Experiments to Investigate the Ability of Speedy Breedy to Simulate Pasteurisation

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Background

Speedy Breedy confirms microbial contamination by the sensitive monitoring of pressure changes within a closed vessel. Samples are added to a vessel containing a culture medium which promotes rapid replication if micro-organisms are present. Any microbial respiration leads to changes in gas composition in the vessel which can be monitored using Speedy Breedy. An internal algorithm defines a significant pressure event associated with detection of contamination and the length of time from inoculation of sample to pressure event is indicative of the degree of contamination. This length of time is referred to in this study as the Time to Detection (TTD).

In the event of a positive result, as Speedy Breedy makes use of growth methods to promote microbial replication and identification, a Speedy Breedy culture vessel will contain a rich broth of micro-organism. It is important that in disposing of culture vessels, they are rendered sterile.

The recommended protocol for disposal of culture vessels upon completion of a test (whether a positive result has been recorded or not) is to pass the vessels through a standard autoclaving procedure of 121°C at 15 PSI for 15 minutes.

Autoclaves are available from suppliers in varying dimensions and costs, from large scale systems used in laboratories and hospitals, to bench-top designs. In some circumstances, access to an autoclave may not be practical or appropriate and with this in mind an alternative to rendering material sterile would be of value.

Hypothesis

Our hypothesis was that the temperature control capabilities of Speedy Breedy would enable us to produce a pasteurisation procedure which samples might automatically undergo to render them sterile (with the exception of organisms known to tolerate pasteurisation temperatures such as the spore-forming *Bacillus spp.* and *Clostridium spp.*).

Aim of Study

The aim of this study was to generate typical pasteurisation conditions through a modified Speedy Breedy protocol and to assess their efficacy by challenging with heavy inocula of several bacterial species, assessing culture viability both before and after the process.

Materials & Methods

Using the Speedy Breedy computer software supplied when purchasing Speedy Breedy, a typical 36°C incubation protocol was modified to incorporate additional steps at the end of the protocol. The standard protocol would dictate that Speedy Breedy conditions would reach 36°C for 24 hours. A modification was made in which an additional pasteurisation phase automatically commenced following the end of the 24 hour incubation.

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Several approaches to pasteurisation exist in industry, including the so-called 'High Temperature Short Time' processes that use higher temperatures for periods of only seconds in length (employed, for example, in the dairy industry) however the typical time and temperature approach to pasteurisation is to utilise temperatures of 63°C for at least 30 minutes. The modified Speedy Breedy protocol incorporated a step to mimic these time and temperature conditions, including adequate time for vessel temperature to elevate from operating temperature to 63°C.

An example industry in which access to an autoclave following testing may not be practical is the ballast water treatment sector. Regulations exist to control the threat of invasive species being transported from port to port in ballast water taken on-board and subsequently disposed of by ships. A variety of treatment systems exist to reduce the risk of contamination of port waters during de-ballast and one method for assessing the efficacy of these systems is to test samples of ballast water for levels of three indicator bacteria – *Escherichia coli*, faecal enterococci and *Vibrio cholerae.*

With this in mind, 1ml of rich suspension of *E. coli*, a representative member of the faecal enterococci (*Enterococcus faecalis*) and a representative member of the Vibrio genus (*Vibrio parahaemolyticus*) were used to inoculate separate Speedy Breedy culture vessels containing growth medium to a working volume of 50ml as per the operating instructions. These vessels were then tested using the modified Speedy Breedy protocol, including an incubation phase and a pasteurisation phase.

Before commencing testing, samples were taken from each of the three bacterial suspensions to perform agar plate counts to determine the microbial load present in each. Upon completion of testing and pasteurisation, triplicate samples were taken from each culture vessel to perform further agar plate counts to determine the microbial load present after the pasteurisation phase was completed.

In addition, upon completion of testing and pasteurisation, each culture vessel was maintained at 36°C in an incubator for a further 48 hours and further triplicate samples were taken from each culture vessel to perform agar plate counts. This was in order to determine if there would be any re-growth following pasteurisation.

Control vessels were also tested, wherein the vessel was subjected to the standard 36°C incubation but without a pasteurisation phase to confirm that any reduction in numbers of organisms would be attributed only to the pasteurisation phase. Once again, subsequent triplicate samples were taken for agar plate counting.

Results

Table 1 below shows a summary of the agar plate count results obtained from the experiments described above. All plate counts given are averages of triplicate sets of plate counts performed.

Table 1: *Agar plate counts (CFU per vessel) before and after pasteurisation testing using a modified Speedy Breedy protocol. "Post Pasteurisation 1" samples were taken following termination of the pasteurisation phase and return of vessel to ambient temperature. "Post Pasteurisation 2" samples were taken following a further 48 hours incubation at 36°C.*

Organism	Microbial Load of Inoculum (CFU)	Microbial Load Post Pasteurisation 1 (CFU)	Microbial Load Post Pasteurisation 2 (CFU)
E. coli (Vessel 1)	4.01×10^6	No Growth	No Growth
E. coli (Vessel 2)	4.01×10^6	No Growth	No Growth
E. faecalis (Vessel 1)	2.19×10^6	No Growth	No Growth
E. faecalis (Vessel 2)	2.19×10^6	No Growth	No Growth
V. parahaemolyticus (Vessel 1)	1.77×10^6	No Growth	No Growth
V. parahaemolyticus (Vessel 2)	1.77×10^6	No Growth	No Growth

Control vessels that did not undergo pasteurisation were inoculated using the same inocula as described above. In each case, whilst the starting microbial load in each vessel was the same as those described in the above table, the plate counts performed following completion of testing all returned counts in excess of 1.00×10^{10} CFU in each vessel.

These post-testing CFU values are higher than the values at the start of the experiment, which reflects the replication of organisms during the 36°C incubation phase.

These post-testing CFU values also highlight the fact that at the time at which the pasteurisation process begins in the pasteurisation experiments, the numbers of organisms present will be even higher (probably greater than 1.00×10^{10} CFU) than those in the initial inocula, given that a 36°C incubation phase was first carried out, providing therefore an even greater challenge to the pasteurisation process if complete sterility is to be achieved.

Interpretation

The lack of microbial activity in vessels following the pasteurisation phase and the lack of re-growth following subsequent incubation for 48 hours at 36°C suggests that the pasteurisation phase of the Speedy Breedy protocol has sterilised the contents of the culture vessel used.

The continued microbial activity seen in control vessels that have not undergone a pasteurisation phase confirms that the reduction in activity is associated with the inclusion of the pasteurisation steps to the Speedy Breedy test protocol.

Conclusions & Observations

As per our hypothesis, a modified Speedy Breedy protocol can be used to replicate pasteurisation conditions and can be used to incorporate a sterilisation step for vegetative cells to a Speedy Breedy test protocol in situations where access to appropriate autoclaving systems is limited.

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