Thermal Sterilisation Kinetics of Bacteria as Influenced by Combined Temperature and pH in Continuous Processing of Liquid

by

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Errata

Additional references to be included:

Page	Line	Reference
104	8	Cerf, O. (1977) Tailing of survival curves. *Journal of Applied Bacteriology* **42**: 1-19
105	7	Daudin, J. D. and Cerf, O. (1977) Influence of sudden changes of temperatures when bacterial cells are subjected to almost instantaneous heating or cooling. *Lebensmittel-Wissenschaft und Technologie* **10**: 203-207 Hermier, J., Begue, P. and Cerf, O. (1975) The combined effect of heat shock and RTD. *Journal of Dairy Research* **42**: 437-444
117	21	Buchanan, R. L. and Edelson, S. G. (1999) Effect of pH-dependent, stationary phase acid resistance on the thermal tolerance of *Escherichia coli* 0157:H7. *Food Microbiology* **16**: 447-458

This work contains no material which has been accepted for the award of any other degrees or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has made in the text.

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SUMMARY

The thermal sterilisation of various bulk liquids is an essential process step in the food and fermentation industries. However present models for process simulation of the continuous sterilisation of liquid are limited in that they are based on gross assumptions regarding the kinetics of bacterial inactivation. Of particular interest are kinetic models that can be reliably used to predict the combined effect of process parameters, principally, exposure temperature and pH (T-pH), in addition to exposure time (t), and; which are of a form that can be readily integrated with equations describing liquid rheology and hydrodynamics of liquid flow. An adequate kinetic model is essential to process optimisation, and longer term, to optimal processing in real time.

Analysis of carefully obtained sets of bench-scale survivor data of selected bacteria with the combined effect of T-pH and limited published continuous steriliser data ($n_T = 708$) has demonstrated that assumptions of first-order (ie log-linear) kinetic dependence are largely inadequate. This finding challenges widely held views. Two new, non-linear kinetic model forms were synthesised from these data. These new model forms are titled "nOE" (n^{th} order rate equation) and "CDT" respectively. Both involve a time dependent rate for thermal inactivation. The CDT form was selected following validation tests on bench-scale data and the rejection of the nOE model. A pilot-scale continuous steriliser was built to experimentally test the underlying hypothesis of the new CDT model. Extensive analyses of residuals were used together with the *per cent variance accounted for* ($\%V$) and mean square error (MSE) as criteria of goodness of fit for validation tests of the log-linear, nOE, CDT and other selected model forms.

The carrier liquid used throughout was a 2 kg m^{-3} mucilage of Carbopol® 934. This mucilage was selected because of the stability of its viscosity over the range of temperature and pH values of interest and its resistance to bacterial attack and growth. The viscosity and value of the pseudoplastic index of this mucilage closely simulates that of a wide range of liquid foods. It is transparent and therefore suitable for dye-trace studies to investigate the adequacy of hydrodynamic flow assumptions in the continuous steriliser.

Three vegetative bacteria were selected: *Escherichia coli* (ATCC 25922), *Listeria monocytogenes* (SLCC 5764) and *Pseudomonas fluorescens* (172). These represent common micro-organisms known to spoil liquid and solid food. The thermal sensitivity of vegetative bacteria conveniently permitted exposure temperatures below liquid boiling (and consequent high pressures) thereby simplifying the pilot steriliser. Findings however can be extrapolated to behaviour of thermally less sensitive contaminant micro-organisms. Up to six levels of exposure temperature (52°C, 54°C, 56°C, 58°C, 60°C and 62°C) in combination with up to eight levels of pH (4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5) with exposure times ranging from 10 *s* to 5 min, were used in experimental designs to cover a wide bio-kinetic range of interest. The effect of pH on the rate of thermal inactivation was significant for all three bacteria, especially at the lower exposure temperatures. Concave-up tails appeared in the survival data for *P. fluorescens* and *E. coli* and both concave-up and concave-down tails appeared in the survival data for *L. monocytogenes*. These findings indicate a departure from log-linear kinetics.

The *n*OE model for combined *T*-pH was assessed using the data determined for *L. monocytogenes*. This model form is new in that it does not appear to have been applied previously to bacterial survivor kinetics. The model explained an overall 89.5% of the *variance accounted for* compared with 71.5%*V* for the log-linear form. The *n*OE model has two terms (1 / *T* and pH), and permits a practical procedure for the evaluation of the microbiological safety of liquid foods processed at a mild temperature regime in combination with different pH. A major drawback with *n*OE model however is that it is of a form that is not readily integrated with equations describing liquid rheology and hydrodynamics of liquid.

The new CDT form explained between 82.9% and 93.0% of the variance across all the *T*-pH data and 94.6% for the published *T* only data from a continuous steriliser. Because of this good fit of the model, together with no apparent systematic features in the plots of residuals and its easier integration with other equations, it was selected for pilot steriliser studies.

The continuous steriliser was sized using predictions of the CDT model for *E. coli*. Direct steam injection heating was employed. The experimental design for the tubular steriliser involved three levels of T (54°C, 56°C and 58°C) in combination with three levels of pH (4.5, 6.0 and 7.5) and inactivation times ranging from 61.1 *s* to 181.4 *s*. Careful effort was made to ensure all the assumptions of the CDT model were practically implemented in the experimental steriliser. This included photographic evidence to confirm velocity profiles and an absence of steam bubbles (ie condensation of the steam), and the effect of dilution by the condensing steam on rheology of the carrier Carbopol liquid.

At low values of T (54°C) inactivation of *E. coli* numbers in the (*dynamic*) flow of the continuous steriliser was greater than in the (*static*) bench data at all levels of pH. As T increased (56°C and 58°C) however the reduction in bacterial numbers was greater in the capillary tubes than in the pilot continuous steriliser. The thermal inactivation kinetics of *E. coli* in the Carbopol carrier liquid differed significantly therefore depending on whether samples were heated in the capillary tubes (*static*) or in the continuous steriliser (*dynamic*). A significant portion of this difference between the CDT model predictions of reduction in the number of viable bacteria and that which was experimentally observed in the pilot continuous steriliser is ascribed to the effects of dispersion of viable bacterial cells. Established steriliser performance equations include consideration of both the liquid flow hydrodynamics and rheology of the carrier liquid but do not presently include a term for dispersion. With increasing steriliser length and consequent holding time, the effects of dispersion become significant. The effect of dispersion has been quantitatively estimated to give rise to one additional order of magnitude in predicted reductions in the number of viable bacteria in the continuous steriliser.

The systematic synthesis and testing of the new CDT model outlined in this thesis importantly points to the need for a directional rethink in both static and flow systems and away from widely used log-linear approaches that imply constant thermal rates of inactivation over a range of combined T-pH. Because shear conditions that normally pertain to processing of liquid foods were used, findings should be of interest to larger-commercial scale sterilisation by heat.

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TABLE OF CONTENTS

CHAPTER

ONE

INTRODUCTION

The food industry is generally a nation's largest manufacturing sector and one of its most stable (Davey 1993a). World-wide media reporting of food poisoning outbreaks has increased public awareness and consumer expectations of a safe and wholesome food supply. Thermal sterilisation is an essential tool that is widely employed to ensure the microbiological safety of a wide range of foods. Thermal processing is more reliable, flexible and economical than other means of sterilisation, such as filtration and chemical disinfection (Aiba, Humphrey and Millis 1973, Hugo 1991). Thermal sterilisation can be carried out as either a batch or continuous process for a wide variety of bulk liquid media. The advantages of a continuous process over a batch process include an improved product quality (because of a greater nutrient retention), a greater production rate with simplified quality control, aseptic handling and reduced costs (Aiba, Humphrey and Millis 1973, Bailey and Ollis 1986). These advantages result from steady-state operation in which greater control of process temperature and exposure time is possible than with batch processes.

A typical continuous steriliser consists of three main sections: a heating section, a holding section and a cooling section (Bailey and Ollis 1986, Aiba, Humphrey and Millis 1973).

Sterilisation[1] is defined as "that process where an apparent death of the cells may be due to the inability of the cells to germinate or, after germination, the inability for outgrowth to occur with the production of more cells" (Banwart 1979). A working definition however is "a reduction in the number of viable cells to some specified number" which is referred to as a sterility requirement.

The objective of sterilisation is to achieve a necessary sterility requirement with minimum concomitant thermal damage to food quality. Because of the risks of a public health significance associated with error, sterilisation processing is generally conservative. This results in over treatment however that is wasteful in both costs and damage to food quality, especially vitamins (Davey and Cerf 1996).

[1] *see* Appendix A for a definition of important terms used in this study

Formulation of adequate mathematical models describing thermal inactivation of bacteria therefore has a central place in planning and design and is an essential pre-requisite to process optimisation. In addition to the exposure temperature (T) and holding time (t), the pH of liquid foods is known to significantly affect sterilisation effectiveness.

Mathematical models for predicting the combined effect of these two process variables T and pH in addition to time are therefore of major interest. Current analyses are limited to the effect of temperature alone, or, are fragmented in that predictions are based on unrelated values of physical properties selected from a range of values given in the literature.

The principal aims of this study are to evaluate and develop mathematical models for sterilisation, undertake experimental studies for determining thermal inactivation effects on continuous processing of a liquid containing contaminant bacteria and compare the data obtained with those predicted by a selected model. The bacteria, methods and liquid are chosen with a view to applying findings to realistic problems related to commercial sterilisation by heat.

A logical and stepwise approach was adopted as a research strategy.

The relevant literature is reviewed and a summary appraisal is presented in Chapter 2. A major shortcoming identified is the widespread, and usually untested, assumption of log-linear thermal kinetics. Based on this review a systematic approach to testing of assumptions in bacterial survivor data is proposed which involves testing of model forms against published and experimentally derived survivor data determined using standard microbiological techniques in capillary (ie *batch*) studies and limited continuous (ie *dynamic*) flow data.

Chapter 3 outlines the selection and characterisation and maintenance of three selected bacteria, choice of carrier liquid and experimental procedures for determining essential inactivation data.

In Chapter 4 a shift from widely assumed log-linear kinetics of thermal inactivation is proposed and demonstrated following analysis with the derived experimental data and data taken from the literature.

In Chapter 5 a number of selected alternative mathematical models are synthesised and presented for predicting the effect of combined *T*-pH on inactivation kinetics. Two are selected for validation tests on the bench-scale data. One of these, a newly synthesised CDT model, is selected for experimental testing in a pilot continuous steriliser.

Chapter 6 describes the continuous steriliser and presents a comparison between the experimental results and the predictions of the selected model for thermal inactivation kinetics with combined process *T*-pH. The kinetics of thermal inactivation in this flow (*dynamic*) system are compared with those from the bench-scale (*static*) studies.

Chapter 7 is a summary of the findings and conclusions of this investigation together with suggestions for further studies.

The definitions of some important terms used in this study are given in Appendix A and all notation used is listed at the back of this thesis. A list of refereed publications arising from this study are presented in Appendix B.

CHAPTER
TWO

LITERATURE REVIEW

Parts of this chapter have been published as:

Chiruta, J., Davey, K. R. and Thomas, C. J. (1997b) Thermal inactivation kinetics of three vegetative bacteria as influenced by combined temperature and pH in a liquid medium, *Transactions of the Institution of Chemical Engineers, Part C, Bioproducts and Food Processing*, **75**: 174-180

2.1 INTRODUCTION

The general principles for continuous thermal sterilisation and practical operation of plant have been reviewed in a number of major texts (Aiba, Humphrey and Millis 1973, Bailey and Ollis 1986). The design of a steriliser is similar to that of a continuous flow reactor in which the relevant reaction is the thermal inactivation of contaminating micro-organisms.

Continuous sterilisation, since its inception some 46 years ago (Whitmarsh 1954), has been seen to be an efficient process for treating large quantities of liquids including fermentation media and foods. Mathematical models of this important process however have only latterly appeared in the literature. One reason for this later development in modelling is attributable to the multidisciplinary nature of the problem, involving as it does biochemical engineering, mathematics and statistics in addition to microbiology.

In the mathematical modelling of thermal sterilisation processing, process parameters describing the resistance of micro-organisms to heat and thermal denaturation of food quality, must be combined with parameters describing the rheology and hydrodynamics of flow of the carrier liquid. Historically, these aspects have been widely studied in separate investigations.

In this chapter the principles involved in continuous sterilisation design and the development of models are examined. The generalised performance equations in continuous sterilisation of homogeneous liquid are then appraised. The model form of Lin (1976) and Davey and Wood (1984) is selected as the most suitable for theoretical extension, modification and experimental testing. Shortcomings in the literature are identified, and the need for a comprehensive evaluation of the effect of combined exposure temperature and pH (T-pH) is highlighted along with inadequacies in the applicability of the log-linear model for thermal inactivation.

2. 2 PRINCIPLES OF CONTINUOUS STERILISATION DESIGN

Continuous sterilisation offers several advantages over batch methods of sterilisation. These include (Bailey and Ollis 1986):

- a simplification of production planning (which permits maximum plant utilisation and minimum production delay)
- reproducible process conditions with negligible heat-up time
- an improved product quality (due to a lower nutrient denaturation)
- ease of automation.

A continuous steriliser consists of three main sections: a heating section, a holding section and a cooling section. The holding section is usually a tube of uniform circular cross section maintained at isothermal conditions. Continuous sterilisers differ mainly in their heating and cooling characteristics (Deindoerfer and Humphrey 1959).

For sterilisation it is necessary to have a suitable holding tube length (L) so as to ensure that the liquid has sufficient residence time (t) at a given exposure temperature for the inactivation of contaminant micro-organisms in the liquid medium. The residence time in the holding tube is given by:

$$t = L / u \tag{2.1}$$

Heat can be applied to continuous sterilisers in either of two ways: direct injection, or, indirect heating through an intermediate heat transfer surface. Direct steam injection is better suited to the practical realisation of rapid heat up. Advantages of direct steam injection over indirect methods include:

- a greater heat transfer coefficient
- elimination of heat exchange surfaces in the heating section.

The value of the heat transfer coefficient for a number of injected media – including air, water, and condensing steam - are given by Jones and Larner (1968).

Condensing steam acts to dilute the liquid. Model analyses must therefore take account of this, especially as dilution effects the rheological properties of the liquid and consequent hydrodynamics of liquid flow and residence times. Steam injection can also introduce instabilities in the flow of liquid in the holding tube if not all the steam bubbles condense rapidly. Generally however, well-designed direct injection systems may be used for products such as milk and fresh dairy (de Jong 1997).

2.3 PROCESS MODEL DEVLOPMENT

2.3.1 Process Models

Process models for continuous sterilisation have developed from contributions by various investigators. A chronological listing is given in Table 2.1. All investigators have, reasonably, implied that the contaminating micro-organism will be uniformly distributed throughout the liquid. Further, all have employed the power law equation (Skelland 1967, Holdsworth 1971) – see equation 2.2 - to describe the rheology and residence time distribution of the carrier liquid.

The analysis of Deindoerfer and Humphrey (1959) is perhaps the earliest attempt at modelling continuous sterilisation. This analysis was based on an assumption of turbulent flow of liquid in the holding section. Changes in temperature were allowed for through use of an overall heat transfer coefficient.

For many liquid foods however, laminar flow is the only economical regime as noted by Manson and Cullen (1974) because of prohibitive pumping costs associated with viscous liquids. Importantly, because a velocity gradient occurs in laminar flow not all portions of the liquid will be subjected to the same thermal exposure.

Table 2.1 Summary of model development for bacterial survival in batch and continuous sterilisation of liquid

Authors	Year	Proposal / Model
Esty & Meyer	1922	Log-linear thermal batch survival data
Deindoerfer & Humphrey	1959	Turbulent flow analysis
Charm	1966	Integration of lethal effects with laminar flow velocity profile
Ruyter & Brunet	1973	Heterogeneous food processing
Manson & Cullen	1974	Foods with discrete particulate matter
Simpson & Williams	1974	HTST heating with laminar flow and intermediate heat transfer surface
Lin	1975	T dependent-dispersion model
Han, Zhang & Krochta	1976	Batch model for innate heterogeneity theory
Lin	1976	HTST analysis
Davey, Lin & Wood	1978	T-pH dependent HTST analysis
Guariguata, Barreiro & Guariguata	1979	Laminar flow of Bingham plastic
Ashley	1982	Design optimisation
Davey & Wood	1984	Comprehensive experimental testing of continuous steriliser
Davey	1993b	Generalised sterilisation chart for T-pH effect
Coker, Davey & Kristall	1993	Combined T-pH dependent denaturation of accompanying vitamin
Cole et al	1993	Combined T-pH non-linear batch-kinetic model
Davey, Hall & Thomas	1995	Combined T-pH thermal inactivation – need for non-linear survivor kinetics
Cerf, Davey & Sadoudi	1996	Predictive model for combined T-pH-a_w effect
Davey & Cerf	1996	Concomitant vitamin denaturation influenced by combined T-pH

HTST High - Temperature - Short - Time

Charm (1966) first demonstrated the concept of integrating parameters that describe thermal inactivation kinetics with the effect of laminar velocity profile through a steriliser holding tube. Procedures for calculations were reported and a final process evaluation was expressed in terms of probability of product spoilage. The analysis is applicable to the isothermal flow of a homogeneous liquid with instantaneous heating and cooling. Instantaneous heating can be practically realised with direct steam injection. Flash cooling – ie a sudden drop in process pressure - can be employed to practically realise rapid cool down of the heated liquid.

The presence of a residence time distribution in continuous flow reactors has been thoroughly investigated and is well documented by, among others, Levenspiel (1972).

de Ruyter and Brunet (1973) outlined the theory on which the processing of liquid containing discrete particulate matter could be based, but the effect of residence time distribution was not considered.

In a comprehensive study, Simpson and Williams (1974) analysed the indirect heating and cooling of a liquid in a shell and tube heat exchanger. A shortcoming is that this type of design relies on conductive heating of the liquid with its low value of overall heat transfer coefficient when compared to the direct injection method. The analysis is applicable to sterilisation of homogeneous liquids in laminar flow. Simpson and Williams concluded that optimisation of product quality required the smallest possible diameter holding tubes and a compact heat exchanger design. However a short length of holding tube of a large diameter is a better practical compromise because of the resulting lower liquid velocities and shear stresses. Too great a shear could rapidly result in a time dependency of the liquid known as liquid *aging*.

Dispersion of the bacterial cells during thermal sterilisation in a long holding tube was investigated by Lin (1975). An allowance was made for variation in temperature (earlier models had assumed a constant temperature in the holding tube). In such models, the mixing characteristics of the molecules of the liquid apply equally to the behaviour of the

bacteria suspended in the liquid.

Lin (1976) later proposed an instantaneous heating and isothermal holding model similar to that of Charm (1966). The model is applicable to the High Temperature / Short Time (HTST) sterilisation of a homogeneous liquid in laminar flow. Illustrative predictions were made for total steriliser length (holding and cooling sections) at different processing temperatures, pseudoplastic indices and sterility requirements for the pathogen spores of *Clostridium botulinum*. Process evaluation was based on the average or bulk concentration of viable cells at the end of the steriliser.

Two proposals for cooling of the sterilised liquid were made:

- instantaneous cooling
- non-instantaneous cooling modelled on the shell and tube exchanger type of Simpson and Williams (1974).

Guariguata, Barreiro and Guariguata (1979) developed a model for continuous sterilisation of foods displaying Bingham plasticity properties, with laminar, isoviscous and no-isoviscous flow. Ashley (1982) discussed design optimisation of the continuous steriliser. The analysis inferred log-linear inactivation kinetics of the contaminant micro-organism and an Arrhenius dependence on exposure temperature.

Davey and Wood (1984) presented a detailed review of the relevant theory and a comprehensive experimental evaluation of an experimental continuous steriliser based on the instantaneous heating and cooling model of Lin (1976). *Escherichia coli* American Type Culture Collection (ATCC) 25922 was selected as the indicator micro-organism. Direct steam injection heating was used to practically achieve instantaneous heating of a flowing liquid stream containing the bacteria. Flash cooling was used for near-instantaneous cooling of the liquid.

Coker, Davey and Kristall (1993), Davey (1993a) and later Davey and Cerf (1996)

developed a model for simulating the combined thermal and pH effect on concomitant vitamin denaturation during sterilisation. This model was based on a linear-Arrhenius kinetic equation for thermal inactivation of a bacterium and, the residence time distribution of a non-Newtonian liquid. The pH of the food was shown to significantly affect the denaturation of both thiamin (B_1) and ascorbic acid (C) vitamins.

Of interest, but not the direct thermal inactivation of contaminant micro-organisms, is the work of McKellar et al (1994) and Muriana (1997) in approaching thermal denaturations. McKellar et al (1994) developed an analysis that described the inactivation of alkaline phosphatase in whole milk in a pilot plant HTST steriliser. *Listeria monocytogenes* was the bacterial contaminant. A computer program was presented to calculate the integrated lethal effect of exposure temperature in the holding tube. For model development, a standard alkaline phosphatase assay was used. The thermal inactivation of alkaline phosphatase was used to simulate inactivation of bacterial cells.

A bench-top flow-injection steriliser was used by Muriana (1997) to investigate the combined effect of pH and hydrogen peroxide on the inactivation *L. monocytogenes* in egg white at a fixed time-temperature regime. The results were graphically presented and discussed. However, no mathematical models were synthesised or appropriated for the experimental data to predict thermal inactivation and the proportion of surviving bacteria.

2.3.2 Performance Equations

From the viewpoint of experimental testing of steriliser performance equations, Davey and Wood (1984) have pointed out that the near instantaneous heating and cooling model is the most convenient. Both Charm (1966) and Lin (1976) proposed this model form. Because the model of Lin (1976) is based more strictly on chemical reactor principles and fluid mechanics it is selected as suitable for theoretical modifications and experimental testing.

The Lin model in its most simple form involves a coupling of the equations for the:

- isothermal velocity distribution of a power law liquid in laminar flow in a tube of circular and constant cross section
- thermal inactivation of viable bacterial cells carried on streamlines of the laminar.

Process evaluation is based on the resulting average (ie bulk) concentration (number) of viable bacterial contaminants at the end of the steriliser.

The isothermal velocity distribution for a power law liquid in laminar flow in a tube of circular cross section is well established in the literature and is given by (Wilkinson 1960, Lin 1976):

$$u / \langle u \rangle = [(3\,n + 1) / (n + 1)]\,(1 - R^{(n+1)/n}) \qquad (2.2)$$

The number of viable bacterial cells at the end of an isothermal holding tube as derived by Lin (1976) with laminar flow is given by:

$$\langle N \rangle / \langle N_0 \rangle = 2 \int_0^1 \exp(-k\,L / u)\ (u / \langle u \rangle)\,R\,dR \qquad (2.3)$$

The laminar flow assumption requires that the velocity in the radial direction is zero. Any deviation from laminar flow will alter the residence time distribution. Any increase in the residence time will decrease the number of viable bacterial cells at the end of the steriliser.

Bulk sampling of sterilised liquid is practical to implement. Standard microbiological methods can readily be applied for enumeration of viable bacterial cells in the bulk samples of control (unheated) and sterilised liquid.

Equation 2.2 implies that as the pseudoplastic index, n, decreases the local velocity near the centre of the holding tube decreases and the velocity near the tube wall increases. In the limiting case where n = 0, plug flow results and the equation reduces to an expression for conversion in a plug flow reactor (Levenspiel 1972):

$$\langle N \rangle / \langle N_0 \rangle = \exp(-k L / \langle u \rangle) \quad (2.4)$$

The reader should note that equations 2.3 and 2.4 do not permit prediction of viable cell numbers to zero survivors.

The effect of temperature on the rate of thermal inactivation, k, is widely expressed in an Arrhenius form, that was used both by Lin (1976) and Charm (1966), namely:

$$k = A \exp(-E / R T) \quad (2.5)$$

In this study the factors A and E are considered independent of temperature.

The concept of a thermal death time, D, is used in thermal sterilisation processes. D is the time required to reduce the number of viable cells to $1/10^{th}$ of the original number (Aiba, Humphrey and Millis 1973, Bailey and Ollis 1986). The decimal reduction time is given by the relation:

$$D = 2.303 / k \quad (2.6)$$

The decimal reduction time is therefore merely a form of the reciprocal of k. As D has the dimension of *time*, it is often more readily understood, especially in industry, than the rate ($time^{-1}$) of k.

2.3.3 Liquids with a Yield Stress

Many liquid foods such as fruit juices and purees (Charm 1966, Holdsworth 1971), margarine and chocolate mixes (Skelland 1967), and mustard and sauces (Holdsworth 1971) exhibit a yield stress. The yield stress is the value of shear that must be exceeded before a liquid flows (and is non-directional). This phenomenon can be accounted for in models of heat inactivation in sterilisers.

For example, Davey, Lin and Wood (1979) outlined a procedure to modify the Lin (1976) continuous steriliser model to include predictions for liquid foods with a yield stress based on the Herschel-Bulkley equation. This provided a flexible, hydrodynamic model to include a wide range of time-independent liquids. Consideration of liquids with a yield stress is outside the scope of this study however and is not considered further.

2.3.4 Effect of Combined Temperature and pH

Davey, Lin and Wood (1978) demonstrated a theoretical model for the rate coefficient for thermal inactivation to account for the combined effect of temperature and pH on survivor kinetics in a continuous steriliser.

Predictions of the number of surviving viable cells at the end of a steriliser were based on selected data from Xezones and Hutchings (1965) for the thermal behaviour of the pathogen *Clostridium botulinum*. Simulation indicated the significant effect of low values of pH in reducing the exposure time at a given temperature and stated sterility requirement for a range of foods.

Importantly, no experimental studies were carried out however.

