (Millipore, UK).

Growth of bacteria within cassettes was in TSBYG prepared at twice the normal concentration and solidified with gelatin. The gelatin (*c*. 225 bloom from bovine skin, Type B, Sigma) was prepared as a 20% (w/v) solution and adjusted to pH 7·0 by the addition of NaOH, 1 mol l^{-1}. It was sterilized by autoclaving at 121°C for 15 min, cooled to 32°C and combined with the doubly-concentrated TSBYG solution in equal volumes. The culture medium therefore contained 10% (w/v) gelatin and was at pH 7·0 and contained 0·5% (w/v) NaCl.

Bacteria were grown successively in TSB (10 ml volume) at 25°C for 24 h, and then at 20°C for 24 h. The absorbance at 650 nm (A_{650}) of the second culture was measured by a spectrophotometer (SP600, Pye Unicam, UK) and the concentration of viable bacteria within determined from an optical-density calibration curve. Inocula were prepared by dilution of a sample of the culture in peptone salt dilution fluid (PSDF, Anon. 1978).

The gel cassette comprised an acetal frame (130 mm×145 mm, 2 mm thick) with a window within the frame (100 mm×100 mm) sealed within a sleeve of 15 μm thick polyvinyl chloride (PVC) packaging film (TSB11, Borden, Southampton, UK). Cassettes were obtained by the method described in Brocklehurst et al. (1995a). These were sterilized (autoclaving at 121°C for 15 min) and the PVC on either side of the cassette made taut using a directed stream of hot air.

An appropriate volume of inoculum was added to a batch of TSBYG/gelatin medium to give approximately 10^3 viable bacteria ml^{-1}, mixed thoroughly and constantly using a magnetic stirrer, and pumped into the cassettes. The cassettes were clamped in a supporting frame that had a perspex front to prevent distortion of the window of the cassette. This ensured that the thickness of gel within it was 2 mm. The clamps were pre-cooled (to 4°C) to lower the temperature of the growth medium to <25°C as this entered the cassettes in order to accelerate gelation, thereby preventing sedimentation of the bacteria and ensuring that they were immobilized and evenly distributed throughout the gel (Brocklehurst et al. 1995a). The filled cassettes were stored in the clamps at 4°C for 5 min to ensure solidification of the gelatin. The cassettes were then removed from the clamps and incubated at 20°C for 18 h. This resulted in colonies in the exponential phase of growth.

Temperature transients applied to gels

The controlled temperature apparatus (Fig. 1) comprised a block of sandwich construction with an upper layer of copper with a raised (by 0·5 mm) central section that matched the central film—covered medium within the frame of the gel cassette. A cassette frame was placed directly above the filled cassette and a plastic block (400 g) was placed above this. The copper plate was separated from an aluminium block containing the heating and cooling elements by a stainless steel layer. This sandwich construction improved the measured uniformity of temperature over the copper plate (experimentally to better than 0·5°C) without greatly increasing the heat capacity of the block. Heating was by three electrical resistance elements connected in parallel. The cooling was based on a water-bath (Grant Se10U, Cambridge, UK) that was modified to allow set points below 0°C (water and 8% ethylene glycol by volume). A refrigeration unit (500 W) comprising a bath of 25% by volume ethylene glycol in water at −7°C and a pump (magnetically coupled centrifugal, RS Components, UK) was used to pass the liquid through a copper coil submerged in the bath.

This apparatus was used with sinusoidally varying temperatures of periods 12 and 32 min in the range of 12–22°C, uniformly applied to inoculated gelatin gels in the cassettes. A small quantity of water maintained thermal contact between the block and the cassette. Initially one dimensional modelling using Fourier's law for the heat flux through a layered composite comprising gel (modelled