More recent theoretical work of Davey (1993a), and Coker, Davey and Kristall (1993) and Cole et al (1993) has aimed at illustrating the inadequacy of the present industry sterilisation models, which are temperature dependent only, to a range of liquid foods. A modified Arrhenius equation has been successfully widely applied to sterilisation of a range of bacteria and foods (Coker, Davey and Kristall 1993, Davey, Hall and Thomas 1995, Davey and Cerf 1996) and is of the form:

$$\ln (k) = C_0 + C_1 / T + C_2 \, \mathrm{pH} + C_3 \, \mathrm{pH}^2 \qquad (2.7)$$

In an attempt to provide sufficient experimental data for the combined effect of process temperature and pH on sterilisation kinetics for model synthesis, Davey, Hall and Thomas

(1995) experimentally investigated heating of samples in capillaries of small diameter. A block experimental design consisting of five exposure temperatures and eight pH values together with five exposure times was used. The test liquid selected had a value of the pseudoplastic index comparable to a range of liquid foods (Davey and Wood 1984). The test micro-organism was *E. coli* ATCC 25922. The effect of pH on inactivation was shown to be highly significant.

A modified Arrhenius model of the form of equation 2.7 was formulated to fit these data. However model predictions at the greater exposure times consistently under predicted the number of surviving bacterial cells. This is apparently due to the occurrence of tails in the bench-scale survivor data. It was concluded that the formulation of an adequate non-linear (ie other than first order) inactivation model that includes the dependence of combined exposure temperature and liquid pH on inactivation rates should be studied and the implications for performance of a continuous steriliser investigated.

2.4 SUMMARY

From the review of the literature the following important factors emerge which are relevant to this study:

1. A number of mathematical models have been proposed to simulate continuous sterilisation of liquids and allow for the effects of changing process parameters, principally the exposure temperature and holding time. Because the values of the parameters used in predictions with the generalised steriliser performance equations have been considered in isolation, these published models are of limited value. Such models can only be used as a guide for broad comparative purposes.

2. Limited theoretical studies of continuous sterilisation and very limited published experimentally determined bench-scale survivor data have highlighted the highly significant effect of pH in addition to exposure temperature (T) and holding time on

the thermal inactivation kinetics of contaminating bacteria. With two exceptions, Davey, Hall and Thomas (1995) and Cole et al (1993) there are not many published data that demonstrate the combined effect of both process temperature and liquid pH (T-pH) on the survival of vegetative bacteria that are amenable to steriliser design and optimisation. Reasons for this limited data include the "... monumental amount of work..." and the "... painstaking effort required..." to generate the data (McMeekin et al 1993) and the move worldwide to *in confidence* research results.

3. Comprehensive experimental evaluation of process models for the combined T-pH effect that would permit a comparison of experimental results with predictions in a dynamic system are not available in the published literature. An evaluation of the effect of combined exposure temperature and pH is an essential step to process understanding and process optimisation.

4. Published models imply first-order or log-linear inactivation kinetics of contaminating bacterial cells. Limited bench-scale data of the combined effect of T-pH suggest non-linear inactivation kinetics. The implications of this in the generalised steriliser performance equations have not been investigated.

5. Because the model form of Lin (1976) and Davey and Wood (1984) is based on chemical reactor principles and fluid mechanics it is selected for theoretical extension, modification and experimental testing.

In the following two chapters, the inactivation kinetics of bacteria as effected by combined process T-pH are reviewed in detail and an extensive experimental investigation is undertaken to determine sufficient bench-scale data to asses the nature of the kinetics of survival of a number of typical liquid food contaminants. The implications of possible confirmation of non-linear inactivation kinetics on the generalised performance equations for sterilisation are investigated and suitable criteria to test for an adequate model are established.

CHAPTER
THREE

MATERIALS AND METHODS

Parts of this chapter have been published as:

Chiruta, J., Davey, K. R. and Thomas, C. J. (1997b) Thermal inactivation kinetics of three vegetative bacteria as influenced by combined temperature and pH in a liquid medium, *Transactions of the Institution of Chemical Engineers, Part C, Bioproducts and Food Processing*, **75**: 174-180

3.1 INTRODUCTION

This chapter describes a bench-scale apparatus and experimental procedures used to determine new survivor data of the kinetics of thermal inactivation of two selected bacteria as affected by combined exposure temperature and pH (T-pH). These data, together with available published data for a third micro-organism, are analysed for an appropriate model form for thermal inactivation kinetics.

The aim is to determine the value of model parameters that can be used in the generalised performance equations for continuous sterilisation to predict the length of holding tube of a pilot steriliser in which the thermal effects on liquid containing bacteria can be observed. The implications of possible confirmation of non-linear inactivation kinetics on the generalised are investigated. Suitable criteria to test for an adequate kinetic model for thermal inactivation are established.

Consequently this chapter deals with:

1. selection and rheological characterisation of a suitable carrier liquid
2. selection of test micro-organisms
3. establishing criteria for testing for an adequate kinetic model.

3.2 TEST LIQUID

A sterile (autoclaved), aqueous mucilage of 2 kg m^{-3} Carbopol® 934 resin neutralised with AR sodium hydroxide was selected as the test liquid. This liquid has a number of characteristics advantageous to this study as pointed out by Davey, Hall and Thomas (1995) and Davey and Wood (1984), that include it:

- is not bactericidal and is non-toxic
- is resistant to degradation by bacteria and does not support the growth of bacteria

- exhibits an appropriate value of the pseudoplastic index (n) that simulates a wide range of time-independent liquid foods
- is transparent and therefore suitable for dye-trace studies to investigate the adequacy of hydrodynamic flow assumptions in the proposed continuous steriliser
- has an excellent product uniformity and well defined rheological properties and does not exhibit aging effects associated with carboxymethylcellulose solutions (Naik, Lee and Richardson 1977)
- is available in quantity.

The pseudoplastic index of the mucilage is n = 0.37 as determined from some 1470 shear stress shear rate data pairs, each the mean of 21 data points with 14 applied shear rates at each of five temperatures (Davey and Wood 1984). This value is approximately a mid-range value between 0.25 and 0.65 that characterises a wide range of liquid foods (Holdsworth 1971, 1992, 1993).

Mucilages with a range of pH values for study were obtained with selected adjustment using a (sterile) solution of (AR) sodium hydroxide (18% w/v), or by the addition of hydrochloric acid or sodium chloride. Rheological testing of the mucilage has shown that the viscosity did not differ significantly across the entire selected pH range (Davey, Hall and Thomas 1995, Davey 1980). Mucilages were prepared using the protocol established by Davey and Wood (1984) and Davey, Hall and Thomas (1995) who reported that the viscosity of the mucilage was not effected by the presence of dispersed bacterial cells of *E. coli*.

Results obtained in this carrier liquid would therefore be applicable to interpretation for the processing of a wide range of liquid foods. Other test liquids might have included tomato puree, condensed milk or orange juice. These were not used because of short shelf life, cost in quantity, irreproducible liquid uniformity, ability to support micro-organism growth and the impracticality for dye-trace studies.

3.3 TEST MICRO-ORGANISMS

3.3.1 Selection Criterion

The strains of microorganism selected for the experimental tests would necessarily have to satisfy a number of essential requirements, namely:

- be well documented
- have a significant sensitivity to temperature
- exhibit simple growth requirements
- be easily dispersed as individual cells.

3.3.2 Selected Strains

Pure strains of three vegetative bacteria were selected as the test micro-organisms: *Escherichia coli*, 'Seattle strain' American Type Culture Collection (ATCC) 25922, *Listeria monocytogenes*, Seelieger Listeria Culture Collection (SLCC) 5764, and *Pseudomonas fluorescens* 172, obtained from the Department of Microbiology and Immunology, University of Adelaide. These represent common micro-organisms known to spoil liquid and solid food. They are non-spore forming and are characterised as thermo-tolerant to mild thermal processes.

The thermal sensitivity of these bacteria conveniently permitted exposure temperatures below liquid boiling (and avoided high pressures) thereby simplifying the consequent pilot continuous steriliser. Findings however can be extrapolated to behaviour of thermally less sensitive contaminant cells in the hydrodynamics of a continuous steriliser as pointed out by Davey and Wood (1984) and Davey, Hall and Thomas (1995).

3.3.3 General Characteristics

3.3.3.1 *Pseudomonas fluorescens*

Pseudomonas spp. are ubiquitously distributed in soil and water and are commonly associated with spoilage of foods including milk. They are strictly aerobic, Gram-negative bacteria. Microscopically, they appear as straight or curved rods and cocci shapes. The cell has a diameter of 0.7μm to 0.8 μm and a length of 2 μm to 3 μm. *Pseudomonas* spp. have an optimum temperature of growth of 25°C to 30°C. However, they grow over the temperature range of 4°C to 43°C. They are motile by one or more polar flagella and never fermentative. Some species produce yellow-orange cellular pigments (Palleroni 1984).

The heat resistance of *Pseudomonas* spp. is not well documented. Dabbah, Moats and Mattick (1969) and Moats (1971) reported that some cells could recover post thermal exposure and grow in a suitable liquid medium.

Most of the recent literature associated with *P. fluorescens* however is related to lability and stability of extra-cellular lipases and proteases (Tsuji et al 1982). Kalchayanand et al (1998) investigated the effect of interactions of hydrostatic pressure, time and temperature on inactivation of *P. fluorescens*. They found that in general cell death increased as the pressure, time, or temperature increased. However, the death rate at high pressure and high temperature (50°C) combination followed first-order kinetics, and at lower pressure and temperature combinations it showed a tailing effect.

3.3.3.2 *Listeria monocytogenes*

Members of the genus *Listeria* are facultatively anaerobic to microaerophilic (growth enhanced under reduced oxygen and 5% to 10% CO_2), Gram-positive bacteria. Microscopically they appear as rod shaped with rounded ends and measure 0.4 μm to 0.5 μm in diameter by 0.5 μm to 2.0 μm in length. The rods have a tendency to produce chains of three to five or more cells. They are motile by flagella when grown at 22°C to 25°C. Growth occurs between 4°C and 38°C, particularly when the medium contains small amounts of glucose (Seeliger and Jones 1986).

L. monocytogenes is reported to be more thermally resistant than many other non-sporing pathogens associated with food such as *Salmonella* (Mackey and Bratchell 1989, Farber 1989). A large outbreak of listeriosis associated with the consumption of pasteurised milk initiated efforts to obtain accurate *D* values for thermal inactivation of *L. monocytogenes* in milk in order to assess the margin of safety for different pasteurisation regimes (Mackey et al 1990). Karaioannoglou and Xenos (1980), Harrison and Carpenter (1989a,b), Carpenter and Harrison (1989) showed that *L. monocytogenes* can survive in red meat or poultry cooked to temperatures as high as 82°C. The work of Cole et al (1993) indicated that the heat resistance of *L. monocytogenes* is affected by a wide variety of environmental conditions. The micro-organism tolerates widely ranging salt concentrations (up to 10% to 12%), pH (5.5 to 9.5) and a wide temperature range (-0.4°C to about 44°C). It is, however, capable of growing well at ordinary refrigerator temperatures, and this property gives it a competitive advantage over any accompanying mesophilic micro-flora (Varnham and Evans 1991).

Usually infection ranges from a mild influenza-like condition to severe infections in the blood and brain. The consequences of infection with *L. monocytogenes* are abortions, encephalitis and meningitis in animals and humans (Grau 1996).

3.3.3.3 *Escherichia coli*

E. coli is a well documented and a widely studied bacterium. Most of the information about the genetic organisation of procaryotic cells is derived from studies with *E. coli*. The bacterium is facultative, Gram-negative and appears as a colourless rod about 1µm to 2 µm in liquid media. It is a component of the normal intestinal flora and gives rise to disease only under exceptional circumstances. This bacterium is often used as an indicator strain for fecal contamination of waters and foods.

The effect of heat and other environmental factors on thermal inactivation of this bacterium can be found in literature over many years. Jordon, Jacobs and Davies (1947) studied the lethal effect of different temperatures on *E. coli* at pH 7.0. They found different combinations of temperature and exposure time that had a 99.99% (ie $\log_{10}4$) lethal effect

on *E. coli*. Their study showed that the lethal temperature for a particular micro-organism depends on the time of exposure (Hawker and Linton 1979). Earlier, Jordon and Jacobs (1944, 1945) had studied the relationship between the logarithms of survivors and time when exposed to various concentrations of phenol (between 3.5 g l^{-1} and 6.01 g l^{-1}) at a temperature of 35°C.

Aiba, Humphrey and Millis (1973) presented experimental data for *E. coli* in buffer, as proportion of survivors against time over a range of temperatures between 54°C and 60°C. The observations suggest that a 5-$\log_{10}$ reduction of survivors was obtained at 60°C and about 3.5 min, or at 58°C for about 6.5 min (Bailey and Ollis 1986). The decimal reduction time value calculated from the data of Aiba, Humphrey and Millis (1973) is D = 16.25 *s* at 60°C, this compared with about D = 3.8 *s* from the data of Davey, Hall and Thomas (1995). These differences might be attributed to both differences in the strains used in the different studies. Lemaire, Cerf and Audurier (1989) also reported differences in the heat resistance among the strains of *L. monocytogenes*.

The effect of the suspending carrier medium should also not be ignored. It is noteworthy in this context however that for *E. coli*, the decimal reduction time values obtained in the study of Davey, Hall and Thomas (1995) were in very good agreement with those reported by Davey (1980) and Davey and Wood (1984), who used identical strain and carrier.

3.4 THERMAL INACTIVATION KINETICS

Because the data for *E. coli* in the study of Davey, Hall and Thomas (1995) were in very good agreement with those reported by Davey and Wood (1984) and Davey (1980) these published data were confidently appropriated to be used for analyses of survivor kinetics as influenced by combined T-pH. Comparative data were similarly experimentally determined for both *P. fluorescens* and *L. monocytogenes*.

The thermal inactivation kinetics of both *P. fluorescens* and *L. monocytogenes* in the selected carrier liquid were determined using the method described by Davey, Hall and Thomas (1995) which was itself a modified form of that outlined by Stern and Proctor (1954). This method uses samples in small diameter glass capillary tubes. The advantage is the rapid heating and cooling of a number of samples that can be treated simultaneously. The investigation involved the:

- maintenance of the bacterial cultures
- production and harvesting of the cells
- heat exposure using the micro-method
- counting of viable cells by the spread plate technique.

3.4.1 Maintenance of Bacterial Strains

Stock cultures of bacteria were routinely maintained as suspensions in glycerol at -70°C.

3.4.2 Production and Harvesting of the Bacteria

P. fluorescens were routinely cultivated on nutrient agar or nutrient broth (Oxoid®). *L. monocytogenes* was cultivated on brain heart infusion agar or broth (Difco®). Cells of *P. fluorescens* were sub-cultured from stock cultures and grown in 50 ml of Nutrient Broth with incubation with shaking at 37°C for 18 hours, and, 25°C for 48 hours, respectively. *L. monocytogenes* was routinely cultivated in Difco Brain-Heart Infusion broth (BHI) and incubated with shaking at 37°C for 18 hours.

The overnight cultures were harvested by centrifugation (4000 x g for 10 minutes), washed and re-suspended in sterile, distilled water (Davey and Wood 1984). All cell suspensions were subjected to the same environmental conditions before and after experiments. To minimise the influence of the age of cells, the experiments were conducted within a short time (about 2 hours) of harvesting. In this way any differences in the number of cells

inactivated during heating can be attributed to the effect of temperature, or other environmental factors.

3.4.3 Micro-Method for Heat Treatment

Harvested cells were added aseptically to a solution of pH-adjusted Carbopol (see section 3.2) to achieve cells densities of between 10^7 - 10^8 cells per ml. Volumes (10 µl) of these cell suspensions were loaded into the centre of sterile, thin-walled glass micro hematocrit tubes (1.1 mm inner diameter with a 1.5 mm outer diameter and a length of 75 mm, Clay Adams, Parsipanny, Nj, USA) using an auto pipette. The ends of the tubes were heat-sealed in the flame of Bunsen-Burner (Davey, Hall and Thomas 1995). Hematocrit tubes were used because of the combination of thin glass wall (0.2 mm) and small suspension volume that gave a practical negligible time for heat up and cool down.

Sealed tubes carrying test suspensions were chosen at random and immersed in a temperature controlled water bath. After exposure the treated tubes were placed in an ice bath for rapid cooling.

The experiments at a given temperature were carried out in random order. Control tubes were treated in an identical manner but without exposure. The numbers of viable bacteria in the heat-treated and control tubes were then determined.

3.4.4 Enumeration of Viable Bacteria

The content of each capillary tube was flushed into sterile plastic tubes using 200 µl of sterile diluent (0.85% w/v sodium chloride). This fluid was serially diluted and 100 µl volumes of each dilution plated on nutrient medium.

P. fluorescens was plated on nutrient agar. *L. monocytogenes* was plated on BHI agar. Inoculated plates were then incubated at 37°C for 24 hours. Plates with between 30-300 colonies were counted and these counts used to determine the number of surviving bacteria

(Meynell and Meynell 1970). Counts of bacteria present in unheated suspensions were also determined to two replicates of each raw sample counted. Any (occasional) contaminant was easily differentiated on the basis of colonial morphology and was not therefore counted.

3.4.5 Experimental Design

For each of the selected bacteria, an experimental block design that covered up to six levels of exposure temperature in the range of 52°C to 62°C (52, 54, 56, 58, 60, 62°C), and up to eight levels of pH values (4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5), together with exposure times from 10 *s* to five minutes, was employed. The experiments at each exposure temperature and time were carried out in a random order. This design permitted quantification at the extreme rates of thermal inactivation.

3.5 CRITERIA FOR AN ADEQUATE MODEL

An adequate mathematical model is an essential step to longer-term process control and process optimisation (Davey 1993a). The criteria for an adequate model include:

- accurate predictions against observed data
- relative complexity (ie parsimony see McMeekin et al 1993)
- ease of use

and, arguably, the potential for physiological significance and interpretation of model parameters (Davey 1993a).

3.6 CONCLUDING REMARKS

The apparatus and experimental procedures described in this chapter have been used to provide robust bench-scale thermal inactivation data for testing of appropriate survivor kinetics. The criteria adopted for testing has been established.

The following chapter presents the evaluation of thermal inactivation kinetics of the test micro-organisms in the test liquid. These data are analysed for an appropriate model form for thermal inactivation kinetics, together with available published data for a third micro-organism. The aim is to determine values of an appropriate kinetic model that can be used to size a pilot continuous steriliser.

CHAPTER

FOUR

INACTIVATION KINETICS OF BENCH-SCALE DATA

The findings of this chapter have been published as:

Chiruta, J., Davey, K. R. and Thomas, C. J. (1997b) Thermal inactivation kinetics of three vegetative bacteria as influenced by combined temperature and pH in a liquid medium, *Transactions of the Institution of Chemical Engineers, Part C, Bioproducts and Food Processing*, **75**: 174-180

4.1 INTRODUCTION

Results obtained from the experimental investigation described in Chapter 3 of the bench-scale thermal inactivation data for *P. fluorescens* and *L. monocytogenes*, as affected by combined temperature and liquid pH (*T*-pH), are presented. These data are analysed together with published data for the bench-scale thermal inactivation of a third micro-organism, *E. coli*, as affected by combined temperature and liquid pH.

Thermal survival kinetics are examined using all three data sets in a comparative study. The effect of pH is shown to be highly statistically significant and a decimal reduction time $D_{°C,pH}$ is defined.

Major findings are summarized and the need for non-linear kinetic models for thermal inactivation is highlighted. The implications arising from the use of non-linear survivor kinetics as influenced by combined exposure temperature and liquid pH to food processing are discussed.

In the following chapter, Chapter 5, the data are analysed for an appropriate model form for thermal inactivation kinetics.

4.2 RESULTS

4.2.1 Survivor Curves

The experimental designs used to derive bench-scale thermal inactivation data resulted in 20 survivor curves for *P. fluorescens* and 48 for *L. monocytogenes*. These compare with the 40 survivor curves for *E. coli* of the data of Davey, Hall and Thomas (1995).

However the number of survivor curves for *P. fluorescens* is targeted over a narrower range of pH that is specific to liquid foods (eg purees, juices).

Sample raw data for *P. fluorescens* are presented in Table 4.1 for a mid-range exposure temperature of 56°C. Presented in Table 4.2 is a sample set for *L. monocytogenes* for a (approximate) mid-range exposure temperature of 58°C.

The complete raw data for *P. fluorescens* and *L. monocytogenes*, and *E. coli* are presented in Appendix C.

4.2.2 Value of the Rate Coefficient (k) at Each Temperature

The value of the rate coefficient, k, was determined for each survivor curve from a linear regression (Snedecor and Cochran 1969) of a plot of $\log_{10} \langle N \rangle / \langle N_0 \rangle$ versus time (t) (equation 2.4) at each T-pH.

4.2.3 Corresponding $D_{°C,pH}$ Values at Each Temperature

Corresponding values of a decimal reduction time defined by both exposure temperature (°C) and pH, $D_{°C,pH}$, were calculated using equation 2.6. These results are presented in Table 4.3 and Table 4.4, respectively, for *P. fluorescens* and *L. monocytogenes*.

For comparison, decimal reduction times for *E. coli* reported by Davey, Hall and Thomas (1995) are summarised in Table 4.5.

4.2.4 Value of k at Each T-pH

Figures 4.1 to 4.3 show the dependence of the rate coefficient for thermal inactivation expressed as ln k versus pH at each exposure temperature over the experimental temperature range of 52°C to 62°C for *P. fluorescens*, *L. monocytogenes* and *E. coli*, respectively.

4.3 VALUE OF *k* FOR COMBINED *T*-pH

The value of the coefficients for the model for combined temperature and pH (equation 2.7) were obtained from a standard linear regression of the survivor data for each bacterium to give, respectively, for *P. fluorescens* and *L. monocytogenes* :

$$\ln(k) = 116 - 41685 / T + 2.56\ \mathrm{pH} - 0.250\ \mathrm{pH}^2 \qquad (4.1)$$

$$\ln(k) = 130 - 41160 / T - 2.73\ \mathrm{pH} + 0.194\ \mathrm{pH}^2 \qquad (4.2)$$

Substitution for T degree absolute gives the rate coefficient for thermal inactivation in s^{-1}.

Equations 4.1 and 4.2, respectively, explain 94.3 $\%V$ and 85.8 $\%V$. For *E. coli*, Davey, Hall and Thomas (1995) reported the following model:

$$\ln(k) = 139 - 44908 / T - 1.52\ \mathrm{pH} + 0.124\ \mathrm{pH}^2 \qquad (4.3)$$

where substitution for T absolute, gives the rate coefficient for thermal inactivation in s^{-1} with 92 $\%V$ explained.

These apparent high values of $\%V$ suggested by equations 4.1, 4.2 and 4.3 are however misleading and must not be used in isolation. The misleading nature of the apparent good fit is clear from directly comparing predictions with the raw data as detailed in the following discussion.

4.4 DISCUSSION

The use of decimal reduction time (D) was criticised by Davey (1982) because this, and other commonly used industry definitions, "confuse, or at least obscure, what should be the ... simple mathematics of a first order equation, where the inactivation kinetics are ...

totally, defined by k and the activation energy. An additional statement about the range of (temperature) and N_0 from which they were determined may also be very important to the designer as the thermal kinetics are often pseudo-first-order from which extrapolation might not be confidently done".

Further, because either degree Fahrenheit or Celsius may be used, a careful check on the calculation is necessary. Davey, Hall and Thomas (1995) demonstrated that in sterilisations, both the pH and temperature are necessary in defining values of the decimal reduction time.

Assuming first-order kinetics for inactivation, an apparent good fit - implied through the high values of $\%V$, equations 4.1, 4.2 and 4.3 - is obtained for k. However, this is very misleading because a good fit to k does not imply, especially with these data, a good fit to the survival level expressed as $\log_{10}(N / N_0)$. This is revealed clearly in Figures 4.4 and 4.5, respectively, for *P. fluorescens* and *L. monocytogenes,* where a direct comparison of predictions with the raw data is made.

Consequently, the decimal reduction time ($D_{°C,pH}$), and thereby k (equation 2.6), is of little practical use for the three bacteria studied. This is because there is no systematic variation in the difference between predicted and observed reductions (residuals) in viable cell numbers. This can be seen for *P. fluorescens* where, from Table 4.3 at a mid-range temperature of 56°C and pH 6.0, $D_{56,\ 6.0}$= 142.9 *s*. From the raw data of Table 4.1, however, the actual reduction at 143 *s* would be about 0.51-$\log_{10}$ (obtained through interpolation) - and not the one $\log_{10}$, (90%), reduction implied by D. The result is an under-treated sample with possible implications of a public health significance. However, at T = 56°C and pH 6.5, $D_{56,\ 6.5}$ = 200.0 *s*, whereas from Table 4.1 with t = 200.0 *s* the actual reduction would be greater at about 1.26-$\log_{10}$. The result of using D is an over-treatment of the liquid that is wasteful in energy and denatured quality (eg vitamin retention) (Davey and Cerf 1996).

For *L. monocytogenes* at T = 58°C and pH 6.0, Table 4.4 gives a value $D_{58,\ 6.0}$ = 227.3 *s* whereas from the raw data of Table 4.2 an actual reduction of 1.24-$\log_{10}$ would be expected. In this case there is an over-treatment that would result in a conservative thermal treatment of the contaminated Carbopol liquid. At the same temperature of 58°C but at a pH 5.0, $D_{58,\ 5.0}$ = 73.5 *s* (from Table 4.4). The raw data of Table 4.2 however shows that a 0.29-$\log_{10}$ reduction in viable cells would be obtained, implying a serious under-treatment of the liquid.

At a T of 56°C and pH 5.0, the reduction in $\log_{10}$ (N / N_0) survivors of *P. fluorescens* ranged between 0.116-$\log_{10}$ and about 5 $\log_{10}$ (with 15 *s* ≤ exposure time ≤ 300 *s*) (Table 4.1), and for *L. monocytogenes* at 58°C and pH 5.0, between 0.03-$\log_{10}$ and about 4-$\log_{10}$ (with 10 *s* ≤ t ≤ 240 *s*) (Table 4.2). These reductions compared with reductions of about 1.90-$\log_{10}$ to 5.41-$\log_{10}$ for the more thermally sensitive (ie more easily inactivated) *E. coli* at 58°C and pH 5.0 (with 8 *s* ≤ t ≤ 40 *s*) of Davey, Hall and Thomas (1995). This reduction in viable cell number for *E. coli* is obtained in the much shorter exposure of 40 *s* compared with the longer times of between 240 *s* and 300 *s*, respectively, for the more thermally resistant *L. monocytogenes* and *P. fluorescens*.

Inspection of the plots of raw data ($\log_{10}$ (N / N_0) versus exposure time) of Figures 4.4 and 4.5, reveals that survivor data cannot be regarded as first-order as implied in equations 2.4 and 2.6. Davey, Hall and Thomas (1995) demonstrated this for the bacterium *E. coli*. Figure 4.4 shows concave-up tails in the survivor curve for *P. fluorescens*. Figure 4.5 shows concave-down tail deviations and concave-up tails for *L. monocytogenes*. Some curves in these figures however appear not to cover a wide enough range to give definitive information about the shape of the survival curve. This is especially true for the survival data obtained at lower temperatures - where reductions are small compared with those obtained at higher temperatures. Nevertheless the survival data taken for all log-reductions at both the higher temperature - where reductions are greater - and lower temperatures at various T-pH combinations clearly do not conform to classical first-order kinetics. The assumption of log-linear (ie first order) inactivation kinetics is not valid therefore for these three bacteria.

For all three data sets therefore, the survivor curves deviate significantly from first-order, ie log-linear kinetics. This finding taken together with that from Cole et al (1993) for the bacterium *L. monocytogenes* - the only other published data for the combined effect of both exposure temperature and liquid pH on thermal survivor kinetics - demonstrates the need for a re-assessment of the assumptions of the kinetics of thermal inactivation as was outlined by Davey, Hall and Thomas (1995).

In this circumstance the use of the decimal reduction time D, or k, the rate of coefficient of thermal inactivation, are not appropriate (unless restricted to demonstrated linear sections of the survivor curve) because of a non-systematic deviation in predictions from experimental values. However, the overall mean value of decimal reduction time shown in Tables 4.3, 4.4 and 4.5, can be used to rank the three bacteria in order of thermal resistance ie *P. fluorescens* (D_{mean} = 267.18 *s*) is more thermally resistant than *E. coli* (D_{mean} = 18.6 *s*) and less thermally resistant than *L. monocytogenes* (D_{mean} = 490.5 *s*).

It is of interest to note that for *L. monocytogenes* these D values are significantly lower at the higher temperatures studied than those reported in the literature. For example, Farber et al (1989) studied the heat resistance of a mixture of 10 different strains of *L. monocytogenes* inoculated into ground meat and ground meat plus cure. They reported that D values for ground meat ranged from 1.01 min at 62°C to 13.18 min at 56°C. Comparative values from our study (at pH 5.0 to pH 6.0) are similar at the lower temperature of 56°C (12.82 min, 769.2 *s* Table 4.4, *cf* 13.18 min) but appreciably lower at the higher temperature (21.3 *s cf* 60.6 *s*). That is, at the higher temperature the published D value is about three times greater than obtained in the present study with *L. monocytogenes*.

Ben-Embarek and Huss (1993) reported a study of the heat resistance of two strains of *L. monocytogenes* in *sous-vide* cooked fillets of cod and salmon. D values at 60°C ranged between 1.95 min and 4.48 min - depending on the strain and fish type (cod or salmon). Both strains were one-four times more heat-resistant in salmon than in cod. Differences in

D value were attributed to a higher fat content in salmon as compared to cod. These values are about 3.7 to 8.5 times greater than values shown in Table 4.4 for *L. monocytogenes*.

Using the graphical data of Aiba, Humphrey and Millis (1973) for *E. coli* - presented as k versus T - it is possible to show from equation (2.6) that the D value at 60°C is 16.25 *s*. This compares with about 3.8 *s* shown in the data of Table 4.5. Therefore the published D value of Aiba, Humphrey and Millis (1973) appears to be about four times than reported earlier (Davey, Hall and Thomas 1995). These differences might be attributed to the behaviour of the suspending carrier medium in the strains used in the different studies. Lemaire, Cerf and Audurier (1989) report "a significant difference in thermal resistance among strains" of *L. monocytogenes*. This appears certainly the case with the effect of the fat content of the two fish species of Ben-Embarek and Huss (1993). The Carbopol carrier medium used in the present study appears less protective than the sausage and fish meats of Farber et al (1989) and Ben-Embarek and Huss (1993). It is noteworthy in this context however that for *E. coli* the D values obtained in the present study are in very good agreement with those reported by Davey (1980) and Davey and Wood (1984) who used identical strains and carrier fluids. At 60°C for example, respectively, D values are 3.8 *s* and 4.3 *s*. Because the mechanism of inactivation of the cell is likely to be a concert of complex interactions involving mechanisms for protein repair and unfolding as well as molecular systems designed to help, the bacterial cell survive under conditions different from the optimum. It is therefore unlikely that one mechanism of inactivation prevails.

There are significant implications for non-logarithmic survival for food processing. These uncertainties mitigate against long term optimisation. Formulation of non-linear models is therefore seen as necessary for accurate prediction and process control of thermal sterilisations. It would appear that, as a first step to formulation, the rate coefficient for thermal inactivation might be regarded as time-dependent.

This study's findings have a direct application to minimally processed foods, including fruit juices, milk and purees where over-treatment will result in lower product quality

because of concomitant denaturation of nutrient, such as vitamin, and caramelisation of sugars with consequent fouling of heat exchange surfaces.

4.5 CONCLUSIONS

1. The effect of pH on the rate coefficient for thermal inactivation is significant for all three bacteria, especially at the lower exposure temperatures.

2. The survivor curve for all three bacteria showed tailing. Concave-up tails appeared for *P. fluorescens* and *E. coli* and, concave-down for *L. monocytogenes*. The assumption of log-linear (ie first-order) inactivation kinetics is therefore not valid over the entire curve for these three bacteria. Consequently, widely used D values and k values, calculated using the log-linear model, are therefore not appropriate for these data, unless used judiciously in demonstrated linear sections of the survivor data.

3. Formulation of a model for non-linear survivor data should be undertaken and demonstrated. This might involve a model form with a time-dependent rate of inactivation.

In the following chapter, the bench-scale data for the effect of combined T-pH on thermal inactivation of the three test micro-organisms in the test liquid, together with the published data of Davey and Wood (1984) that were obtained in continuous steriliser (but with the effect of T only), are analysed for an appropriate non-liner model form for thermal inactivation kinetics.

Table 4.1 Sample raw data for *P. fluorescens*: $\log_{10}$ (N / N_0) (mean of two replicates) at a mid-range value of exposure temperature of 56°C and a range of exposure times and pH

Exposure time	log (N / N_0)			
(*s*)	**pH 5.0**	**pH 5.4**	**pH 6.0**	**pH 6.5**
15	-0.116	-0.192	-0.141	-0.074
30	-0.220	-0.202	-0.174	-0.096
45	-0.227	-0.209	-0.210	-0.150
60	-0.453	-0.230	-0.217	-0.158
90	-0.431	-0.333	-0.300	-0.289
120	-0.511	-0.203	-0.413	-0.516
180	-0.921	-0.435	-0.695	-1.300
240	-2.141	-1.870	-1.577	-1.203
300	-5.317	-4.921	-2.889	-1.426

Table 4.2 Sample raw data for *L. monocytogenes*: $\log_{10}$ (N / N_0) (mean of two replicates) at a mid-range value of exposure temperature of 58°C and a range of exposure times and pH

Exposure time	log (N / N_0)							
(*s*)	**pH 4.0**	**pH 4.5**	**pH 5.0**	**pH 5.5**	**pH 6.0**	**pH 6.5**	**pH 7.0**	**pH 7.5**
10	-0.630	-0.613	-0.030	-0.103	-0.106	-0.028	-0.040	-0.045
20	-0.714	-0.686	-0.060	-0.136	-0.099	-0.087	-0.142	-0.214
30	-0.691	-1.432	-0.158	-0.339	-0.168	-0.079	-0.178	-0.405
60	-1.400	-1.158	-0.207	-0.433	-0.107	-0.141	-0.335	-0.566
120	-4.053	-2.156	-0.577	-0.619	-0.305	-0.219	-0.698	-0.821
180	-5.727	-3.531	-2.089	-2.022	-0.430	-0.831	-1.329	-1.159
240	-6.427	-5.501	-4.326	-2.617	-1.464	-0.618	-1.794	-2.172

Table 4.3 Decimal Reduction Time for *P. fluorescens* (expressed in *second*) as affected by combined exposure temperature and liquid pH

T **(°C)**	***D*** **(*s*)**				***D*** **Mean (*s*)**
	pH 5.0	**pH 5.4**	**pH 6.0**	**pH 6.5**	
52	454.5	500.0	555.5	1190.5	675.1
54	370.4	434.8	400.0	526.3	432.9
56	83.3	100.0	142.9	200.0	131.6
58	52.6	66.7	45.5	77.0	60.5
60	31.3	40.0	32.3	40.0	35.9
D **Mean**	198.4	228.3	2.35.2	406.8	**267.2**

Table 4.4 Decimal Reduction Time for *L. monocytogenes* (expressed in *second*) as affected by combined exposure temperature and liquid pH

T **(°C)**	***D*** **(*s*)**								***D*** **Mean (*s*)**
	pH 4.0	**pH 4.5**	**pH 5.0**	**pH 5.5**	**pH 6.0**	**pH 6.5**	**pH 7.0**	**pH 7.5**	
52	214.1	471.7	900.0	826.4	1960.6	2381.0	1470.6	8333.3	2069.8
54	58.3	79.9	127.9	167.5	613.5	294.1	1123.6	666.7	391.4
56	32.2	59.5	357.1	92.0	769.2	344.8	294.1	185.1	266.8
58	34.2	47.2	73.5	98.0	227.3	434.8	138.9	126.6	147.6
60	18.0	33.2	51.8	39.5	31.5	64.5	95.2	44.8	47.3
62	10.1	18.8	29.5	23.1	19.5	21.3	18.1	18.2	19.8
D **Mean**	61.2	118.4	256.8	207.8	603.6	590.1	523.4	1562.5	**490.46**

Table 4.5 Decimal Reduction Time for *E. coli* (expressed in *second*) as affected by combined exposure temperature and liquid pH

T **(°C)**	***D*** **(*s*)**								***D*** **Mean (*s*)**
	pH 4.0	**pH 4.5**	**pH 5.0**	**pH 5.5**	**pH 6.0**	**pH 6.5**	**pH 7.0**	**pH 7.5**	
54	19.6	31.0	43.3	71.4	93.7	132.3	83.5	49.1	65.5
56	12.3	13.3	15.2	18.2	20.2	19.6	17.1	13.2	16.1
58	4.2	5.6	6.0	6.8	6.7	5.7	6.1	5.0	5.8
60	3.1	3.8	3.0	3.7	3.8	3.3	3.6	3.1	3.4
62	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.1	2.0
D **Mean**	8.2	11.1	13.9	20.4	25.3	32.6	22.5	14.5	**18.6**

Figure 4.1 Summary of experimentally determined data describing the combined effect of exposure temperature and pH on inactivation of *P. fluorescens,* and a comparison with model predictions of equation 4.1 (□ = 60°C, Δ = 58°C, O = 56°C, ◊ = 54°C, ∇ = 52°C)

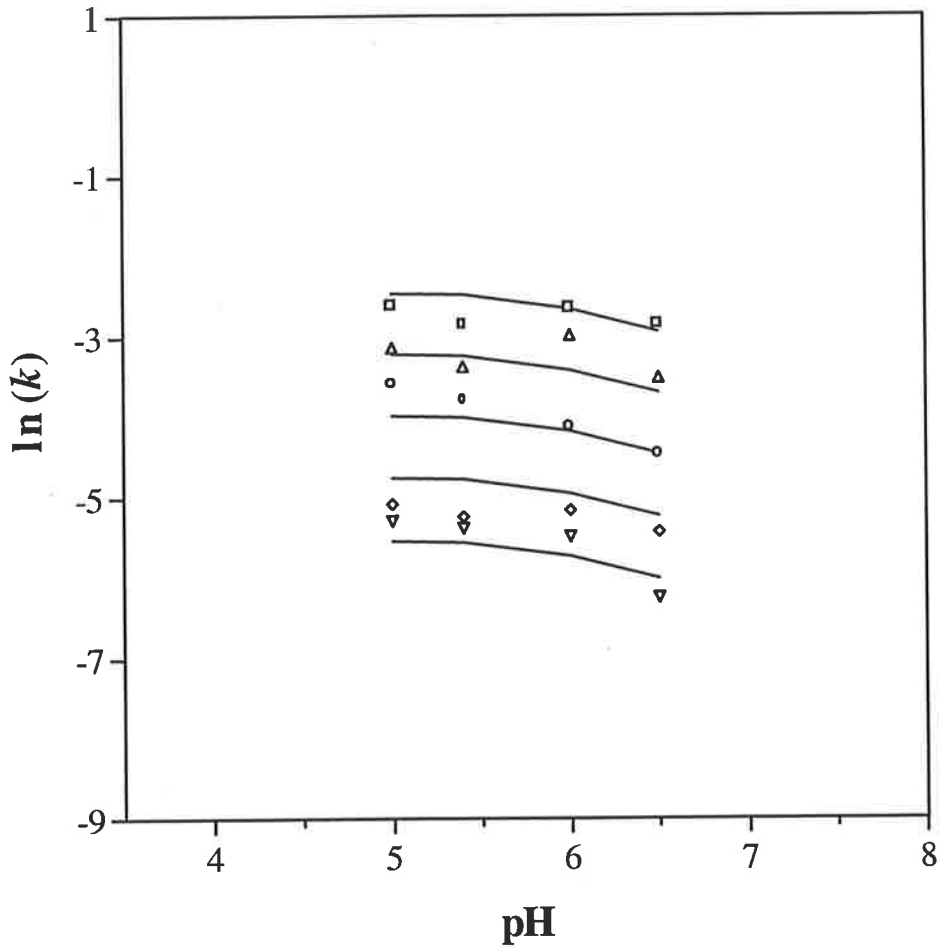


Figure 4.2 Summary of experimentally determined data describing the combined effect of exposure temperature and pH on inactivation of *L. monocytogenes,* and a comparison with model predictions of equation 4.2 (■ = 62°C, □ = 60°C, Δ = 58°C, O = 56°C, ◊ = 54°C, ∇ = 52°C)

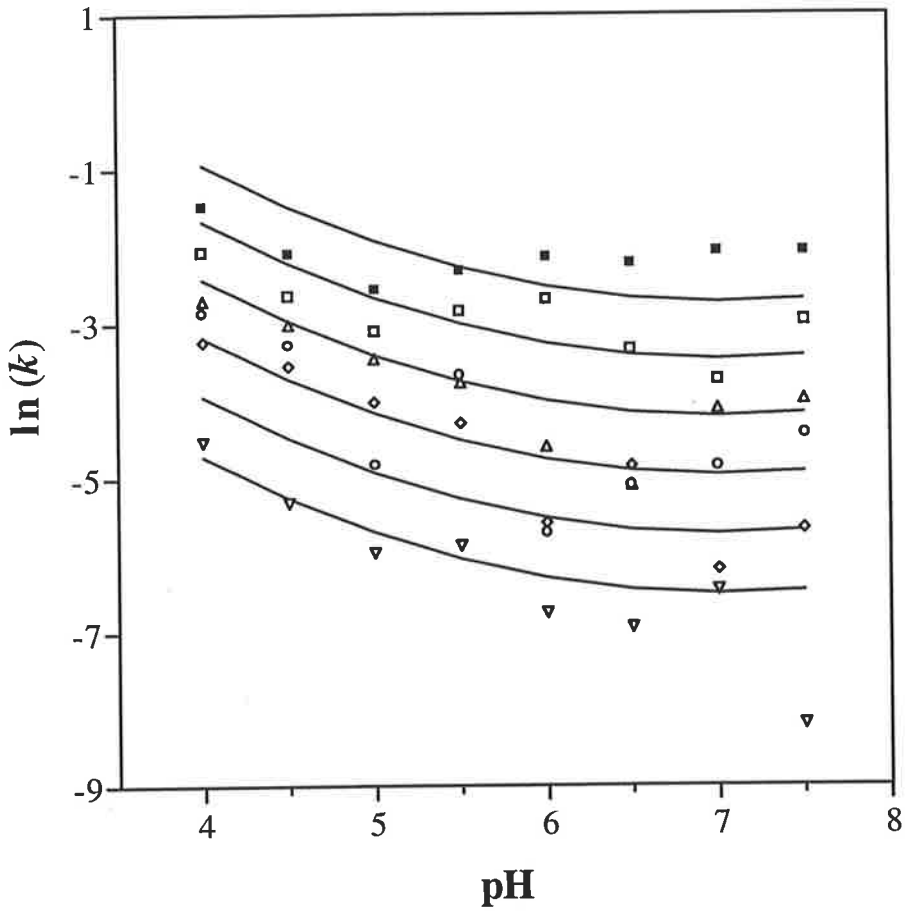


Figure 4.3 Summary of experimentally determined data describing the combined effect of exposure temperature and pH on inactivation of *E. coli,* and a comparison with model predictions of equation 4.3 (from Davey, Hall and Thomas 1995) (■ = 62°C, □ = 60°C, Δ = 58°C, O = 56°C, ◊ = 54°C)

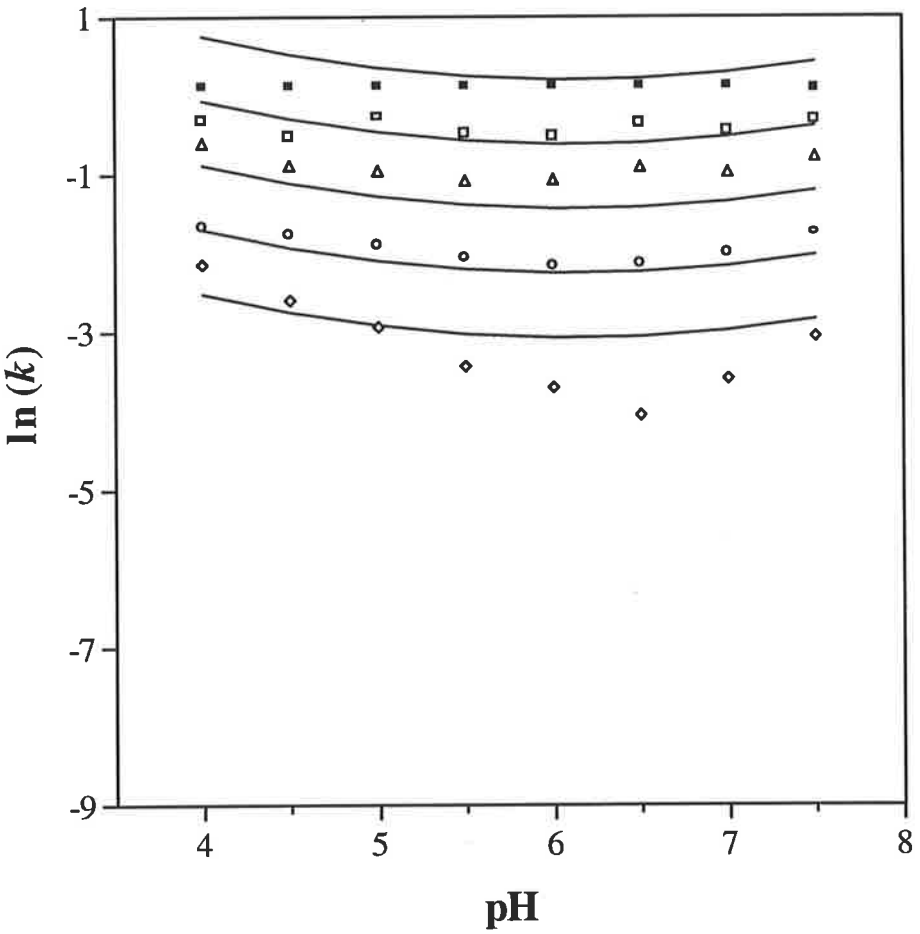


Figure 4.4 Selected plots of $\log_{10}(N / N_0)$ versus *exposure time* for *P. fluorescens* at an exposure temperature of 56°C and four values of medium-range pH compared with log-linear predictions (◊ = pH 5.0, □ = pH 5.4, Δ = pH 6.0, O = pH 6.5)

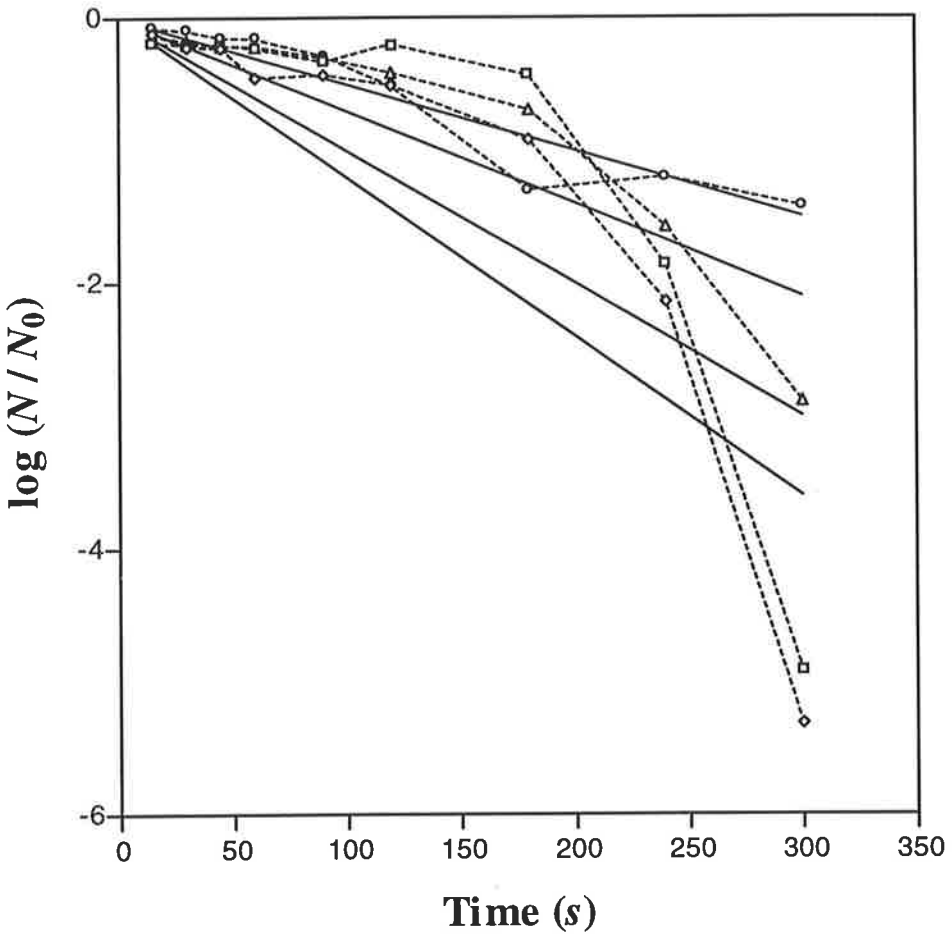
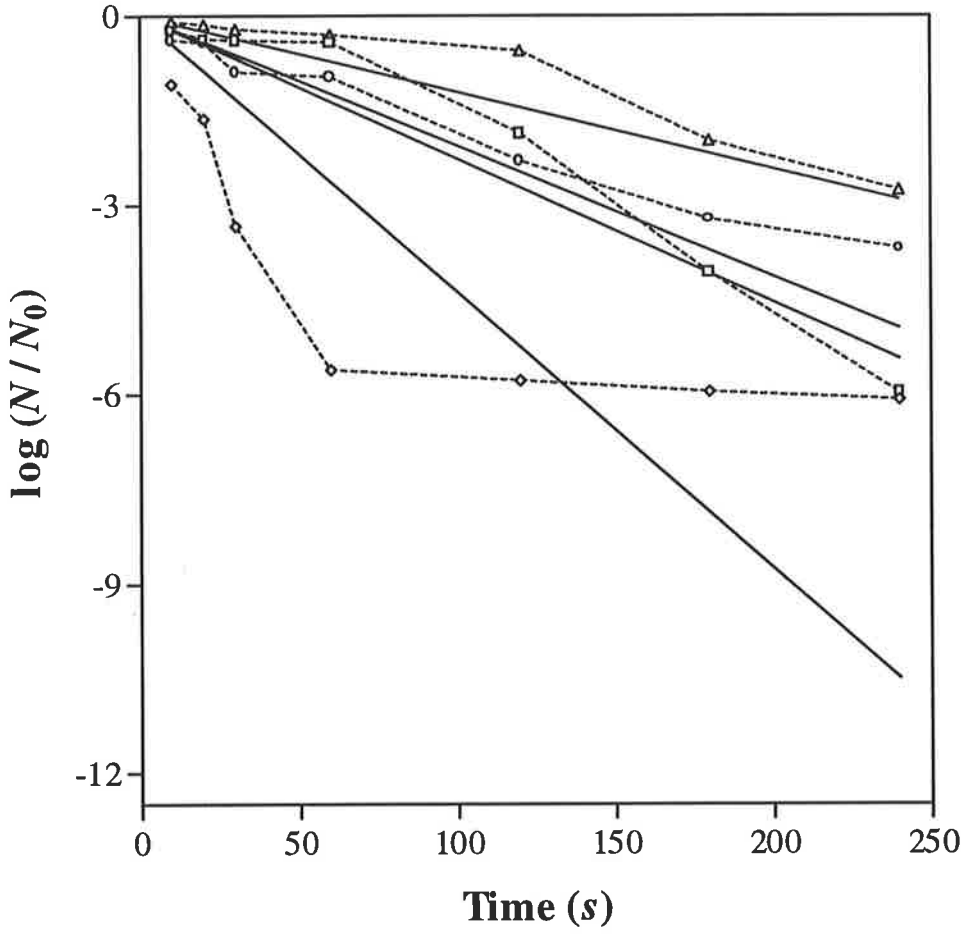


Figure 4.5 Selected plots of $\log_{10}$ (N / N_0) versus *exposure time* for *L. monocytogenes* at four different exposure temperatures and medium-range values of pH compared with log-linear predictions (◊ = 62°C pH 5.0, □ = 60°C pH 7.5, Δ = 58°C pH 5.5, O = 56°C pH 4.5)



CHAPTER

FIVE

SYNTHESIS AND VALIDATION OF TWO NON-LINEAR THERMAL INACTIVATION KINETIC MODELS

The results presented in this chapter have been published as:

Chiruta, J., Davey, K. R. and Thomas, C. J. (1999) A new non-linear kinetic model for survival of bacteria in liquid as affected by combined temperature - pH in continuous sterilisation, *Transactions of the Institution of Chemical Engineers, Part C, Bioproducts and Food Processing* (*submitted*)

Davey, K. R., Thomas, C. J. and Chiruta, J. (1998a) Mathematical evaluation and design for a new time-dependent thermal inactivation of bacteria in liquid as influenced by combined temperature and pH, *14th Australasian Biotechnology Conference*, 19-23 April, Adelaide, Australia (*in press*)

Davey, K. R., Thomas, C. J. and Chiruta, J. (1998b) A new non-linear kinetic model for design of thermal inactivation of bacteria in liquid as effected by combined temperature and pH in a continuous steriliser, *26th Australasian Chemical Engineering Conference, CHEMECA '98*, 28-30 September, Port Douglas, Queensland, Australia, Paper 68 (ISBN 1 85825 683 5)

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Chiruta, J., Davey, K. R. and Thomas, C. J. (1997b) Thermal inactivation kinetics of three vegetative bacteria as influenced by combined temperature and pH in a liquid medium, *Transactions of the Institution of Chemical Engineers, Part C, Bioproducts and Food Processing*, **75**: 174-180

Chiruta, J., Davey, K. R. and Thomas, C. J. (1997c) An *n*th order reaction model for thermal inactivation of *Listeria monocytogenes* in liquid with combined temperature and pH, *Proceedings of the 3rd International Conference on Modelling and Simulation*, October 29 - 31, Victoria University of Technology, Melbourne, Australia, 343-346 (ISBN 1 86 272 4903)

Chiruta, J., Davey, K. R. and Thomas, C. J. (1996a) Modelling the combined effect of temperature and pH on continuous thermal sterilisation of liquids, *The 2nd International Conference of Predictive Microbiology*, February 18 - 22, Hobart, Australia, Paper 6

Chiruta, J., Davey, K. R. and Thomas, C. J. (1996b) Modelling the combined effect of process temperature and pH in continuous sterilisation of liquids, *24th Australian and New Zealand Chemical Engineering Conference, CHEMECA '96*, September 30 -October 2, Sydney, Australia, **3**: 129-134 (ISBN 0 85825 658 4)

5.1 INTRODUCTION

In this chapter the synthesis of an appropriate non-linear model form for the thermal inactivation kinetics of bacteria is undertaken. The bench-scale *T*-pH data presented in Chapters 3 and 4 for the three test micro-organisms in the test liquid, namely *P. fluorescens*, *L. monocytogenes* and *E. coli*, together with the published data of Davey and Wood (1984) that were obtained in continuous steriliser (but with the effect of *T* only), are used.