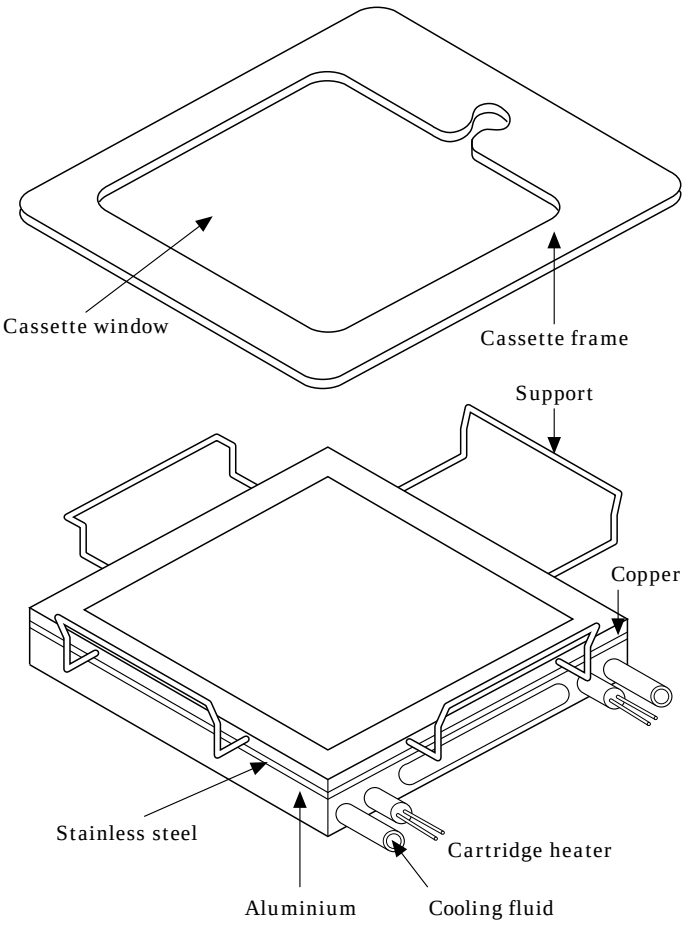

Figure 1. Schematic diagram of the gel cassette on the controlled temperature block.

as water), stainless steel, copper and aluminium had shown that the temperature through the thickness of the gel-filled cassette follows the waveform but lags by about 0·3 min and its amplitude is reduced by less than 0·5°C for sinusoidally-varying temperatures for periods as short as 4 min. At shorter periods the amplitude is attenuated for this thickness of gel.

In practice, uniformity of temperature to better than 0·5°C through the thickness of the cassette was obtained using platinum resistance foil thermocouples at the cassette surfaces and centrally within an uninoculated gel cassette. This also provided a calibration between the block thermocouple temperature, which could be readily monitored, and that within the gel. On changing the gel material or thickness, or polymer film, and preferably for each series of measurements a new calibration curve is necessary.

The temperature at the upper surface of the control block was monitored every 20 s using a combined PC-based controller and logger (based on a Viglen 386 PC; Viglen, Alperton, UK). The control program was written in VIEWDAC (Keithley Asyst, Reading, UK), which also provided a real time display and logging of the temperature. The computer applied the heating and cooling pulses of controlled duration to generate a sinusoidal temperature cycle in the copper plate. Although the temperature in the gel can be predicted approximately by heat conduction equations, direct calibration of the gel temperature against the copper plate thermocouples was carried out for the cycles of interest as described above.

The gel cassettes were weighed before and after the application of the temperature sequence and the weight loss was never greater than 0·14 g, 0·5% of the mass of the gel.

Growth rate of bacteria in the gel cassettes

At intervals during temperature cycling or isothermal incubation the PVC film was removed from one window of the cassette and a 10 mm wide strip of gelled growth medium removed. This was mixed with 90 ml of warm (32°C) PSDF and blended (Stomacher Lab-blender) for 1 min. The number of viable bacteria in the resulting suspension was determined by spreading 100 µl of this dilution (or further dilutions made in PSDF) onto the surface of duplicate plates of plate count agar (PCA, Oxoid, CM325) that were incubated at 32°C for 24 h. The same procedure was followed for constant temperature incubation for 12, 16 and 20°C.

Modelling growth rate

Because the methodology resulted in colonies in the exponential phase of growth, the growth rates under isothermal conditions were modelled assuming constant exponential growth

$$\ln N = \ln N_0 + \mu t \qquad (1)$$

where N is the number of viable bacteria per millilitre of culture at time t (h), N_0 is the initial viable count at t=0 and μ is the specific growth rate (h^{-1}). Growth in gelatin under non-isothermal conditions was calculated assuming the principle of time–temperature integration that the instantaneous growth rate for a particular temperature was equal to the steady isothermal growth rate at that temperature. Growth curves and mean growth rates were calculated by integrating the growth rate expression (Eqn 1) to give:

$$\ln N = \ln N_0 + \int_0^t \mu\,(T(t))\,dt \qquad (2)$$

where T is temperature (°C).