The log-linear kinetic equation for thermal inactivation is employed as a convenient starting point in synthesis, and a number of selected alternative models are synthesised for predicting the effect of combined *T*-pH on bacterial inactivation kinetics.

The aim is to determine an adequate but significant non-linear kinetic model form, and the appropriate values of the coefficients of the model that can be reliably used to size a pilot continuous steriliser for later experimental validation.

Two new models are selected for validation tests on the survival data. The first of these is titled *n*OE (n^{th} order reaction model). The alternative, titled CDT, is selected for experimental testing in a pilot continuous steriliser.

A key conclusion of this chapter is that the thermal inactivation of bacteria does not follow the generally applied log-linear (ie first-order) model and that the rate coefficient of thermal inactivation is *time* dependent. This finding represents an important change in the direction of development of models for thermal sterilisation.

The following chapter, Chapter 6, describes the sizing and experimental testing carried out in a pilot continuous steriliser and presents a comparison between the experimental results and the predictions of the selected CDT model for thermal inactivation kinetics with combined process *T*-pH. The kinetics of thermal inactivation in this flow (*dynamic*) system is compared with those from the bench-scale (*static*) studies.

5.2 VALIDATION TESTS FOR MATHEMATICAL MODELS

The criteria established for an adequate model is given in Chapter 3.5. A particular criterion is that the model should be of a form that can be easily used.

The practical understanding of this criterion is that the model form for thermal inactivation kinetics must be readily integrated with those equations describing the rheology and hydrodynamics of flow ie the performance equations for the sterilisation unit operation that appear in section 2.3.2.

The fit of mathematical models to experimental data is determined from linear regression analyses (Snedecor and Cochran 1969).

A widely used test of goodness of fit of kinetic models in the literature is the *per cent variance accounted for*, $\%V$ (Snedecor and Cochran 1969). This is a measure of the difference between the observed and the predicted value and is given by:

$$\%V = [1 - (1 - r^2)\,(n_T - 1) / (n_T - N_T - 1)] \times 100 \qquad (5.1)$$

The $\%V$ is considered a stringent test of goodness of fit when there are few data, or, a large number of terms in the model, and in which case the multiple regression coefficient (r^2) gives the appearance of a better than actual fit (see Davey and Cerf 1996).

From equation 5.1 it can be seen that as the number of terms increases (N_T), the $\%V$ decreases and at $n_T >> N_T$, $\%V$ approximates r^2. As a result, a high value for r^2 does not show a necessary good fit of an actual model but $\%V$ criterion helps to do so.

An alternative measure of goodness of fit that is also widely used is given the Mean Square Error (MSE) (Snedecor and Cochran 1969). The MSE is defines as:

$$\mathbf{MSE} = \Sigma\,(\text{observed} - \text{predicted})^2 / n_T \qquad (5.2)$$

An additional important test in model validation is the analyses of any systematic features in the plots of *residuals* (Weisberg 1985) where:

$$\text{Residual Value} = \text{Observed Value} - \text{Predicted Value} \qquad (5.3)$$

Plots of observed value versus predicted value and plots of predicted value versus residual value are extremely useful in diagnosing and highlighting the effectiveness of inherent assumptions in models.

Together, all four tests for validation outlined above are demonstrably comprehensive and are used in the following sections in assessing the two new model forms for non-linear thermal inactivation kinetics as effected by combined T-pH. The first of these models is the n^{th} order inactivation model (nOE).

5.3 AN n^{th} ORDER INACTIVATION MODEL FOR THE THERMAL INACTIVATION OF *LISTERIA MONOCYTOGENES* IN LIQUID WITH COMBINED T-pH

Because of the widely used assumption of first-order reaction (n = 1) kinetics for thermal inactivation of bacteria, the significance of the implied order of has not been fully investigated.

In this section a non-linear, empirical n^{th} order rate equation (nOE) is investigated as a possible alternative model form to describe accurately thermal inactivation of kinetics of bacteria as influenced by T-pH. The thermal inactivation data for the vegetative pathogen *L. monocytogenes* are used for model validation tests. The implications of nOE for process sterilisations with liquids are briefly discussed.

5.3.1 Synthesis

Assuming the thermal inactivation of bacteria follows a law of general irreversible reaction of dissociation - where the mechanism of reaction is not known - the change in number of viable bacterial cells is given by an empirical rate equation of n^{th} order (Aiba and Toda 1967, Levenspiel 1972), that can be written as:

$$dN_t / dt = - k N_t^n + e_t \tag{5.4}$$

where e_t is a residual error. The model assumes that e_t is a statistically independent error at each time point and distributed as normal with zero mean and $e_t \sim N(0, \sigma^2)$.

Separation and integration of equation 5.4 yields for all values except $n = 1$ (Aiba and Toda 1967, Aris 1969):

$$N_t^{1-n} - N_0^{1-n} = (n - 1) k t + e_t \tag{5.5}$$

or

$$N = N_0 [1 + k N_0^{n-1} (n - 1) t]^{-1/(n-1)} + e_t \tag{5.6}$$

The rate coefficient for thermal inactivation, as affected by combined T-pH, can be defined in terms of a reduced linear-Arrhenius model (Cole et al 1993, Davey, Hall and Thomas 1995, Daughtry et al 1997) such that:

$$\ln (k) = C_0 + C_1 / T + C_2 \text{ pH} + e^{(k)} \tag{5.7}$$

where T is in degree absolute.

Taking logarithms of both sides of equation 5.4 yields

$$\ln(-dN_t / dt) = \ln(k) + n \ln N + e_t \quad (5.8)$$

From equation 5.8 the classical log-linear model ($n = 1$) is given by the expression:

$$k_{n=1} = -[\ln(N_t / N_0)] / t + e_n^{(k)} \quad (5.9)$$

Similarly, where $n \neq 1$, a nOE model for the rate coefficient, from equation 5.6, is:

$$k_{n=n} = [(N_t / N_0)^{(1-n)} - 1] / [(n-1)\, t\, N_0^{(n-1)}] + e_n^{(k)} \quad (5.10)$$

If we let $a = n$-1, equation 5.10 can be simplified to:

$$k_{n=n} = [(N / N_0)^a - 1] / (a\, t\, N_0^a) + e_n^{(k)} \quad (5.11)$$

Two important features of the model form of equation 5.11 are that it is based on a realistic approach to evaluation of survival kinetics and is of a relatively simple form as it involves only two terms: $1 / T$ and pH.

5.3.2 Fit of the nOE Model to Data for *Listeria monocytogenes*

Linear regression analyses (Appendix E.3) resulted in the following overall values of the model coefficients for *Listeria monocytogenes*:

$$\ln(-dN / dt) = 69.7 - 24294 / T - 0.338\ \text{pH} + 1.074 \ln(N) \quad (5.12)$$

Equation 5.12 can be used to predict the combined effect of temperature and pH on the rate of thermal inactivation of *L. monocytogenes* in the carrier liquid. The variance accounted for by this new nOE was 89.5%V. This compared with 71.5%V for the log-linear model. Importantly, additional terms (ie pH^2 and $1 / T^2$) in the nOE model were found not to be statistically ($p > 0.05$) significant.

5.3.3 Discussion

Table 5.1 presents a comparison of the average values of r for the nOE model on a range of pH at each of the five temperatures. The value of $n_{average}$ varies between 1.08 and 2.26. The range of values for n in Table 5.1 suggests that n might be both temperature and pH dependent - with T being more significant than pH. Table 5.2 presents a comparison between experimentally observed reductions at 58°C and pH 5.0 against predictions from both the log-linear and nOE models. Calculations from the models show that the nOE model gives a better fit to the observed survival levels of *L. monocytogenes*.

The value of MSE of 0.56 for nOE model (compared with that of 1.74 for log-linear) further suggests that the log-linear form is inappropriate for these experimental data. Other predictions in addition to those of Table 5.2 can be generated for a range of T and pH values eg at 56°C-pH 7.0 MSE values are 0.80 and 1.68 for, respectively, nOE and log-linear models. (A comparison of the observed data with predictions from the log-linear model is shown, for *L. monocytogenes*, in Figure 4.5 of Chapter 4).

5.3.4 Concluding Remarks and Rejection of the nOE Model

The nOE model is a better predictor for the time necessary for sterilisation of *L. monocytogenes* than the log-linear model. It permits a practical procedure for the evaluation of the microbiological safety of liquid foods processed at mild thermal conditions and pH, and highlights the evidence of non-linearity of survivors. Further work with additional survival data is necessary to determine whether the nOE model form is applicable to pathogens universally.

Although the nOE model does give a better fit to the experimental data for *L. monocytogenes* than does the log-linear model form, it is seen that a draw back is that it is not readily integrated with the performance equations for the sterilisation unit operation. For this reason synthesis of an alternative non-linear model was investigated (Chapter 5.4).

Table 5.1 Values of n and r for the fit of the nOE ($n \neq 1$) model for *L. monocytogenes* at each of five temperatures

T (°C)	n								$n_{average}$	$r_{average}$
	pH									
	4.0	**4.5**	**5.0**	**5.5**	**6.0**	**6.5**	**7.0**	**7.5**		
54	1.16	0.93	1.29	0.55	2.73	1.40	7.74	2.31	2.26	0.72
56	1.11	1.18	1.20	1.78	-	3.00	2.01	2.53	1.83	0.88
58	0.98	1.12	0.92	0.99	1.49	0.88	1.03	1.21	1.08	0.86
60	1.25	2.23	0.93	1.04	1.29	0.86	1.07	0.99	1.21	0.95
62	-	1.56	1.24	1.04	1.38	1.15	1.14	1.14	1.24	0.96
$n_{average}$	1.13	1.40	1.12	1.08	1.72	1.46	2.60	1.63	**1.52**	0.88

Table 5.2 Comparison between experimentally observed reductions at $T = 58$°C and pH 5.0 with predictions from both the log-linear and nOE models ($N_0 = 3.27 \times 10^8$ number ml^{-1})

t (s)	ln (- dN / dt)		
58°C / pH 5.0	**observed**	**predicted**	
		log-linear	**nOE**
10	16.19	16.19	16.31
20	16.03	15.87	16.09
30	14.20	15.27	15.86
60	14.99	13.94	15.73
120	14.67	12.03	14.82
180	11.20	10.11	11.07
	MSE	1.74	0.56

5.4 SYNTHESIS OF A NEW CDT MODEL FOR THERMAL INACTIVATION OF LIQUID WITH COMBINED *T*-pH

5.4.1 Introduction

The synthesis of a new, empirical and non-linear model for predicting bacterial survival kinetics as influenced by combined process *T*-pH is presented. Synthesis is based both on published continuous steriliser data of Davey and Wood (1984) for *T* only effect and the batch bench-scale data described in Chapter 3 for three vegetative bacteria as affected by combined *T*-pH.

Synthesis of the new CDT model form is evolved from testing of selected models in a logical progression. The starting point was the classical log-linear model. Other models included that of Han, Zhang and Krochta (1976) (HZK) and a proposed exponential model (EXP) (Table 5.3).

The rate coefficient for thermal inactivation is shown to be time dependent. Predictions of the new CDT model are contrasted with those of the selected models. Extensive analysis of residuals is used together with *%V* as criteria of the goodness of fit for comparison and validation of models.

5.4.2 Synthesis

The first-order, log-linear model (equation 2.4) for thermal inactivation can, on integration, be written as:

$$\ln (N / N_0) = -k\,t \qquad (5.13)$$

Temperature Effect

The effect of temperature (T) only on the rate coefficient for thermal inactivation (k) can be predicted from either of two model forms.

1. The more widely used of these is the empirical Arrhenius, or, more aptly titled linear-Arrhenius model (Davey 1993a, Holdsworth 1992, Davey, Hall and Thomas 1995) where:

$$\ln k = C_0 + C_1 / T \tag{5.14}$$

The Arrhenius equation 5.14 can be written in the more familiar form, using activation energy:

$$k = A \exp [-E / (R\,T)] \tag{5.15}$$

2. A general form of the temperature dependence of k was developed by Eyring (Daughtry, Davey and King 1997) and is given by:

$$k = A\,T^n \exp [-E / (R\,T)] \tag{5.16}$$

Equation 5.16 can be expressed more generally as:

$$\ln k = C_0 + C_1 / T + C_2 \ln (T^n) \tag{5.17}$$

For $n = -1$ equation 5.17 becomes:

$$\ln k = C_0 + C_1 / T + C_2 \ln (1 / T) \tag{5.18}$$

Combined Temperature-pH Effect

The effect of combined T-pH on the rate coefficient for thermal inactivation has been modelled by the Davey linear-Arrhenius equation (Davey, Lin and Wood 1978, Davey 1993b, Davey 1994, Davey, Hall and Thomas 1995, McMeekin et al 1993), namely:

$$\ln k = C_0 + C_1 / T + C_1 / T^2 + C_3 \text{ pH} + C_4 \text{ pH}^2 \qquad (5.19)$$

A generalised model form for two or more combined environmental factors was illustrated by Cerf, Davey and Sadoudi (1996) for the combined effect of three environmental factors using the extensive T-pH-a_w data of Reichart (1994) for *E. coli*. The generalised model is given by: $\ln k = C_0 + \sum_{i=1}^{j} (C_{2i-1} F_i + C_{2i} F_i^2)$. This model suggests that in addition to inactivation T, another environmental factors (F), pH, a_w, etc, act in combination and affect cell destruction.

New Model

An empirical non-linear equation of the following general form was selected from initial study of the data to describe a time-dependent rate coefficient for the thermal inactivation of bacteria in liquid:

$$Y = \varepsilon + \omega f(t) + \Omega f(t)^2 \qquad (5.20)$$

where

$$Y = \ln [1 - \log(N / N_0)] \qquad (5.21)$$

and it is assumed ω and Ω can be modelled by the Davey linear-Arrhenius equation to give:

$$\omega = C_0' + C_1' / T + C_2' / T^2 \qquad (5.22)$$

$$\Omega = C_0'' + C_1'' / T + C_2'' / T^2 + C_3'' \text{ pH} + C_4'' \text{ pH}^2 \qquad (5.23)$$

The function $f(t)$ is characterised by the natural logarithm of sterilisation time:

$$f(t) = \ln t \tag{5.24}$$

From equations 5.20 through to 5.24 a time-dependent rate coefficient for thermal inactivation of viable bacteria, $k(t)$, can be expressed as:

$$k(t) = -2.3\,[1 - \exp(\varepsilon + \omega \ln(t) + \Omega (\ln t)^2]\,/\,t \tag{5.25}$$

The new model form of equation 5.25 is titled the CDT model.

Based on the above, four generalised model forms can be used to predict survival kinetics (Table 5.3).

The reader should note that for computational convenience in fitting of these model forms, temperature is expressed in degree Celsius throughout.

5.4.3 Validation of the CDT Model for the Effect of Temperature

The models presented in Table 5.3 can be re-written as stochastic equations as illustrated in Table 5.4 for the effect of T only. The models described in Tables 5.3 and 5.4 are deterministic equations that imply a stochastic element (e_t) and therefore involve specific assumptions, namely:

- on a logarithmic scale the errors are additive
- the "noise" components are independent at each time, t
- there is equal variance at each time point (ie σ^2 is not dependent on t).

The fitting of a non-linear model to experimental data necessitates sophisticated procedures and, generally, such models have associated problems with inference (Daughtry et al 1997). To obviate this, the fitting of models was carried out in a single process using linear

regression analyses where the patterns of inactivation rate, k, are linear and dependent on T (or combined T-pH).

A summary of the goodness of fit of each of the model forms to the data of Davey and Wood (1984) for the inactivation of *E. coli* in a continuous steriliser is given in Table 5.5. As is seen from Table 5.5 the log-linear and CDT model forms appear to give a better fit to these data than the HZK or EXP forms - with between 89.0% and 94.6% of the variance accounted for (*%V*). The HZK model, however, has not previously been assessed for thermal inactivation data obtained in a flow system such as a continuous steriliser.

Table 5.6 summarises and compares the predictions of the log-linear and new CDT model against the experimental data of Davey and Wood (1984) for the sterilisation of *E. coli* in a continuous steriliser with the effect of T only over each of the four process temperatures (53.5°C, 54.5°C, 56°C and 59°C). The CDT model is seen to give a better fit at each data point than the log-linear model. Table 5.7 presents a summary of the value of the Mean Square Error (MSE) for both the log-linear model and new CDT model fit to these. The new CDT model is seen to give a better fit than the log-linear model to these data expressed as MSE.

The HZK and EXP models were therefore rejected and the log-linear and CDT forms examined further for predicting thermal inactivations with combined T-pH.

Table 5.3 Generalised model forms (including the new CDT model) that could be used to describe thermal inactivation

Model	Equation
Log-linear	$\ln (N_t / N_0) = -k\, t$
HZK	$\ln (N_t / N_0) = -k\, t + \sigma^2\, t^2$
EXP	$\ln (N_t / N_0) = -k \exp (\alpha t)$
CDT	$\ln (N_t / N_0)] = \varepsilon_1 - \varepsilon_2\, t \exp [\omega + \Omega \ln (t)]$

Table 5.4 Model forms of thermal inactivation kinetics for temperature dependent only data

Model	Equation	Critical coefficients
Log-linear1	$\ln (N_t / N_0) = -k\,t + e_t$	$\ln k = C_0 + C_1 / T$
Log-linear 2	$\ln (N_t / N_0) = -k\,t + e_t$	$\ln k = C_0 + C_1 / T + C_2 \ln (1/T)$
HZK1	$\ln (N_t / N_0) = -k\,t + \sigma^2 t^2 + e_t$	$\ln k = C_0 + C_1 / T$ $\ln\sigma^2 = C_0 + C_1 / T + C_2 \ln (1/T)$
HZK2	$\ln (N_t / N_0) = -k\,t + \sigma^2 t^2 + e_t$	$\ln k = C_0 + C_1 / T + C_2 \ln(1/T)$ $\ln\sigma^2 = C_0 + C_1 /T + C_2 \ln(1/T)$
EXP1	$\ln (N_t / N_0) = -k\,e^{\alpha t} + e_t$	$\ln k = C_0 + C_1 / T$ $\ln \alpha = C_0 + C_1 / T + C_2 \ln (1/T)$
EXP2	$\ln (N_t / N_0) = -k\,e^{\alpha t} + e_t$	$\ln k = C_0 + C_1 / T + C_2 \ln (1/T)$ $\ln \alpha = C_0 + C_1 / T + C_2 \ln (1/T)$
CDT1	$\ln [1 - \log (N_t / N_0)] = \omega_1 + \Omega_1 \ln t + e_t$	$\omega_1 = C_0 + C_1 / T + C_2 / T^2$ Ω_1 = constant
CDT2	$\ln [1 - \log (N/N_0)] = \varepsilon + \omega_2 \ln(t) + [\Omega_2 (\ln t)^2] + e_t$	$\omega_2 = C_0 + C_1 / T + C_2 / T^2$ ε, Ω_2 = constant
CDT	$\ln [1 - \log(N/N_0)] = \varepsilon + \omega \ln (t) + [\Omega (\ln t)^2] + e_t$	$\omega = C_0 + C_1 / T + C_2 / T^2$ $\Omega = C_0 + C_1 / T + C_2 / T^2$

Table 5.5 Comparison of the degree of goodness of fit (*%V*) for selected models to the survivor data of Davey and Wood (1984) for sterilisation of *E. coli* as affected by *T* only in a continuous steriliser

Model	*%V*
Log-linear1	88.1
Log-linear2	89.0
HZK1	24.3
HZK2	88.8
EXP1	52.1
EXP2	75.9
CDT1	94.3
CDT2	94.4
CDT	94.6

Table 5.6 Comparison of predicted inactivations (as $\log_{10}(N / N_0)$) from both the log-linear and non-linear CDT models to the observed data of Davey and Wood (1984) for inactivation of *E. coli* in a continuous steriliser with the effect of process T only

time (*s*)	**Observed -$\log_{10}(N / N_0)$**	**Predicted -$\log_{10}(N / N_0)$**	
		Log-linear model	CDT model
	$T = 53.5^{\circ}C$		
14.08	0.126	0.204	0.115
17.69	0.242	0.328	0.303
25.26	0.356	0.684	0.811
34.75	1.794	1.322	1.660
35.41	1.824	1.374	1.728
	$T = 54.5^{\circ}C$		
10.36	0.152	0.196	0.235
14.16	0.660	0.374	0.456
24.22	1.680	1.135	1.321
35.73	3.000	2.534	2.757
	$T = 56^{\circ}C$		
14.19	1.323	0.880	1.129
16.52	1.633	1.205	1.404
22.00	1.975	2.177	2.171
37.52	5.018	6.560	5.272
	$T = 59^{\circ}C$		
10.00	2.022	1.597	1.939
10.94	2.022	1.923	2.120
11.82	1.947	2.257	2.300
12.98	2.745	2.738	2.551
21.46	4.648	7.737	4.842

Table 5.7 Summary value of the Mean Square Error (**MSE**) for both the log-linear model and new CDT model fit to the data of Davey and Wood (1984) for the sterilisation of *E. coli* in a continuous steriliser with the effect of T only

Model			**MSE**		
T(°C)	53	54	55.5	58	**Mean**
Log-linear	0.109	0.120	0.560	1.966	**0.689**
CDT	0.053	0.019	0.352	1.715	**0.535**

5.4.4 Validation of the CDT Model for the Effect of Combined *T*-pH

The selected models (log-linear, CDT1, CDT2 and CDT) were assessed against the three sets of experimental data for the combined effect of temperature and pH on the survival of: *P. fluorescens, L. monocytogenes* and *E. coli* described in Chapter 3. The resulting fits are presented in Table 5.8 together with the overall mean $\%V$ on the three data sets. The table shows the CDT form gives a better fit to each of the three data sets. Also, it can be seen that a greater overall mean value of $\%V$ of 89.2% for the CDT model demonstrates a better fit than that of the log-linear, CDT1 and CDT2 forms, with respectively, 77.0%, 73.3% and 80.0%V.