Least squares regression using a curve-fitting program (FIG P, Biosoft, Cambridge, UK) was used to fit the predicted growth curves to the isothermal experimental data to obtain specific growth rates for the exponential phase. In the manner of Gibson et al. (1988) a quadratic expression was used to interpolate growth rates in gelatin at temperatures between the available isothermal growth rate data points:

$$\ln\mu = a + bT + c\mathrm{T}^2 \qquad (3)$$

The mean growth rate, $\bar{\mu}$, averaged over the duration of the experiment, t_f, is calculated using:

$$\bar{\mu} = \frac{\ln \frac{N_f}{N_0}}{t_f} \qquad (4)$$

where N_f is the final viable count. Average generation times, τ, were calculated using the equation:

$$\tau = \frac{\ln 2}{\bar{\mu}} \qquad (5)$$

Results and Discussion

Solidified gelatin gel at a concentration of 10% (w/v) prevented motility of the bacteria. In its molten form it was sufficiently fluid to be pumped and was stable without syneresis over the temperature (4–22°C), pH (5–7) and NaCl (0·5–3·5% w/v) ranges used (see also Brocklehurst et al. 1995b). The protocol adopted resulted in immobilized cells that grew into colonies evenly distributed throughout the cassette (Brocklehurst et al. 1995a). At an inoculum concentration of 10^3 bacteria g^{-1} the cells were immobilized *c.* 1 mm apart.

The specific growth rates at temperatures of 12, 16 and 20°C in gelatin were found to be 0·15, 0·27 and 0·58 h^{-1}, respectively. By comparison the specific growth rates of *S. typhimurium* LT2 in liquid medium at pH 7 and 0·5% (w/v) NaCl were 0·14 and 0·58 h^{-1} at 12 and 20°C, respectively (Brocklehurst et al.

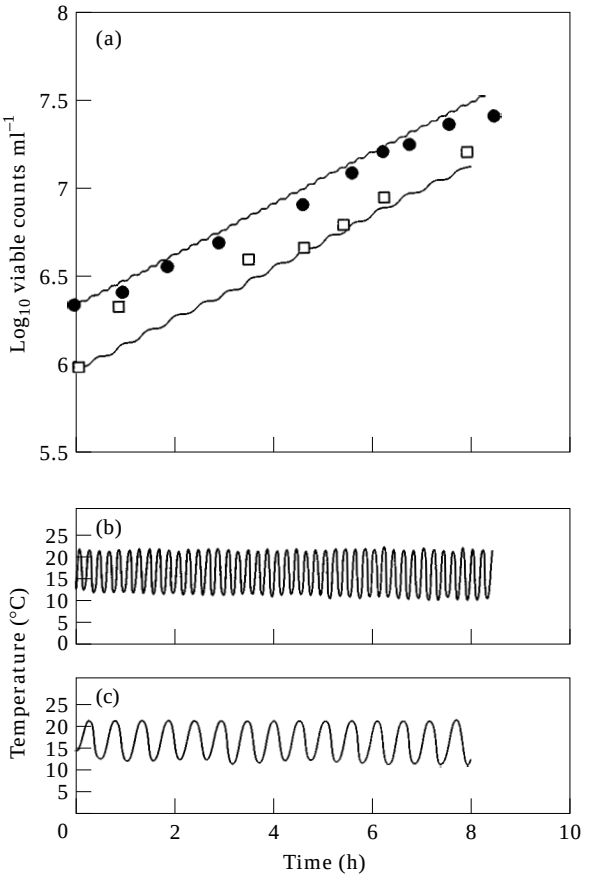

Figure 2. (a)Growth curve of *Salmonella typhimurium* LT2 during incubation at pH 7·0, 0·5% (w/v) NaCl. Temperatures cycled between 12 and 22°C with a period of (b) (●), 12 min; (c) (□), 32 min. Predictions based on integration of isothermal growth rates in gelatin shown as continuous line.

Table 1. Average experimental and predicted generation times (τ_{PL}, liquid; τ_{PG}, gel) for cycling experiments between 12 and 22°C in 0·5% (w/v) NaCl, pH 7·0

Period of cycle (min)	Average generation time (h) Experiment τ_E	Average generation time (h) Prediction[a] τ_{PL}	Average generation time (h) Prediction τ_{PG}	$[\tau_E-\tau_{PL}]/\tau_{PL}$ (%)	$[\tau_E-\tau_{PG}]/\tau_{PG}$ (%)
12	2·4	1·9	2·1	26	13
32	1·9	1·7	2·0	12	–5

[a]Based on isothermal growth data of *Salmonella typhimurium* LT2 in liquid culture given in Brocklehurst et al. (1995b).