Table 5.9 presents a summary comparison of the degree of goodness of fit of the selected model forms to survivor data of all three sets of bacteria at a mid-range value of $T = 58^{o}C$.

Table 5.10 compares the goodness of fit between the two models using the statistical parameter Mean Square Error (MSE) over a range of four values of pH.

Table 5.11 provides a summary of the values obtained for each of the coefficients of the newly derived CDT model for each of the three sets of inactivation data for combined *T*-pH, respectively for, *P. fluorescens*, *L. monocytogenes* and *E. coli.* Substitution of the values of the coefficients from Table 5.11 into the kinetic model for thermal inactivation permits prediction of the numbers of surviving bacteria as effected by combined *T*-pH.

The model summarised in Table 5.11 can therefore be used together with the steriliser performance equations to size a pilot continuous steriliser for the thermal inactivation of the bacteria with the effect of combined *T*-pH.

Plots of observed value versus predicted value and plots of predicted value versus residual value were important to the synthesis and appraisal of the CDT (and log-linear) model and in diagnosing and highlighting the effectiveness of inherent assumptions in the model. A summary of these analyses is presented below.

Analyses of Plots of Observed Value versus Predicted Value and of Predicted Value versus Residual Value

The plots of predicted value versus observed value for both the log-linear and CDT models for the survival of *P. fluorescens*, *L. monocytogenes* and *E. coli* are presented in Figures 5.1 to 5.3, respectively. The spread of these plots underscored the progression of development of the new model from CDT1 through CDT2 to CDT model. The plot for the log-linear model for *P. fluorescens* (Figure 5.1) shows a clear curved trend away from and beneath the axis of symmetry - supporting the notion of a time dependent rate of inactivation kinetics of the bacterium in the test liquid. In Figure 5.2 the same trend in residuals for this model is apparent for *L. monocytogenes*. For *E. coli* (Figure 5.3) it is interesting to note that the residuals from the log-linear model display an opening given by longer exposure times in respect with the axis of symmetry. The pattern in residuals for the log-linear model contrast with the distribution of residuals for the new CDT model in each of the three figures. These patterns in residuals for all three bacteria also suggests (more clearly for *E. coli*) an increase in variance.

Figures 5.4 through 5.7 present plots for the CDT model of predicted value versus residual value for survival, time, temperature, and pH, respectively, for the *E. coli* data; though the other two bacterial strains displayed a similar configuration of residuals. The non-systematic pattern of these residual plots indicates that the CDT model allows for all time, temperature and pH effects that are independently distributed. These results indicate that the increase in the variance may be due to other factors (such as experimental error increasing with the greater exposure times).

Value of the Rate Coefficient

The rate coefficient for thermal inactivation (k) plays a central role in the construction of the CDT model. Figures 5.8 through 5.10 illustrate the effect of combined T-pH and time on the rate coefficient k for *P. fluorescens*, *L. monocytogenes* and *E. coli*, respectively, for a selected range of exposure time and sterilisation temperature values.

Table 5.8 Summary comparison of degree of goodness of fit of selected model forms to survivor data for *P. fluorescens*, *L. monocytogenes* and *E. coli* as affected by combined process *T*-pH in bench-scale (*batch*) experiments

Bacterium	***T*-pH**	n_T	*%V*			
			Log-linear	**CDT1**	**CDT2**	**CDT**
P. fluorescens	5 *x* 4	172	75.8	64.0	77.5	93.0
L. monocytogenes	6 *x* 8	310	74.0	66.5	77.2	82.9
E. coli	5 *x* 8	208	81.3	89.4	88.4	92.3
Mean *%V*			**77.0**	**73.3**	**80.0**	**89.2**

Table 5.9 Comparison of predicted inactivations (as $\log_{10}(N / N_0)$) of the log-linear and non-linear CDT models to the observed data for, respectively, *P. fluorescens*, *L. monocytogenes* and *E. coli* for combined T-pH effect at a mid-range value of $T = 58^{\circ}C$

time	Observed $-\log_{10}(N/N_0)$				Predicted $-\log_{10}(N/N_0)$							
(s)					Log-linear model				CDT model			
pH	5.0	5.5	6.0	6.5	5.0	5.5	6.0	6.5	5.0	5.5	6.0	6.5
					P. fluorescens							
15	0.267	0.240	0.201	0.088	0.285	0.225	0.330	0.195	0.212	0.127	0.104	0.175
30	0.355	0.222	0.300	0.187	0.570	0.450	0.660	0.390	0.296	0.211	0.174	0.218
45	0.452	0.438	0.442	0.343	0.855	0.675	0.990	0.585	0.479	0.393	0.340	0.360
60	0.529	0.510	0.517	0.418	1.140	0.900	1.320	0.780	0.694	0.608	0.538	0.530
90	1.094	0.780	0.532	0.833	1.710	1.350	1.980	1.170	1.175	1.092	0.986	0.911
120	3.068	1.237	2.674	0.842	2.280	1.800	2.640	1.560	1.709	1.636	1.486	1.329
180	4.016	2.924	4.317	1.824	3.420	2.700	3.960	2.340	2.915	2.882	2.627	2.256
240	4.148	4.386	6.113	3.379	4.560	3.600	5.280	3.120	4.300	4.339	3.952	3.298
300	5.625	4.060	6.317	4.583	5.700	4.500	6.600	3.900	5.867	6.010	5.464	4.453
					L. monocytogenes							
10	-0.03	0.103	0.106	0.028	3.920	2.491	1.433	1.203	0.187	0.141	0.113	0.100
20	0.059	0.136	0.099	0.086	0.507	0.280	1.820	1.528	0.321	0.236	0.184	0.162
30	0.152	0.321	0.168	0.079	0.901	0.498	0.317	0.130	0.470	0.349	0.277	0.246
60	0.205	0.416	0.106	0.140	1.262	0.698	0.443	0.231	0.904	0.682	0.553	0.498
120	0.573	0.615	0.302	0.218	2.244	1.240	0.788	0.324	1.715	1.293	1.055	0.956
180	2.089	2.020	0.430	0.827	3.988	2.205	1.401	0.576	2.494	1.863	1.518	1.376
240	4.325	2.592	1.463	0.425	5.584	3.087	1.962	1.024	3.263	2.415	1.959	1.774
					E. coli							
8	1.899	2.254	2.312	2.632	1.213	1.069	1.027	1.076	2.254	2.156	2.118	2.138
16	2.701	2.213	2.533	2.934	2.172	1.915	1.840	1.926	3.406	3.172	3.083	3.130
24	6.047	3.553	5.308	3.879	3.055	2.693	2.588	2.711	4.174	3.816	3.682	3.752
32	5.114	5.000	4.329	4.480	3.891	3.430	3.296	3.453	4.756	4.286	4.111	4.202
40	5.415	5.511	5.073	7.812	4.694	4.138	3.977	4.166	5.226	4.653	4.441	4.552

Table 5.10 Comparison of the value of the Mean Square Error (MSE) for both the log-linear and CDT models to the fit of the batch, bench-scale data for *P. fluorescens*, *L. monocytogenes* and *E. coli* at a mid-range temperature of 58°C and four pH values (5.0, 5.5, 6.0 and 6.5)

Micro-organism	**MSE log-linear / CDT**							
pH	5.0		5.5		6.0		6.5	
P. fluorescens	0.423	0.277	0.314	0.181	0.802	1.436	0.334	0.122
L. monocytogenes	5.093	4.437	1.301	1.224	1.257	0.904	0.781	0.954
E. coli	2.344	0.978	1.317	1.004	2.360	0.962	3.830	0.874

Table 5.11 Summary of values obtained for each of the coefficients of the newly derived CDT model for each of the three sets of inactivation data for combined *T*-pH, respectively for, *P. fluorescens*, *L. monocytogenes* and *E. coli*

$$\ln [1-\log(N/N_0)] = \varepsilon + \omega \ln (t) + \Omega (\ln t)^2$$

$$\omega = C_0' + C_1' / T + C_2' / T^2$$

$$\Omega = C_0'' + C_1'' / T + C_2'' / T^2 + C_3'' \text{ pH} + C_4'' \text{ pH}^2$$

Coefficient	*P. fluorescens* %*V*=93.0 n_T=172	*L. monocytogenes* %*V*=82.9 n_T=310	*E. coli* %*V*=92.3 n_T=208
ε	1.84	0.51	0.0567
C_0'	9.77	7.32	-6.49
C_1'	1.39 E3	-8.92 E2	1.39 E3
C_2'	4.36 E4	2.58 E4	-5.69 E4
C_0''	1.19	0.76	-1.44
C_1''	-58.71	-25.85	99.40
C_2''	0*	0	0
C_3''	0.015	-0.070	-0.104
C_4''	-0.0017	0.0052	0.0086

* not significant if $p > 0.05$

Figure 5.1 Comparative plot of the predicted versus observed survivor value (as $\log_{10}$ (N / N_0)) for both log-linear and CDT models for the thermal inactivation of *P. fluorescens* with combined T-pH dependence (■ = log-linear model, □ = CDT model)

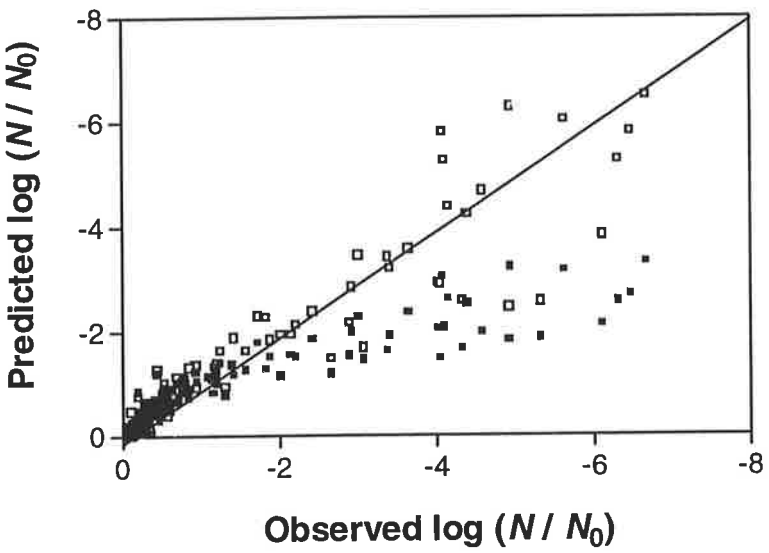


Figure 5.2 Comparative plot of the predicted versus observed survivor value (as $\log_{10}$ (N / N_0)) for both log-linear and CDT models for the thermal inactivation of *L. monocytogenes* with combined *T*-pH dependence (■ = log-linear model, □ = CDT model)

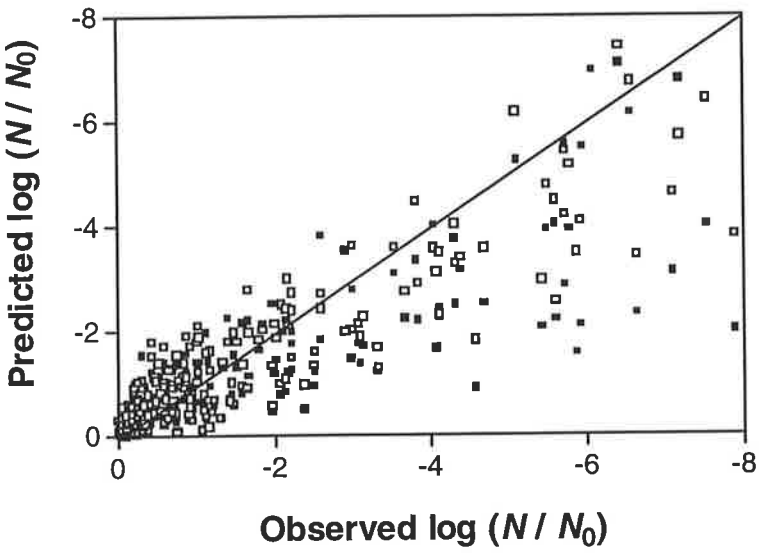


Figure 5.3 Comparative plot of the observed versus predicted survivor value (as $\log_{10}$ (N / N_0)) for both log-linear and CDT models for thermal inactivation of *E. coli* with combined T-pH dependence (■ = log-linear model, □ = CDT model)

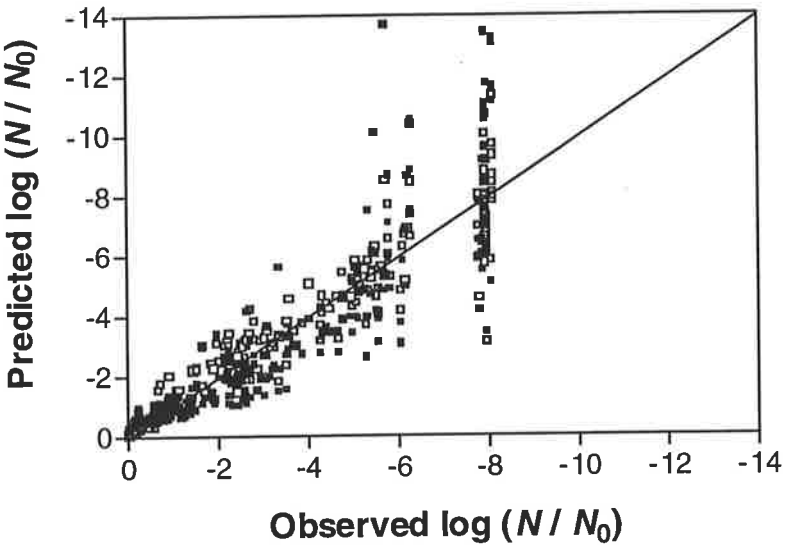


Figure 5.4 Plot of residuals versus predicted **survival** for the CDT model for the thermal inactivation of *E. coli* with combined *T*-pH dependence

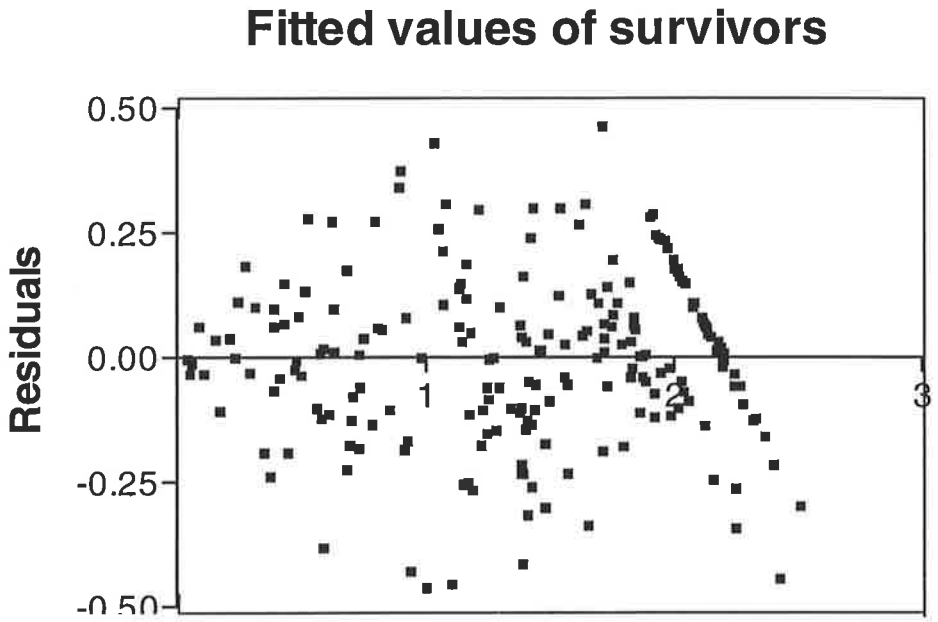


Figure 5.5 Plot of residuals versus **time** for the CDT model for the thermal inactivation of *E. coli* with combined *T*-pH dependence

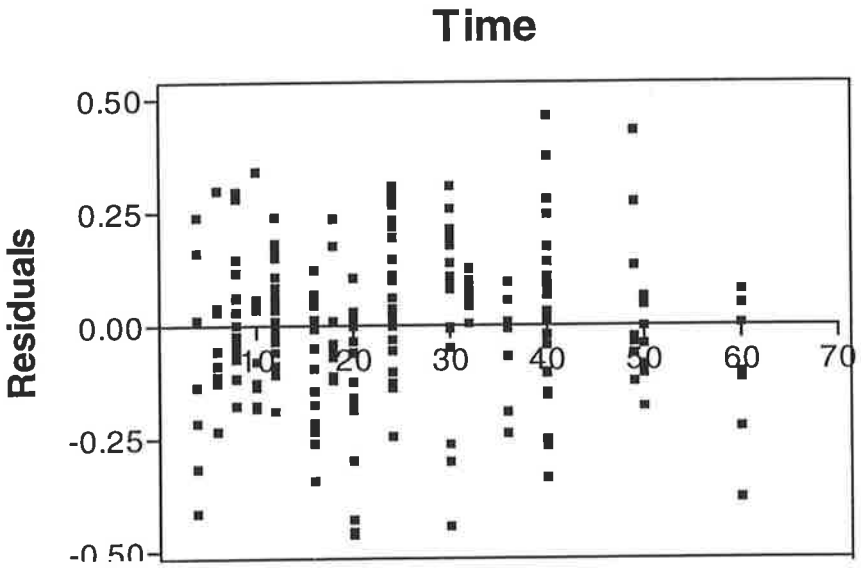


Figure 5.6 Plot of residuals versus **temperature** for the CDT model for thermal inactivation of *E. coli* with combined *T*-pH dependence

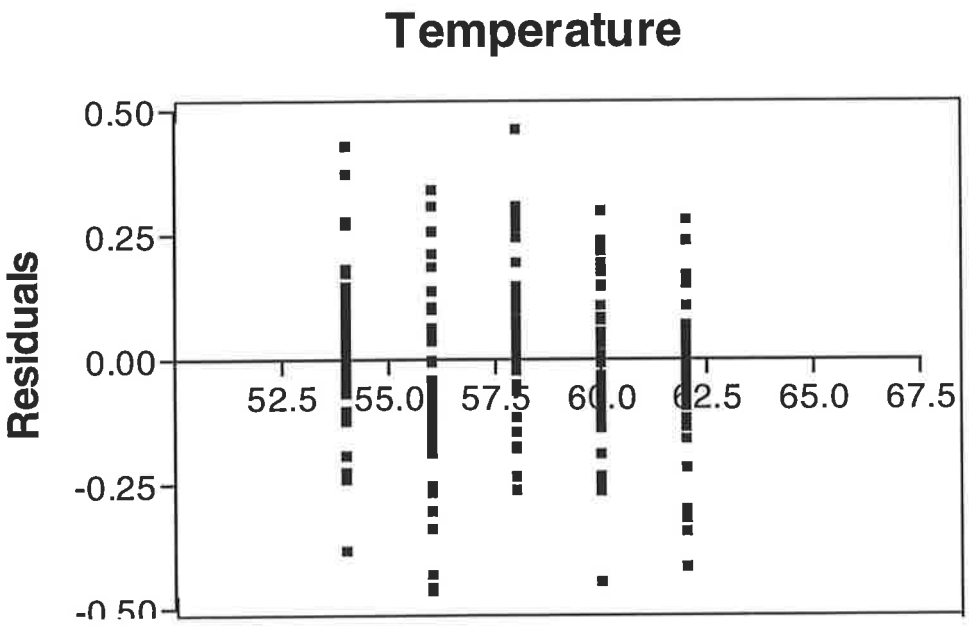


Figure 5.7 Plot of residuals versus **pH** for the CDT model for thermal inactivation of *E. coli* with combined *T*-pH dependence

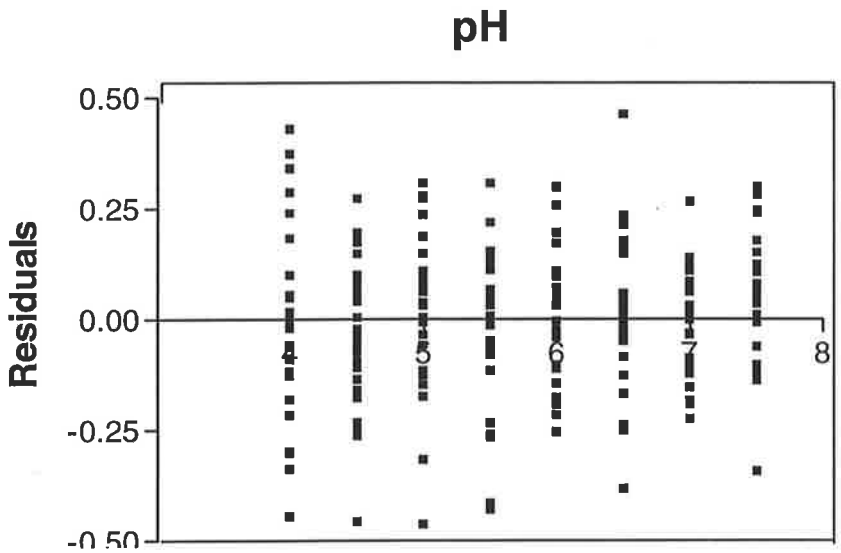


Figure 5.8 Summary plot of the predicted value of the rate coefficient for thermal inactivation versus time for *P. fluorescens* for combined *T*-pH effect (□ = pH 5.0, ◊ = pH 5.5, Δ = pH 6.0, O = pH 6.5)

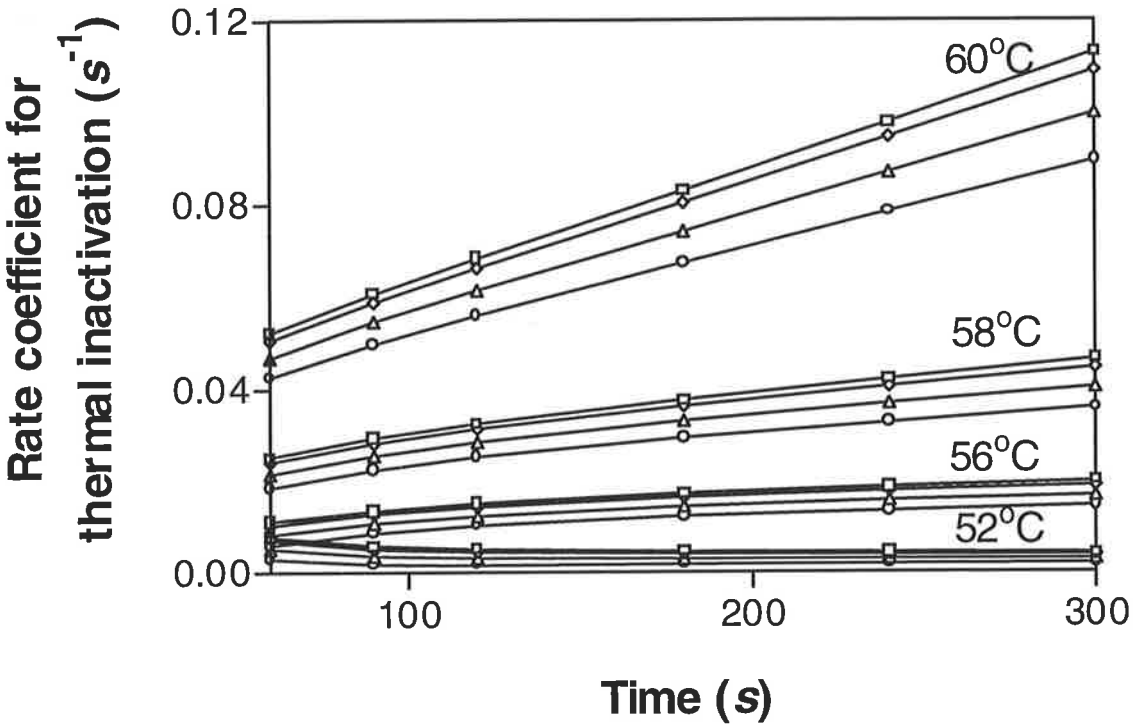


Figure 5.9 Summary plot of the predicted value of the rate coefficient for thermal inactivation versus time for *L. monocytogenes* for combined *T*-pH effect (■ = pH 4.0, ◆ = pH 4.5, □ = pH 5.0, ◊ = pH 5.5, Δ = pH 6.0, O = pH 6.5, ▲ = pH 7.0, ● = pH 7.5)

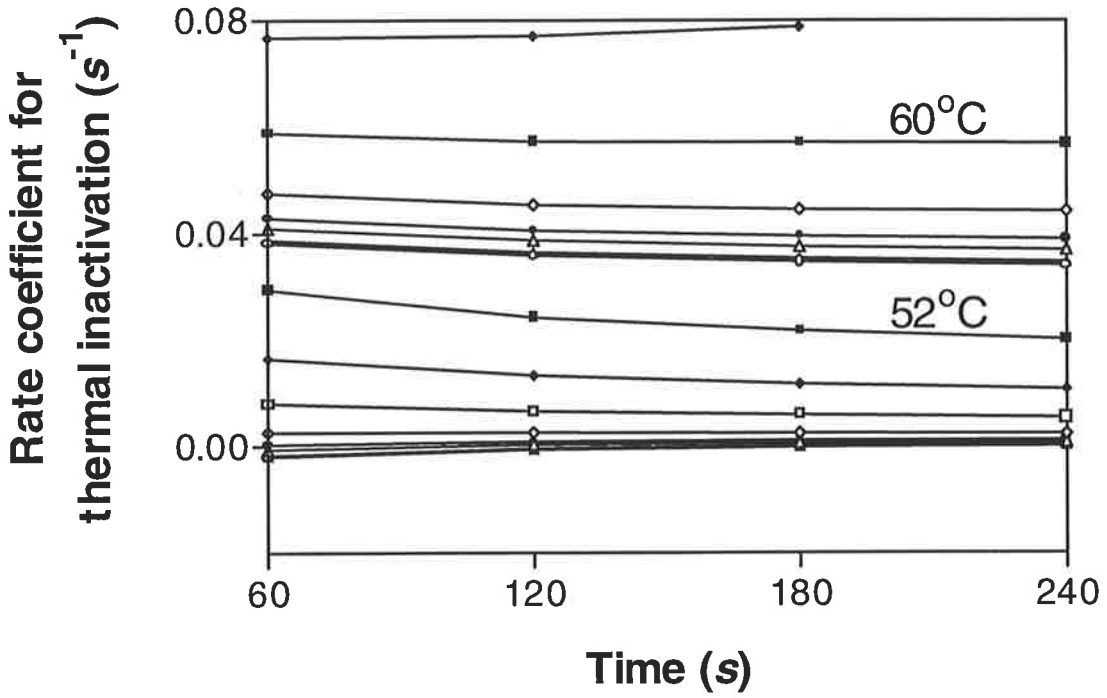
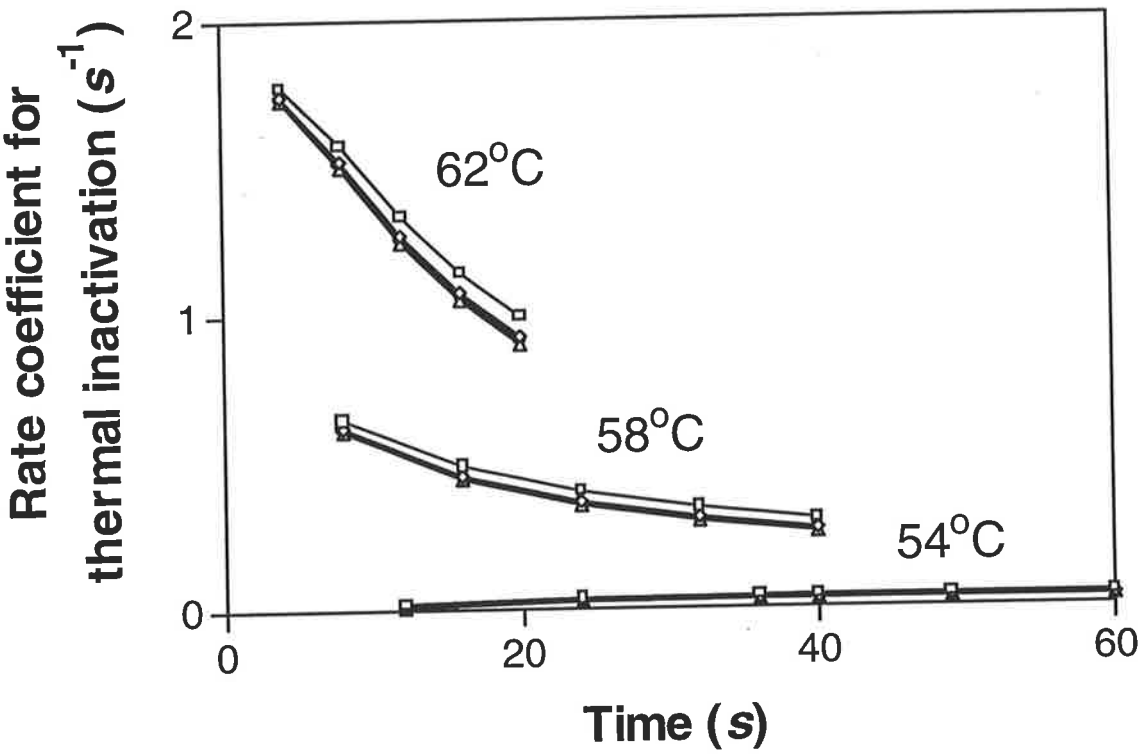


Figure 5.10 Summary plot of the predicted value of the rate coefficient for thermal inactivation versus time for *E. coli* for combined *T*-pH effect

(□ = pH 5.0, ◊ = pH 5.5, Δ = pH 6.0, O = pH 6.5)



5.4.5 Discussion

The new CDT model clearly gives a very good fit to the data for combined effect of T-pH for each of the three test bacterial strains with *per cent variance accounted for* (*%V*) ranging from 82.9% to 93.0%, and with an overall mean over the three of 89.4% (Table 5.8).

The model is appropriate for the range of combined T-pH for, respectively, *P. fluorescens* (n_T = 172) 52°C < T < 60°C and 5.0 < pH < 6.5; *L. monocytogenes* (n_T = 310) 52°C < T < 62°C and 4.0 < pH < 7.5, and; *E. coli* (n_T = 208) 54°C < T < 62°C and 4.0 < pH < 7.5, and for T only for *E. coli* (n_T = 18) 53.5°C < T < 59.0°C (pH = 7.0).

It is clear that the data sets are sufficiently large ($n_T >> 30$) and varied (ie three bacteria) as to underscore a high level of confidence in the analyses and model synthesis despite the CDT form involving eight coefficients (Table 5.11).

Although the CDT model gives a very good fit to each of the data sets, predictions should be extrapolated with care.

Analysis of predictions of thermal inactivation with the effect of combined T-pH generated using the log-linear and CDT models (Table 5.9) demonstrates that reliance on the classical log-linear form would both over- and under-predict the reduction in numbers of viable contaminants following exposure of a suspension in a liquid in a continuous steriliser. At a mid-range temperature of 58°C and pH 5.0 and after 30 *s* residence (exposure) time the reductions in viable cells (as $\log_{10}(N / N_0)$) predicted by the log-linear model are: -0.570 for *P. fluorescens*, -0.901 for *L. monocytogenes*, and -3.891 for *E. coli*; compared with the CDT model of -0.296, -0.470 and -4.756, respectively. The corresponding reductions in viable cell number for the experimental data are: -0.355, -0.152, and -5.114, respectively. These figures imply significant over-treatment (37.7%) for both *P. fluorescens*, and for *L. monocytogenes* (83.1%) and under-treatment for *E. coli* (23.9%) of the liquid in using the log-linear model.

These results together (and other similar predictions with the models) clearly have implications in the delivery of a safe product, without concomitant waste in energy and damage to end-product quality (eg vitamin denaturation or denaturation of colour or structure).

Analysis of the thermal inactivation data of Davey and Wood (1984) from studies in a continuous steriliser with the effect of T only (Table 5.7) confirm the better overall fit of the newly synthesised CDT model where a mean value of MSE of 0.535 was obtained compared with that of 0.689 for the log-linear model.

In Table 5.10 for the effect of combined T-pH on the batch, bench-scale data, the overall mean value of MSE can be shown to be 0.504, 1.880 and 0.955 respectively, for *P. fluorescenes*, *L.* monocytogenes and *E. coli* compared with 0.468, 2.108 and 2.463 for the less appropriate log-linear model.

It is interesting to note from Table 5.11 that the value of the coefficient C_2" is zero for each of the three data sets. This is consistent with other findings (Davey 1993a). Sterilisation happens relatively quickly (seconds) when compared with growth (days).

5.4.6 Concluding Remarks and Acceptance of the CDT Model for Testing in a Pilot Continuous Steriliser

It appears from the very good fit of the new CDT model to experimental data from the continuous steriliser of Davey and Wood (1984) for the effect of T only, and to those experimental data for the combined effect of T-pH from batch, bench-scale experiments with the three bacteria, that *time* plays a significant role in the kinetics of thermal inactivation.

It would be very interesting (and necessary) to test the fit of the new, non-linear CDT model to a wider range of data to assess whether the model has a general, or universal,

application to thermal inactivation kinetics of bacteria suspended in liquid. A bacterium-liquid interaction might be expected if (eg Carbopol, versus fruit juices, purees, milk, etc). Importantly, the form of the new CDT model can be readily integrated with a wide range of equations that characterise the hydrodynamics of liquid flow in a steriliser and with parameters describing the viscous and flow properties of the liquid.

The CDT model therefore was selected for evaluation in a continuous steriliser together with the steriliser performance equations.

5.5 CONCLUSIONS

1. The combined effect of process temperature and pH (T-pH) on (*static*) bench-scale data for the thermal inactivation of *Listeria monocytogenes* SLCC 5764 at near instantaneous heating has been modelled using an empirical n^{th} order rate equation (nOE). The nOE model is a significant departure from usual first-order (n = 1) models and appears to be a new form for bacterial survivor kinetics.

2. The nOE model gave a very good correlation with experimental data with an overall 89.5% of the *variance accounted for*. This compared with 71.5%V for a first-order model. The nOE model has two terms (1 / T and pH). It permits: a practical procedure for the evaluation of the microbiological safety of liquid foods processed at mild thermal T-pH, a general principle that can be used to describe bacterial inactivation for the higher temperatures, and; a basis for more realistic process optimisation. A drawback appears to be however that the model cannot be readily integrated with a wide range of equations that characterise the hydrodynamics of liquid flow in a steriliser and with parameters describing the viscous and flow properties of the liquid.

3. A new non-linear, empirical model for the thermal inactivation of bacteria titled CDT has been synthesised from experimental survivor kinetics of three sets of bench-scale

data of vegetative bacteria - *Escherichia coli* (ATCC 25922), *Listeria monocytogenes* (SLCC 5764) and *Pseudomonas fluorescens* (172) - as affected by combined *T*-pH in which inactivation has been hypothesised as *time*-dependent. The new CDT model explained between 82.9 *%V* and 94.6 *%V*. This compared with the log-linear form which accounted for between 74.0 *%V* and 88.1 *%V*.

4. The improved fit of the CDT form over the classical log-linear form appears to support the hypothesis that the rate of thermal inactivation of viable bacteria is *time*-dependent. A time-dependent rate coefficient for thermal inactivation will effect important changes in design, nutrient losses and minimising of operating costs in steriliser operations.

5. Synthesis of the new model is an important directional change in the development of models for thermal sterilisations. Importantly, the CDT model can be readily integrated with a wide range of equations that characterise the hydrodynamics of liquid flow in a steriliser and with parameters describing the viscous and flow properties of the liquid.

6. The CDT model therefore was selected for evaluation in a continuous steriliser together with the steriliser performance equations.

The following chapter describes the sizing and experimental testing carried out in a pilot continuous steriliser and presents a comparison between the experimental results and the predictions of the selected CDT model for thermal inactivation kinetics with combined process *T*-pH. The kinetics of thermal inactivation in this flow (*dynamic*) system are compared with those from the bench-scale (*static*) studies.

CHAPTER

SIX

PILOT CONTINUOUS STERILISER STUDY

6.1 INTRODUCTION

This chapter describes the sizing and experimental testing of a pilot continuous steriliser and presents a comparison between the experimental results and the predictions of the selected CDT model for thermal inactivation kinetics with combined process *T*-pH.

The main aims of the pilot continuous steriliser were to:

- observe thermal effects on bacteria as effected by combined process temperature and pH (*T*-pH) in the test liquid

- compare the kinetics of thermal inactivation in this flow (*dynamic*) system with those from the bench-scale (*static*) studies.

Consequently the experimental program focussed on:

(1) selection of a suitable micro-organism from those studied

(2) sizing of the pilot steriliser based on the CDT model (equation 5.25) together with the steriliser performance equations developed by Lin (1976) and Davey and Wood (1984) (see equations 2.2 and 2.3)

(3) construction of the steriliser in which the integrated model for the unit steriliser operation based on thermal inactivations predicted from the CDT model could be adequately tested

(4) production of adequate experimental data from trials with the continuous steriliser.

The major findings are summarised and an analysis of differences between the predicted and experimental data is presented. The implications arising from the use of a simple flow model for sterilisation are discussed.

6.2 CONTINUOUS STERILISER

6.2.1 Selection of Test Micro-Organism

Escherichia coli was selected as the test micro-organism because:

- it is a particularly well documented micro-organism
- there was sufficient bench-scale data (*static*) to compare with results obtained in a pilot continuous (*dynamic*) steriliser
- a direct comparison could be made with the published continuous steriliser data for T only of Davey and Wood (1984).

6.2.2 Sizing of the Pilot Continuous Steriliser

A range of lengths of continuous steriliser holding tube were calculated on the basis of the CDT kinetic model synthesised from bench-scale data for *E. coli* inactivation as effected by combined T-pH. These calculations are presented in Appendix F: Sizing the continuous steriliser length from bench-scale data using the CDT model for *E. coli* inactivation as affected by combined T-pH. A reduction in numbers of viable cells of 10^{-5} was selected as a practical steriliser design criterion. Clearly, sufficient numbers of viable cells (10^2 ml^{-1} to 10^3 ml^{-1}) needed to remain after heat treatment in the continuous steriliser to provide a practical basis for reliable plate counts.

A maximum pilot steriliser holding tube length of 15 metre (inclusive of Swagelok® connective fittings) was selected based on an overall average length predicted for all temperatures (54°C to 58°C) at one median pH value (pH 6.0). Steam injection heating was selected as a practical means of realising the (near) instantaneous temperature rise implied in the steriliser performance equations.

Tube of 316 stainless steel, with a smooth bore and internal diameter 9.5 mm, was used for the holding tube. A required length was made by coupling a number of sections of 980 mm (15 in total) together with Swagelok fittings. As well as enabling the lengths and steam

injector (see below) to be easily connected, the Swagelok fitting produces an aseptic seal and avoids gaps between matching tube ends. The holding tube was thermally insulated using 13 mm thick Thermotec® insulation.

6.2.3 Flow Diagram

Figure 6.1a is a flow diagram of the steriliser used in the validation trials. Figure 6.1b is a photograph of the actual steriliser.

The carrier liquid containing the bacterium was pumped from a continuously stirred holding tank (15.7 litre), through a specially designed and constructed steam injector, and into the insulated holding tube.

Total liquid flow – Carbopol solution and condensed steam – was controlled to give bulk velocities within the range 0.05 m s^{-1} to 0.15 m s^{-1} in the holding tube. This range corresponds to flow velocities used in commercial sterilisation (Pfiefer and Vojnovich 1952, Davey and Wood 1984). Corresponding volumetric flow are in the range 3.57 cm^3 s^{-1} to 10.7 cm^3 s^{-1}. The total liquid flow rate was determined by weighing the liquid at the end of the holding tube and recording the time needed for liquid to traverse the 15 m length.

The flow in the holding tube was set at the required rate using a variable speed, magnetically coupled, stainless steel gear pump. The magnetic pump drive provided a flow rate between 0.11 litre min^{-1} and 2.80 litre min^{-1} of water. Flow from this pump was smooth and pulse free. Other advantages of using a variable speed pump were that: it developed a sufficiently large head to create hydrostatic pressure in the holding tube, it could be steam sterilised and cleaned *in situ*, has corrosion resistant wettable parts, and; that it provided a flow rate that could be accurately controlled.

A section of glass tubing could be coupled (with Teflon® fittings) to the holding tube. This was used to check for the presence of uncondensed steam bubbles. Contaminated liquid effluent was collected in a receiving drum that contained disinfectant (Hibicet® 1%).

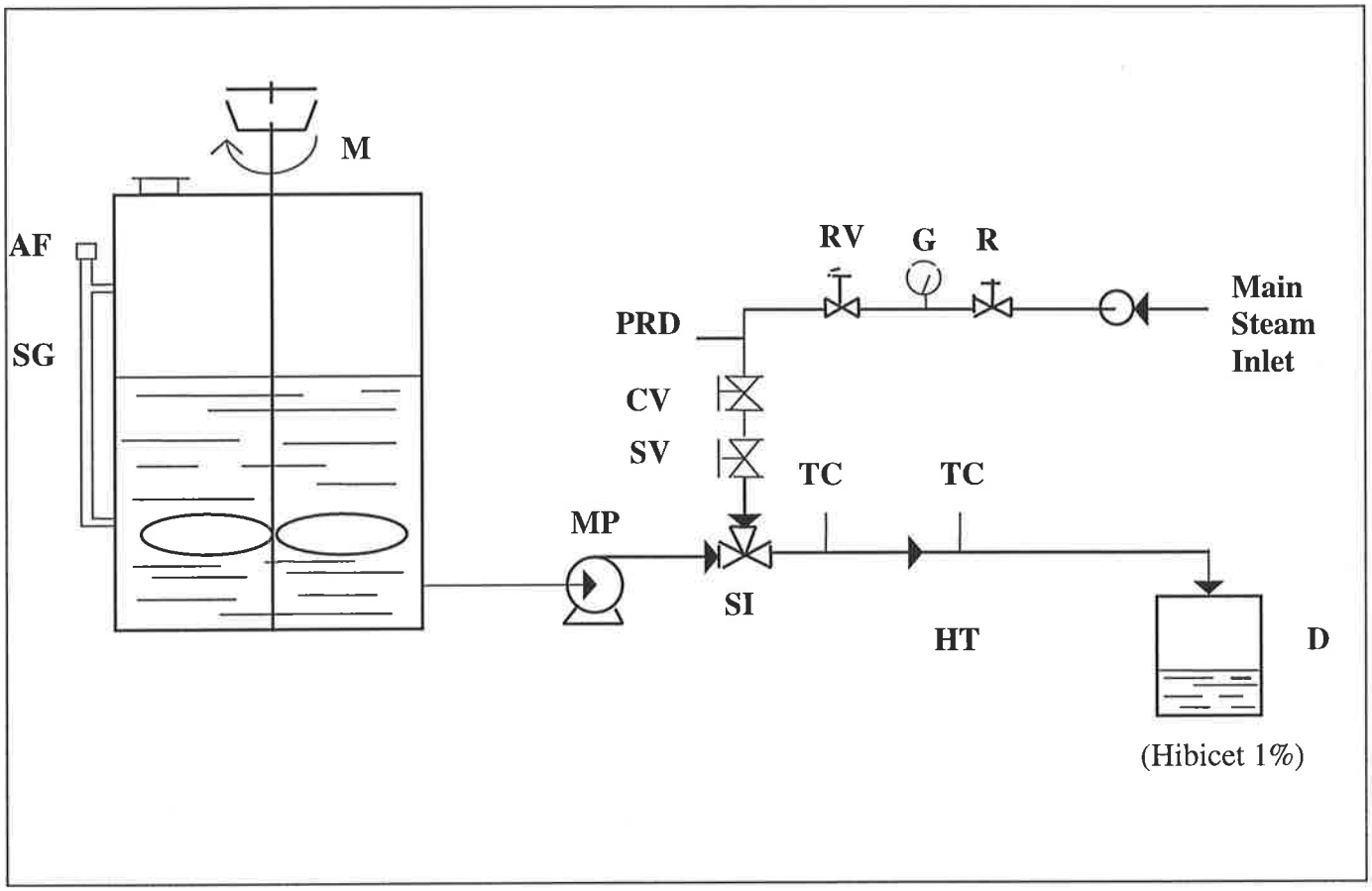
Temperatures were measured with copper-constantan thermocouples placed at 1 m intervals along the holding tube. The thermocouples were attached to the wall of the tube, between the tube and the insulation, and, to minimise heat conduction along the sheath, were aligned so that 5 cm of each thermocouple was parallel with the holding tube. Thermocouples were connected via a 10-way switch box (model Tracker 140), and a Shimaden® digital controller which displayed the temperatures recorded by the thermocouples. The thermocouples were calibrated by immersing them in a constant temperature water bath of known temperature and comparing their temperature reading against the temperature reading of a standard thermometer (Appendix I).

Steam inlets were arranged to allow the whole apparatus to be steam-sterilised *in situ*. All flow connections on the pump inlet were made with autoclavable silicone rubber tubing.

The Reynolds number (Re) in the holding tube was less than 1,100 (dimensionless) over the range of exposure times and corresponding bulk velocities ranging between 8.43 cm s^{-1} (Re = 801) and 11.46 cm s^{-1} (Re = 1,089). This indicates definite laminar flow and corresponding laminar flow profile (Perry 1973, Bailey and Ollis 1986, Holdsworth 1993) as implied in the steriliser performance equations, 2.2 and 2.3.

The Standard Operating Procedures (SOP's) for safe start-up and operation of the pilot continuous steriliser are given as Appendix L.

Figure 6.1a Flow diagram of the pilot continuous steriliser



Key to Symbols:

AF	Air filter	PRD	Pressure relieve devices
CV	Control valve (type needle)	R	Regulator for pressure
D	Drum	RV	Relieve valve
G	Pressure gauge	SG	Side glass
HT	Holding tube	SI	Steam injector
M	Motor driven stirrer	SV	Stop valve
MP	Magnetically driven pump	TC	Digital Controller

Figure 6.1b Photograph of the pilot continuous steriliser

6.2.4 Steam Injector

Of the methods available for heating, direct steam injection was deemed best suited to the practical realisation of the instantaneous heating and cooling assumptions of the performance equations derived for the continuous steriliser. This method gives a higher overall heat transfer coefficient than indirect heating together with the elimination of heat exchanger equipment in the heating section of the steriliser (Jones and Larner 1968, Lin 1976, Ruyter and Brunet 1973, Davey and Wood 1984, Holdsworth 1992).

However, with steam injection heating, dilution of the liquid flow with condensate occurs and provision must be made for this in the design of the steriliser.

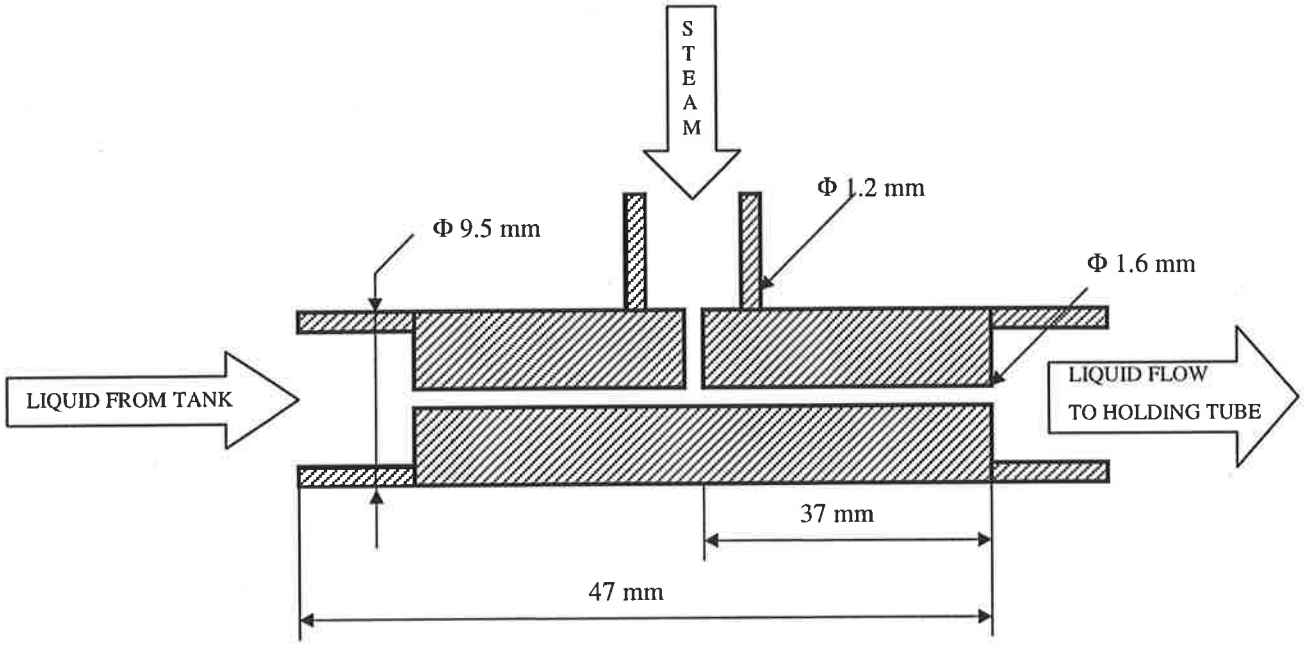
The equations developed for the design of a direct steam injector for heating liquid in a flow system are based on the following assumptions, the:

- bulk temperature of the liquid should rise rapidly to the holding temperature at the steam injector
- flow of the liquid in the holding tube should be laminar and free of vapour bubbles
- residence time of the liquid in the injector should be very short, because the thermal inactivation of bacteria follow a complex kinetic equation and the corrections to the holding time are difficult to estimate.

The equations solved to size the dimensions of the injector used in this investigation are given in Appendix H.

Dilution of the Carbopol by steam condensate was predicted at 6.9% ± 1.5 over the range of flow and temperature conditions used and was assumed constant at 7%. A Bourdon gauge indicted the steam pressure. A schematic diagram of the steam injector is presented in Figure 6.2.

Figure 6.2 The Steam Injector



6.2.5 Experimental Method

The holding tube of the continuous steriliser and carrier liquid were prepared as follows:

The stainless steel holding tank was steam-sterilised *in situ* and allowed to cool. Sufficient Carbopol 934 powder was added to water to give a 2.14 kg m^{-3} concentration when the volume of both the sodium hydroxide solution used for neutralisation and bacterial suspension were taken into account (Appendix J). Dilution (7%w/w) with condensed steam during passage through the steam injector gave the specified 2 $kg.m^{-3}$ Carbopol concentration in the holding tube. The Carbopol solution was left at ambient temperature over night. One hour prior to experimental trials, the sodium hydroxide was added to the Carbopol solution and pH checked after another 30 min. During this time the pump, pump lines and holding tube were steam-sterilised *in situ*. Immediately prior to experiments, 400 cm^3 of bacterial suspension was added into the sterile Carbopol solution in the holding tank and dispersed with continuous stirring. This resulted in between 10^8 to 10^{10} viable bacteria per ml in the Carbopol carrier liquid The steam pressure to the steam injector and the flow rate of liquid were set to the required levels. Temperatures and the steam pressure were continuously monitored, until the system reached a steady-state. Different holding tube lengths were used to coincide with different exposure times to thermal inactivation.