1995b). Fitting the specific growth rates in gelatin in Eqn 3 gave:

$$\ln\mu = -2{\cdot}605 - 0{\cdot}00700T + 0{\cdot}00550T^2 \quad (6)$$

with a coefficient of determination, r^2=1. Fig. 2 shows that the rate of growth of immobilized bacteria incubated at sinusoidally-varying temperatures between 12°C and 22°C at neutral pH and 0·5% (w/v) NaCl was predict-

able from isothermal growth rates in gelatin using Eqn 2. The experimental values of the generation time, τ_E, averaged over the duration of the experiment are given in Table 1 for both the cycle periods, together with the corresponding values for the predicted growth, τ_{PG}. Their normalized difference, expressed as a percentage, is given as an index of how well the model fits the experimental behaviour. The average generation time for a prediction, based on isothermal growth of *S. typhimurium* in liquid culture is also given, using data from Brocklehurst et al. (1995b). The agreement with experiment by using a model based on liquid culture growth is almost as good as that using gel medium growth. However, deviations between experiment and predictions based on liquid growth data have been observed at lower pH and higher NaCl concentration, which resulted from differences in isothermal growth rates between planktonic and immobilized bacteria that were chemically stressed (Brocklehurst et al. 1995b).

The gel cassette system in combination with the temperature-controlled block proved a convenient means for the study of growth rates of immobilized bacteria during the application of transient temperatures. These experiments were carried out at constant water activity because a small relative water loss from the cassette (0·5% w/w), which was unavoidable in an oxygen-permeable film, would not change the water activity of the gel (Niwa et al. 1992). The transparent windows of the gel cassettes facilitated microscopical examination of the colonies and a laser interrogation system has been used to measure the microbial growth rather than the enumeration method used here (Robins et al. 1994). Cassettes may be examined before and after the application of a sequence of transient temperatures. A controlled temperature chamber using the principles described in this paper has been designed for *in situ* use of a cassette with the laser system.

Acknowledgements

We gratefully acknowledge funding from the Ministry of Agriculture, Fisheries and Food. We thank R. Seale and W. Pleass for technical assistance and the engineering staff at the Institute for fabricating the cassette heating apparatus.

References

Anon. (1978) *Micro-organisms in Foods. 1. Their Significance and Methods of Enumeration*. 2nd edn, International Commission for the Microbiological Specifications for Foods. Toronto, University of Toronto Press.

Anon. (1992) *Food Micromodel*. Ministry of Agriculture, Fisheries and Food, London, UK.

Brocklehurst, T. F., Mitchell, G. A., Ridge, Y. P., Seale, R. and Smith, A. C. (1995b) The effect of transient temperatures on the growth of *Salmonella typhimurium* LT2 in gelatin gel. *Int. J. Food Microbiol.*. **27,** 45–60.

Brocklehurst, T. F., Mackie, A. R., Steer, D. C. and Wilson, D. R. (1995a) *Detection of Microbial Growth*. International Patent Application: WO 95/00661.

Gibson, A. M., Bratchell, N. and Roberts, T. A. (1988) Predicting microbial growth: growth responses of Salmonellae in a laboratory medium as affected by pH, sodium chloride and storage temperature. *Int. J. Food Microbiol.* **6,** 155–178.

Mitchell, G. A., Brocklehurst, T. F., Parker, R. and Smith, A. C. (1994) The effect of transient temperatures on the growth of *Salmonella typhimurium* LT2 I. Cycling within the growth region. *J. Appl. Bacteriol.* **77,** 113–119.

Mitchell, G. A., Brocklehurst, T. F., Parker, R. and Smith, A. C. (1995) The effect of transient temperatures on the growth of *Salmonella typhimurium* LT2 II. Excursions outside the growth region. *J. Appl. Bacteriol.* **79,** 128–134.

Niwa, E., Tominaga, Y. and Kanoh, S. (1992) Water activity of some food hydrogels examined by thermal conduction type humidity sensor. *Nippon Suisan Gakkaishi* **58,** 1757–1760.

Robins, M. M., Brocklehurst, T. F. and Wilson, P. D. G. (1994) Food structure and the growth of pathogenic bacteria. *Food Technol. Int. Europe*, 31–36.

Skinner, G. E., Larkin, J. W. and Rhodehamel, E. J. (1994) Mathematical modeling of microbial growth: a review. *J. Food Safety* **14,** 175–217.

Thomas, L. V., Wimpenny, J. W. T. and Peters, A. C. (1991) An investigation of four variables on the growth of *Salmonella typhimurium* using two types of gradient gel plates. *Int. J. Food Microbiol.* **14,** 261–275.