Samples of heated liquid in the holding tube were obtained from a series of sampling valves placed at one metre intervals along the length of the steriliser. Sample volumes of 64 cm^3 of the heated Carbopol were immersed in an ice-bath and rapidly cooled. Estimates of the viable count of bacteria in control (unheated) and heated samples were determined by standard plate count methods (Meynell and Meynell 1970). Importantly, the sampling was started from the end of the steriliser towards the steam injector. This was done in order to eliminate possible errors introduced by disturbing the laminar velocity flow of the contaminated liquid in the holding tube.

6.2.6 Practical Implementation of Inherent Assumptions of Steriliser Performance Equations

The design of the continuous steriliser is influenced by the thermal behaviour of the contaminating micro-organism, and by both rheological and heat transfer characteristics of the medium. The assumptions implied in the continuous steriliser performance equations are:

1. The flow in holding tube is laminar, incompressible, and steady-state
2. There is no slip at tube wall, that is, the velocity of the liquid at the tube wall is equal to the velocity of the wall surface which is zero
3. Isothermal conditions occur at every point in the holding tube
4. End effects and sampling effects are negligible
5. Bacteria are uniformly distributed in the liquid
6. There is no slip between bacteria and liquid, that is, the bacterial cells travel on the laminar streamlines
7. The age of viable cells are the same for both bench-scale (*static*) and dynamic continuous steriliser
8. The bulk temperature of the liquid is instantaneously raised to the holding temperature and is instantaneously cooled to a non-lethal temperature
9. At the shear rates that pertain to food processing, and which are simulated in the pilot steriliser (120 s^{-1} to 706 s^{-1}), the behaviour of the Carbopol solution may be described by the power law equation
10. The pseudoplastic index of the carrier liquid is not significantly temperature dependent (n = 0.37).

Assumptions 1 and 2 are usual in such analyses and are realistic for a liquid in laminar flow. Assumptions 3 through 6 can be reasonably assumed to be practically implemented for a well-mixed solution of test liquid and bacterial cells, and a steam injector with an internal, highly turbulent flow. Assumption 7 is readily practically implemented with the standardised protocols for the bench scale and continuous steriliser studies. The time for heating in the steam injector will be very short and is estimated at 0.0183 *s* (Appendix H).

As the liquid is discharged some flash cooling will occur and together with the ice-bath it is reasonably assumed assumption 8 is practically implemented. More than 1470 shear stress / shear rate data sets (with 21 replicates) were used by Davey (1980) to determine the rheological properties of a Carbopol 934 solution of 2 kg m^{-3} in a Rheomat 15, Cone and Plate viscometer with a vapour shield to prevent evaporation of water from the solution, over a range of temperature of 25°C to 90°C. The rheological properties were not significantly affected by the dispersion of bacterial cells, and the pseudoplastic index (n) was not significantly affected over the temperature range 25°C to 90°C. Assumptions 9 and 10 are therefore practically implemented.

6.3 RESULTS AND DISCUSSION

Results obtained from the pilot steriliser are summarised in this section. Because of the large amount of labour involved in obtaining data, the experimental work was restricted. Replicate data were determined for each of three selected sets of combined *T*-pH together with a range of exposure times.

6.3.1 Results Summary

Figures 6.3 to 6.5 summarise the thermal inactivation kinetics of the test bacterial strain in the Carbopol carrier liquid in the pilot steriliser.

The data are presented as the $\log_{10}$ of the fraction of bacteria that remained viable after thermal exposure. Exposure temperatures of 54°C ± 0.2, 56°C ± 0.2, and 58°C ± 0.2, in combination with a pH of 4.5, 6.0 and 7.5, and with immersion times between 8.73 *s* and 173.35 *s* were used. The data are the mean of two replicates. The standard deviation ranged between 10.9% and 39.0% with an overall mean of 24.6%. The total number of individual data sets was $n_T = 218$.

The initial concentration of *E. coli*, N_0, ranged between 9.41 x 10^6 ml^{-1} and 1.32 x 10^9 ml^{-1}.

Figure 6.3 Summary of the continuous steriliser experimental data for thermal inactivation of *E. coli* at pH 4.5 with three exposure temperatures (Δ = 54°C, $\Diamond$ = 56°C, □ = 58°C) and a range of exposure times

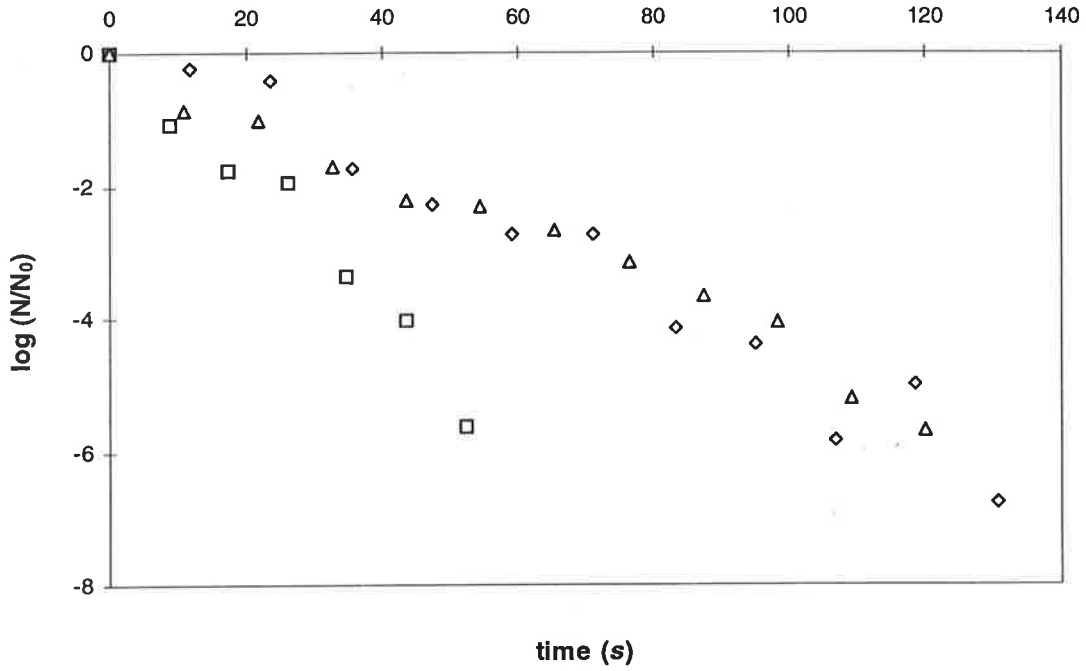


Figure 6.4 Summary of the continuous steriliser experimental data for thermal inactivation of *E. coli* at pH 6.0 with three exposure temperatures (Δ = 54°C, $\Diamond$ = 56°C, □ = 58°C) and a range of exposure times

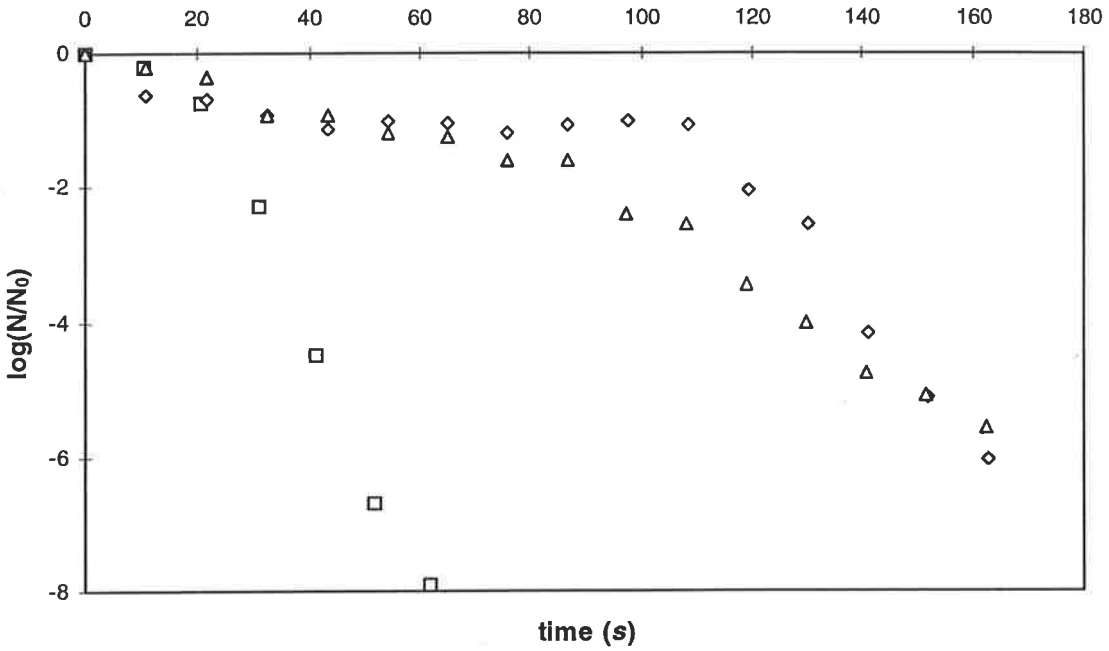
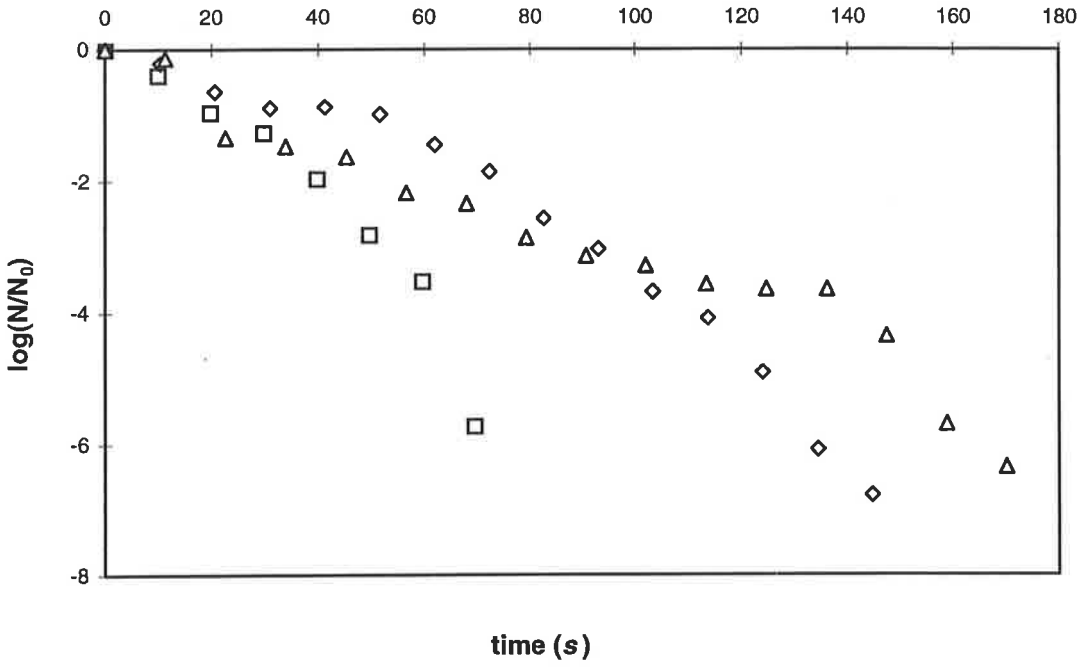


Figure 6.5 Summary of the continuous steriliser experimental data for thermal inactivation of *E. coli* at pH 7.5 with three exposure temperatures (Δ = 54°C, ◊ = 56°C, □ = 58°C) and a range of exposure times



6.3.2 Comparison with Theoretical Predictions

A comparison of the predictions of the CDT-steriliser model with the experimentally determined survivor data in the pilot steriliser are summarised in Figures 6.6a through 6.6i for 54°C, 56°C and 58°C at a pH 4.5, 6.0 and 7.5, respectively.

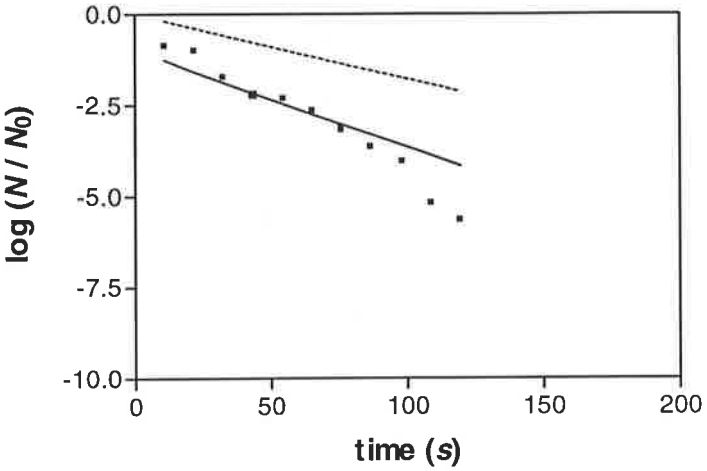
These data are presented as a mean of the $\log_{10}$ of the fraction of survivors remaining viable over a holding time $t = L / \langle u \rangle$.

6.3.3 A Comparison between Survivor Kinetics in the Continuous Steriliser with the Capillary Data

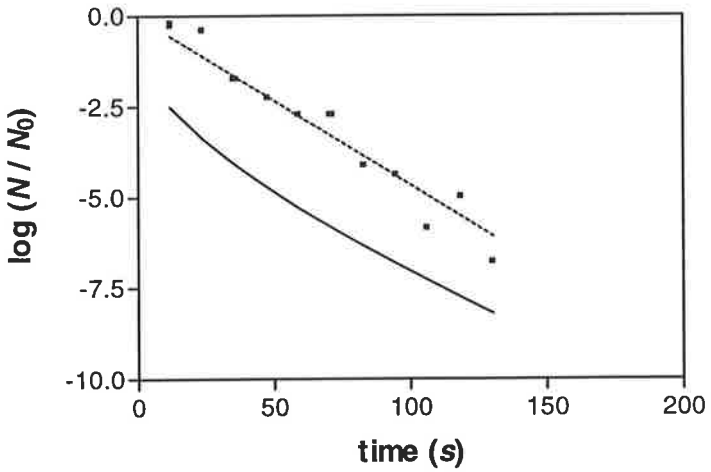
The fraction of survivors of *E. coli* suspended in the Carbopol carrier liquid survived unexpectedly longer periods of heating when exposed in the capillary tubes of the bench scale method at low temperatures (54°C) than in the pilot continuous steriliser. However, at the higher temperatures (56°C and 58°C) bacterial cells were inactivated more rapidly in the capillary tubes at any pH.

This is underscored through a comparison of the two experimental data sets. A comparison of the experimental data, as fraction of survivors, obtained in the capillary tubes (*static*) with the experimental data obtained in the continuous steriliser (*dynamic*) is presented in Table 6.1 for a range of exposure times (40 *s* to 60 *s*) and temperatures (54°C to 58°C) and pH (4.5 to 7.5). Table 6.1 shows that during the initial 60 *s* at 54°C, the reduction in survivors in the continuous steriliser (*dynamic*) is greater than for the capillary tube data (*static*) for all pH values (4.5, 6.0 and 7.5). At 56°C and 58°C, however, after the first 50 *s* and 40 *s*, respectively, the reduction in survivor fraction is much less in the continuous steriliser than in the capillary tubes. These findings demonstrate clearly that predictions based on the capillary tube data do not agree with data obtained in the continuous steriliser at equivalent sterilisation temperatures and exposure times for a given pH.

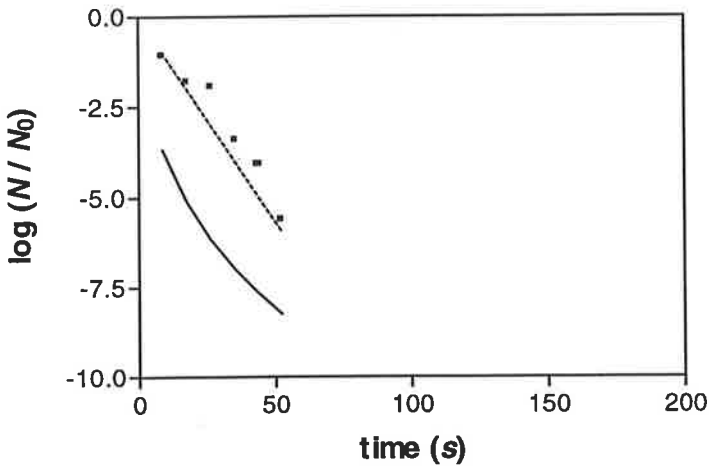
Figure 6.6 Comparison of the predictions of CDT hydrodynamic model (——) and log-linear model (- - - - -) with experimentally determined continuous survivor data (■) for *E. coli* over a range of *T*-pH and exposure times



a) 54°C and pH 4.5

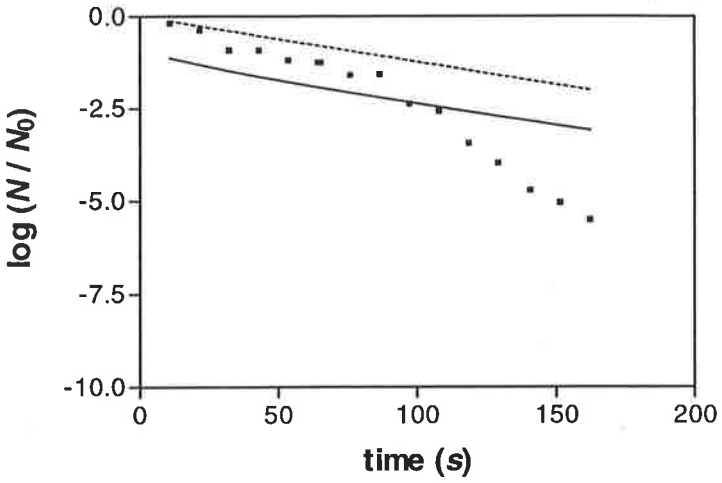


b) 56°C and pH 4.5

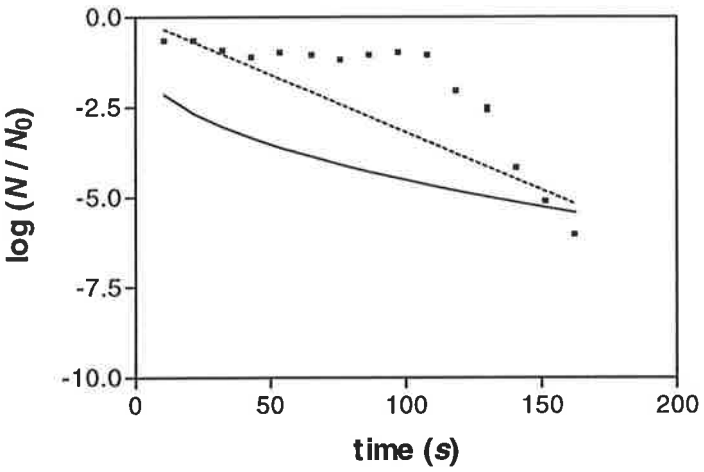


c) 58°C and pH 4.5

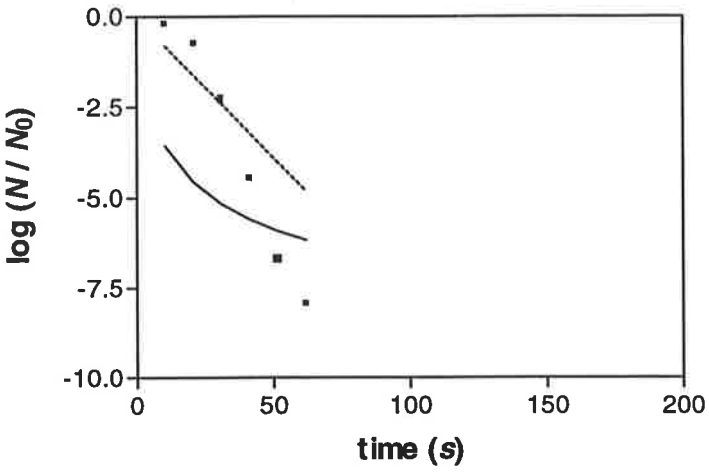
Figure 6.6 continued ...



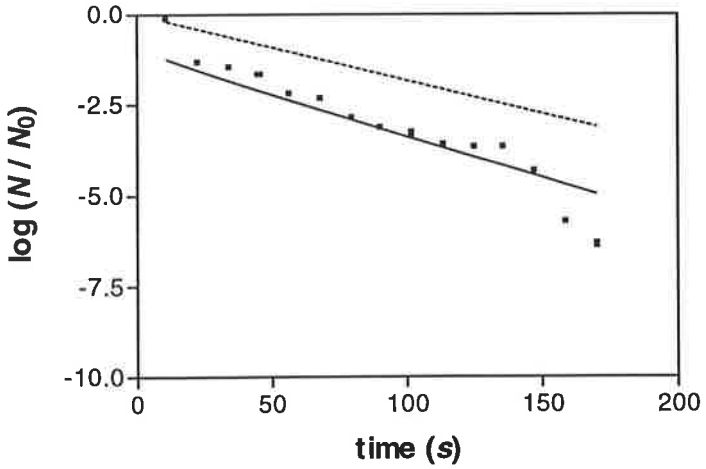
d) 54°C and pH 6.0



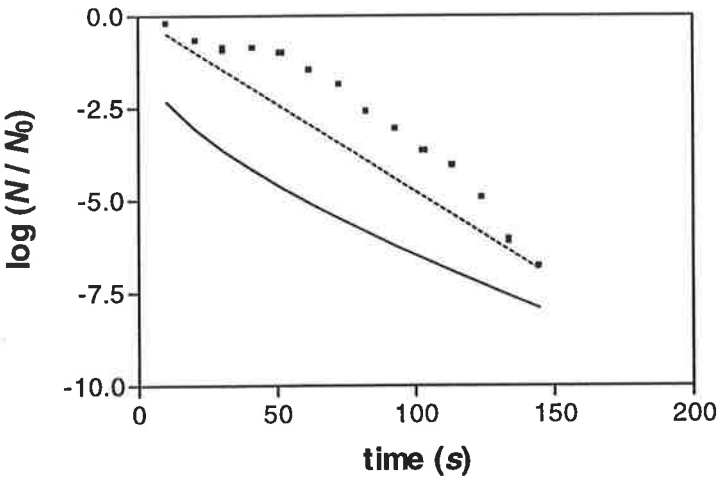
e) 56°C and pH 6.0



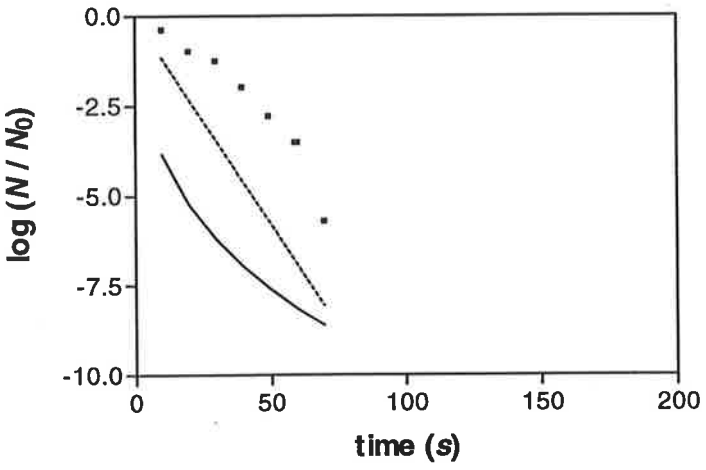
f) 58°C and pH 6.0



g) 54°C and pH 7.5



h) 56°C and pH 7.5



i) 58°C and pH 7.5

6.3.4 Analysis of the Continuous Steriliser Survivor Curves

All curves in Figures 6.6a through 6.6i display a typical shoulder, more or less pronounced, depending of the environmental conditions in which bacterial cells were exposed. It is suspected that Figures 6.6a through 6.6i show a new microbiological phenomenon of bacteria in dynamic flow when compared with batch conditions.

This phenomenon might be related to:

1. denaturation of key proteins
2. stress proteins, that work to repair damaged proteins
3. heat and pH stresses, when damage occurs faster than repair
4. a bi-phasic behaviour of bacteria.

For example, as the T value increases, the CDT model predicts more bacteria are inactivated than experimentally determined. Figure 6.4a through 6.6i show both concave-up and concave-down curves. For a T of 54°C and pH 7.5 (Figure 6.6a), the CDT model predictions follow the experimental data up to a time of 75 s; after this period of time, the model predicts fewer bacteria are inactivated than found experimentally. A similar pattern is observed at 54°C and pH 7.5 (Figure 6.6g). At 56°C and 58°C and the same inactivation time, significantly fewer bacteria are inactivated than predicted especially true as the exposure temperature increases.

Despite the significant difference in CDT prediction of the number of bacteria inactivated and that determined experimentally, a positive aspect of these findings is that the difference is apparently systematic. That is, the predicted inactivation is always greater over the range of T-pH and exposure time that was used. The model predictions therefore imply a greater sterilisation efficacy than was the actual case. Any liquid product processed on the basis of the predictions of the CDT model would therefore contain many more viable bacteria than assumed. This consequence would be of a serious public health significance if used without caution. A short summary comparison between CDT model predictions and the experimental continuous steriliser data reveals that:

1. continuous experimental data indicate a distinctive pattern
2. continuous experimental survivor curves show concave trends, indicating non-linear inactivation kinetics
3. the CDT model predicts more bacteria are inactivated than determined experimentally.

A detailed investigation of Figure 6.6a through 6.6i shows an initial region where the logarithm of the fraction of survivors is approximately linear with time, and a second region of extreme tailing in which the fraction of survivors decreases with time. The time where the survivor trend is turning into an apparent tail is illustrated in Table 6.2, and its variation with T and pH shown in Figures 6.7, respectively. It should be clarified that all the values presented in Table 6.2, and subsequently plotted as Figures 6.7, have a trend of subjectivity, as they have been judged graphically and estimated by eye using a ruler.

A plot of T versus these shoulder lag times is presented as Figure 6.7a. Results show a clear variation of the shoulder lag with T. These variations decrease rapidly as the T increases. For example, for a pH of 7.5, the shoulder lags vary from 135 s for 54°C, to 115 s for 56°C, and to 35 s for 58°C. At 58°C, regardless of the pH of the medium, the cells apparently have less time to react and the shoulder is about 40 s. Most likely this is because at the higher T the heat transfer to the cell will annihilate the abilities of cell self-protection; whereas, at the lower temperature of 54°C the cells are influenced more by both T and pH.

Although the data are limited, the relationship between shoulder lag variation and T appears linear as shown in Figure 6.7a.

Figure 6.7b is a plot of pH versus the shoulder lags. This figure reveals that the length of the linear region is also apparently linear with pH, and almost constant at temperatures of 56°C and 58°C. This behaviour indicates that at the higher temperature the effect of temperature is dominant over pH.

The appearance of the shoulder in survival data may relate to the imposition of two different types of stress on the viable cells. An initial heat shock, when the cells and carrier liquid temperature is raised from ambient to between 54°C and 58°C in the steam injector (in about 0.0183 *s*), and a subsequent cold shock when the samples of the thermally treated cells and carrier liquid are cooled. Damage may result to different types of cell proteins with each of the hot and cold shock stresses. Clearly, any temperature above 40°C for *E. coli* is a significant heat stress to the viable cell.

Extrapolation of Figure 6.7a indicates that the shoulder lag time would be nearly zero at about 60°C. Therefore, the minimum temperature at which the continuous steriliser could operate optimally is 60°C. A practical reality, however, is that the operation of the steriliser at temperatures below this, 54°C to 58°C, was necessary, in order to obtain continuous experimental data that permitted this apparent implication for optimal operation.

A summary comparison of the CDT model and the log-linear model predictions is presented in Figure 6.8. The residuals versus observed data for both models show concavity, or a shoulder in the trend of continuous experimental data. It can be seen that the CDT model under-predicts for the first 2-$\log_{10}$ reductions, but gives good predictions between 2-$\log_{10}$ and 3.7-$\log_{10}$ reductions, but over-predicts after 3.7-$\log_{10}$ reductions. The log-linear model consistently over-predicts for all combined *T*-pH and time.

These findings necessitated an analysis of the experimental methodology in which the assumptions of the experimental procedures, and; of the underlying assumptions of the steriliser performance equations, are examined. This analysis is summarised in the following sections of this thesis.

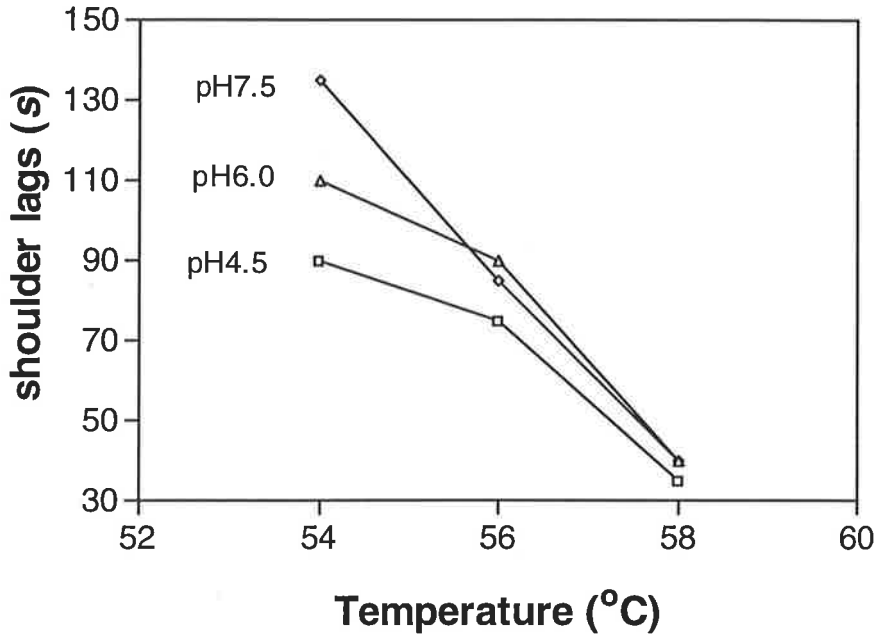
Table 6.1 Comparison of continuous survivor experimental data with survivor data obtained in the bench-scale capillary tubes for *E. coli* for combined *T*-pH at exposure times between 40 *s* and 60 *s*

time (*s*)	***T* - pH**	**-$\log_{10}$ (N / N_o)**	
		Continuous steriliser (*dynamic*)	**Bench-scale capillary tube** (*static*)
60	54°C - pH 4.5	2.7	1.7
	54°C - pH 6.0	1.3	0.6
	54°C - pH 7.5	2.3	1.1
50	56°C - pH 4.5	2.5	3.6
	56°C - pH 6.0	1.1	2.1
	56°C - pH 7.5	1.0	3.7
40	58°C - pH 4.5	4.0	6.1
	58°C - pH 6.0	4.5	5.1
	58°C - pH 7.5	2.0	7.8

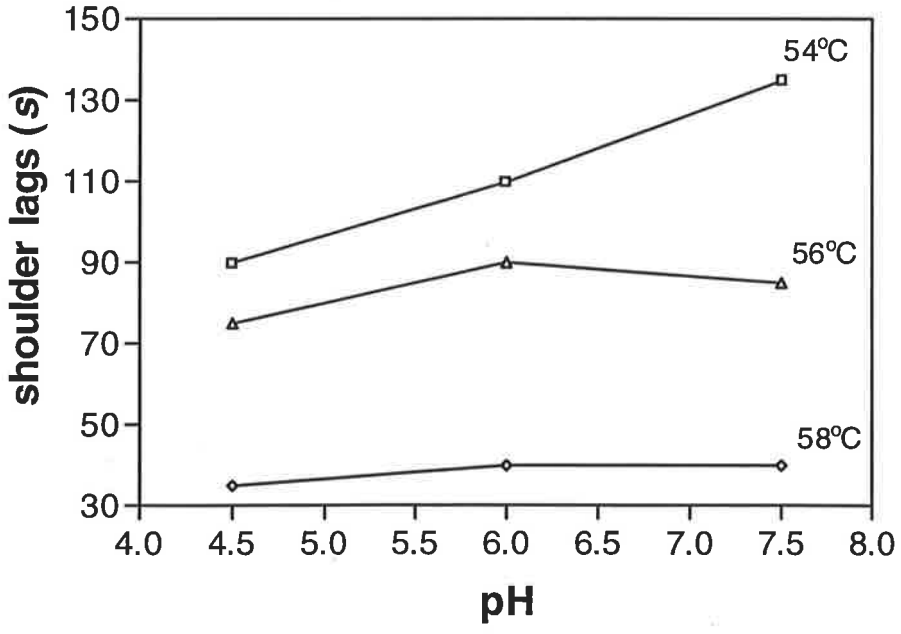
Table 6.2 Comparison of the estimated shoulder lag times at combined *T*-pH for continuous steriliser experimental data for *E. coli*

T (°C)	pH	time (*s*) shoulder lag
54	4.5	90
	6.0	110
	7.5	135
56	4.5	75
	6.0	90
	7.5	85
58	4.5	35
	6.0	40
	7.5	40

Figure 6.7 Variation of the shoulder lags of experimental survivors in continuous steriliser for *E. coli* as a function of *T* and pH

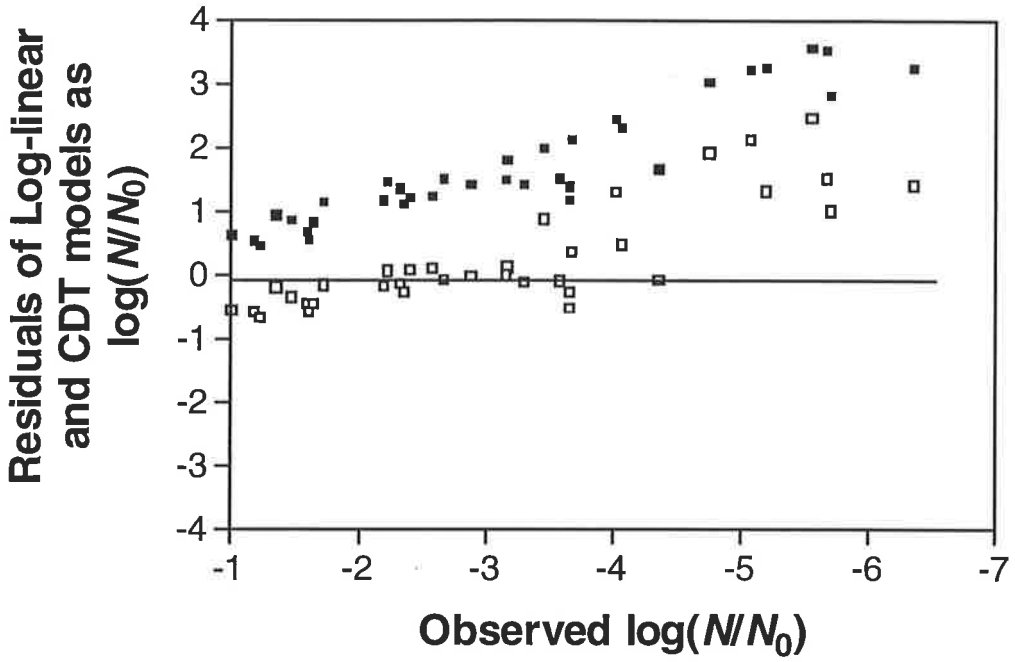


a) *T* dependence at pH constant



b) pH dependence at *T* constant

Figure 6.8 Comparative plot of the residuals versus observed survivor data for both log-linear and CDT models for the thermal inactivation of *E. coli* in a continuous steriliser for combined *T*-pH (■ = residuals of log-linear model; □ = residuals of CDT model)



6.3.5 Analysis of the Assumptions Inherent in the Continuous Steriliser Trials

Possible explanations for the deviation of the model predictions from the experimental data obtained in the continuous steriliser may arise from a combination of errors inherent in:

1. sampling
2. significant temperature gradients along the holding tube
3. a cell population of a heat sensitive bacterium with a more thermally resistant micro-organism (ie contaminated culture)
4. significant temperature gradients within the liquid in the holding tube
5. a failure to practically implement the hydrodynamic flow assumptions of the steriliser performance equations.

The error in the sample volume measurement at the conditions used in the steriliser trials was found to be less than 2%. This was determined by comparing the volumes of 30 control samples collected consecutively along the steriliser length. This small error does not give rise to an approximately one order of magnitude in the experimental data. Hypothesis 1 may therefore be rejected.

Acceptance of hypothesis 2 would imply a significant temperature gradient along the steriliser holding tube. However, the temperature gradient along the length of holding tube was much less than 0.5°C for any experimental trial. This is not sufficient to give rise to the large differences between the predicted and experimental results obtained in the continuous steriliser. Hypothesis 2 is therefore rejected.

Cultures of ATCC 25922 *E. coli* used in all experimental trials were derived from the same stock suspension held at –70°C as used by Davey and Wood (1984) and Davey, Hall and Thomas (1995). Consequently it is unlikely that the culture had become contaminated with another strain that was thermally more resistant. The likelihood of a large variation of thermal responses within the pure cell culture that would give rise to the magnitude of differences in the predictions and experimentally determined numbers of bacterial

survivors is negligible. Further, the colonial morphology of both thermally exposed and control (unexposed) samples was identical. Hypothesis 3 may therefore be rejected.

As the liquid and contaminant bacterial cells are heated in a highly turbulent regime inside the steam injector the possible consequence of a temperature gradient within elements of the liquid as it flows along the holding tube is highly unlikely. In any event, the temperature gradient along the holding tube was negligible, and this reinforces the hypothesis that a significant temperature gradient within elements of the liquid that may favour greater numbers of survivors than was predicted, is unlikely. Hypothesis 4 was therefore rejected.

Deviations from laminar flow (hypothesis 5) will alter the residence time of the elements of the liquid and therefore of the contaminant bacterial cells that are assumed to travel on the streamlines of the laminar flow of the liquid in the holding tube. A significant breakdown in the residence time and assumed hydrodynamic flow in the holding tube could give rise to deviations in the experimentally determined numbers of survivors and those predicted from the CDT model and steriliser performance equations.

An experimental investigation of the hydrodynamic flow in the holding tube and its significance in implementing the assumptions inherent in the predictions was therefore undertaken.

6.3.6 Experimental Testing of the Holding Tube Hydrodynamics

To test that the experimental data in the continuous steriliser were obtained under conditions that satisfy the assumptions inherent in the steriliser performance equations, flow conditions inside the holding tube were examined experimentally.

A glass section of holding tube (1200 mm) was coupled to replace a length of steel holding tube in the pilot steriliser and photographs were taken of the actual velocity profile and hydrodynamic picture inside the holding tube. Dye traces were injected into the centre of the steriliser holding tube immediately downstream of the steam injector (~ 2.5 cm) during

operation of the continuous steriliser. A suitably modified 21-gauge hypodermic syringe was used to inject methylene blue indicator in a 2 kg m^{-3} Carbopol solution of the test liquid at room temperature (~ 20°C). The viscosity of the test liquid was known not to be significantly affected by temperature over the range 25°C to 90°C (Davey 1980, Davey and Wood 1984).

A number of these dye trace studies were carried out for a bulk liquid temperature of 54°C and a bulk velocity of 1.8 cm s^{-1}. Figure 6.7 shows a summary sample photograph of actual velocity profiles.

From this summary photograph it is seen that full laminar flow was obtained in the holding tube after a short distance (~ 13 cm, ie 13.3 holding tube diameters) from the steam injector outlet (Part 1). Importantly, this finding confirms stable operation of the steam injector and the condensation of all steam as no bubbles are observable.

Part 2 of the figure shows the laminar profile at a distance of 70 cm from the steam injector. The profile is observed to be fully laminar. In Part 3, 150 cm from the steam injector, the profile is seen to remain laminar. However this laminar profile has begun to disappear in Part 4, a distance of 200 cm from the steam injector, and the effects of axial dispersion have begun to make an impact. The extent of the effect of axial dispersion on the liquid hydrodynamics cannot be quantitatively assessed however from visual studies along the length of the steriliser holding tube.

Continued investigation using the glass section of holding tube was carried out however. Progressive sections of the tube were replaced with the glass section and dye traces observed to determine the holding tube dynamics at each section of all the 15 lengths. These studies showed conclusively the gradual breakdown of the laminar profile in the holding tube. The liquid profile slowly disappears through the effects of gradual dispersion, that is, a superimposed backmixing or intermixing of the carrier liquid.

Figure 6.7 Summary photograph showing the liquid profile of the carrier liquid in the pilot continuous steriliser and effects of dispersion in the holding tube

Hypothesis 5 – a failure to practically implement the hydrodynamic flow assumptions of the steriliser performance equations – was therefore accepted as being a major contributor to the observed differences between experimental data obtained in the continuous steriliser and the predictions of the CDT model.

An attempt at a quantitative analysis of the likely magnitude of these unaccounted effects of dispersion on the continuous steriliser performance equations as applied to the experimental studies in the pilot continuous steriliser was therefore undertaken.

6.3.7 Effects of Dispersion

A literature search indicated that the seminal work on relevant effects of dispersion is that of Levenspiel (1972). Aiba, Humphrey and Millis (1973) were to (briefly) apply the notion of dispersion to microbiological reactors such as continuous sterilisers. Lin (1975) investigated theoretically the thermal sterilisation of liquid with effective dispersion. He allowed for a variation with temperature, whereas the earlier models assumed a constant temperature. There are no reports of dispersion effects for combined *T*-pH.

Important to understanding the dispersion effects of backmixing and intermixing on the gradual breakdown of a liquid velocity profile in a continuous steriliser is that the behaviour of the bacterial cells suspended in the liquid are considered to follow closely the hydrodynamic mixing characteristics of the particular steriliser. This assumption that bacterial cells would follow the mixing characteristics is a reasonable one, and is implicit in the steriliser performance equations presented in Chapter 2.3.2. That is, the bacterial cells travel on the laminar layers of the velocity profile and that there is no "slip".

The effect of significant dispersion is to increase the holding time required for sterilisation. In a practical sense, this means an added length of holding tube is required for a (fixed) flow rate of liquid.

The treatment of dispersion in the literature appears to be limited to its effect on ideal plug flow in a tubular reactor. This situation is much less complex than when considering the

effects of dispersion on laminar flow in the pilot continuous steriliser. A deal of further complexity is further added since the CDT model is not a simple first-order equation.

However any attempt at an appropriate quantification of the effects of dispersion could provide useful information or insight and was therefore undertaken. If the effect of dispersion is significant, an additional element might be needed in the steriliser performance equations.

A convenient treatment of the effect of dispersion on plug flow in a continuous steriliser is that provided by Lee (1992). It is convenient in that it is built from basic considerations of a steady-state mass balance on viable bacterial cells contaminating a liquid in continuous flow. Typically, the procedure to calculate effects of dispersion is to assume ideal plug flow and determine a suitable length of holding tube (L) for a required reduction in viable cell numbers (ie a design criterion $\log_{10} \langle N \rangle / \langle N_0 \rangle$), then, determine the required additional length of holding tube if the effect of axial dispersion is considered. However, for the present purpose what is required is:

1. *given* L and l $\log_{10} \langle N \rangle / \langle N_0 \rangle$
2. *calculate* the effect dispersion might have had on the extent of thermal inactivation of the numbers of bacterial cells
3. *moderate* CDT model predictions for this effect
4. *appraise* the significance of dispersion in the experimental continuous steriliser.

A procedure was set up to do this, and is presented in detail in Appendix K. The effect of axial dispersion is only indicative at this stage and results obtained are formulated on estimates.
Holding tube lengths of between 6 m and 15 m were selected for comparison with and without effects of dispersion and where survivor numbers of *E. coli* were large enough to be easily identified. This gave a range of holding times of 52 *s* to 170 *s*.

Table 6.3 is a comparative summary. Three temperature values are considered at an averaged range of pH 6.0. The table highlights that the CDT model under-predicts the

efficacy of sterilisation at T = 54°C, but over-predicts at greater temperatures. Over this range of temperature the effect of dispersion estimated appears controlling to give a fixed reduction of $\log_{10}$ = 7.0. Significantly, it can be seen that, overall, the value predicted in reduction of viable *E. coli* cells by the dispersion model agrees better with the experimental values obtained in the pilot continuous steriliser. This is especially true at the greater $\log_{10}$ reductions in viable cells and greater holding times (long sections of holding tube).

A CDT-Dispersion model appears to give a better mechanistic view of the thermal inactivation kinetics, accounting as it does for the effects of:

- environmental factors (T-pH)
- time (t)
- liquid rheology (n)
- dispersion.

Significantly, the new CDT model predicted better the survival of bacteria than the widely used log-linear model.

The work presented in this chapter has addressed some important issues that must be considered when sterilisation is conducted in a (pilot scale) continuous steriliser. The observed behaviour of bacteria heat-treated with different liquid pH demonstrates that actual bacterial kinetics in continuous steriliser are more complex than that implied in the assumptions of the hydrodynamic performance equations.

This is a matter reported only very recently by McMahon et al (2000) for relevant pathogens. For a study of the growth phase on induction of the heat shock response of *Yersinia enterocolitica* and *Listeria monocytogenes*, they reported on exponential and stationary preconditioned cultures that were heat shocked. Heat-shocked cells of these pathogens were able to survive much longer than non-heat shocked cells when heated at 55°C – a temperature very similar to that used in this study. The increase in heat resistance between heat-shocked and non-heat-shocked cells was greater for the exponential culture

than the stationary. This is the overall relative thermal resistance of each pathogen was dependent on cell growth phase.

The work of McMahon et al therefore requires recognition of the presence of potentially injured micro-organisms. It is imperative for accurate interpretation of microbiological data. The public health consequences associated with failure to detect injured pathogens that subsequently repair "could be substantial" (McMahon et al 2000).

Differences observed between the survivor kinetics in capillary (*static*) and continuous steriliser (*dynamic*) heat treatments may therefore relate to the thermal history of the bacteria. This effect might need to be quantitatively assessed in future studies of the sterilisation of vegetative pathogens.

Table 6.3 Comparison of the experimental continuous steriliser data for *E. coli* survival with predictions of the CDT model with hydrodynamic flow and with dispersion effects at an average value of pH 6.0

T (°C)	$-\log_{10}(N/N_0)$		
	Experimental Data	**CDT Model Predictions**	
		Hydrodynamic Flow	**Dispersion Effects**
54	5.9	4.1	7.0
56	6.5	7.2	7.0
58	6.4	7.7	7.0

6.4 SUMMARY

1. Kinetic data obtained from bench-scale studies in capillary tubes (*static*) of thermal inactivation of *E. coli* in a Carbopol carrier liquid did not satisfactorily agree with those obtained in a pilot scale continuous (*dynamic*) steriliser.

2. A newly synthesised CDT model based on these capillary data and used to size the continuous steriliser predicted a greater reduction in the number of viable cells in the liquid than was actually realised, especially at higher temperatures.

3. A significant portion of the difference between predicted reductions in the number of viable bacteria by the CDT model, based on data obtained from the capillary tubes, and that which was experimentally realised in the pilot continuous steriliser is associated to the effects of dispersion.

4. A model for the simulation of thermal inactivation of bacteria in liquid in a continuous steriliser must involve a consideration of the likely significant effects of dispersion, especially at longer holding tube lengths and corresponding holding times.

CHAPTER
SEVEN

CONCLUSIONS

1. Thermal inactivation curves from bench-scale (*static*) studies for three selected bacteria, namely, *Pseudomonas fluorescens*, *Escherichia coli* and *Listeria monocytogenes*, and continuous steriliser (*dynamic*) data for *E. coli*, showed tailing. The assumption of log-linear kinetic dependence is therefore inadequate over the entire survivor curve. Widely used D values are not appropriate to describe thermal inactivation of these three bacteria unless used carefully and judiciously in demonstrated linear sections of the survivor curve.

2. The effect of pH over the pH range 4.0 to 7.5 on the rate coefficient for thermal inactivation is significant for all three selected bacteria, especially at lower exposure temperatures of 54°C. Significant concave-up tails appeared in the survivor data for *P. fluorescens* and *E. coli*, and both concave-up and concave-down tails appeared in the survivor data of *L. monocytogenes*. The reason(s) for different tailing behaviour exhibited by the bacteria is unknown, but this has important implications for development of predictive models applicable to industry. For these data, a decimal reduction time that is dependent on combined temperature and pH (T-pH), $D_{°C,pH}$, has therefore been defined for use with appropriate linear sections of the survivor curve.

3. An uncritical use of the log-linear model will result in a serious under- or over-estimate of survivors for these three bacteria over a range of combined T-pH.

4. Two new and non-linear kinetic models, n^{th} order rate equation (nOE) and CDT, were therefore synthesised from these substantial experimental data (n_T = 708) to better predict the effect of combined T-pH on thermal survival kinetics. Both models involve a time dependent rate for thermal inactivation. This contrasts sharply with the time independent form of the log-linear kinetic model.

   The nOE model has two terms (1 / T and pH) and explained an overall 89.5%V, compared with 71.5%V for the classical log-linear model. Although nOE permits a practical procedure for the evaluation of the microbiological safety of liquid foods processed at mild T-pH, a major drawback is that it is of a form that is not readily

integrated with equations that characterise liquid rheology and hydrodynamics of liquid flow to simulate a steriliser process unit operation.

An advantage of the CDT form is that it can be readily integrated to simulate a steriliser unit operation. This new model explained 94.6%V for the T data, and between 82..9%V and 93.0%V for combined T-pH data. This compares with, respectively, 88.5%V and 74.0%V and 81.3%V for the log-linear form.

5. Outside a range of between 2-$\log_{10}$ to 3.7-$\log_{10}$ reduction in the number of viable bacterial cells however, the CDT model consistently under-estimated the extent of thermal inactivation. A detailed and careful analysis suggests that these under-estimates are primarily dependent on the effect of axial dispersion within the liquid flow in the continuous steriliser. The effect of axial dispersion was highlighted through visual and dye trace studies. It was shown to be more pronounced with longer holding tube lengths and corresponding holding times. A contributing factor also, to differences in predicted and observed sterilisation values, the magnitude which is difficult to ascertain, is that bench-scale (*static*) data determined from glass capillary studies appear limited in application to sizing and design of a continuous steriliser (*dynamic*) at equivalent T-pH and t.

   Bench-scale studies are therefore viewed as a necessary design approach but require a more cautious application than implied in the literature.

6. Established performance equations for simulating continuous thermal sterilisation of liquid are inadequate. Whilst these include consideration of thermal inactivation kinetics of bacteria, liquid rheology and hydrodynamic and flow considerations, they do not include a term for dispersion. The effect of dispersion has been quantitatively estimated to give rise to an order of magnitude difference in predicted reductions in the number of viable bacterial cells in a continuous steriliser. Dispersion is therefore seen to be highly significant in thermal sterilisation of liquid.

7.1 RECOMMENDATIONS

1. Studies should be carried out to further develop an accurate process model that includes rigorous, quantitative effects of axial dispersion in the steriliser performance equations.

2. Apparent differences in observed thermal inactivation kinetics of bacteria subjected to heat in glass capillaries (*static*) compared with the continuous steriliser (*dynamic*) at equivalent *T*-pH require further investigation.

APPENDIX A

DEFINITION OF SOME IMPORTANT TERMS USED IN THIS STUDY

LOG-LINEAR

A mathematical model that describes the thermal inactivation of bacteria following a first-order kinetic equation; the rate of the inactivation process is proportional to the number of the viable cells present at a defined time

NON-LINEAR KINETIC MODEL

A mathematical model that describes the survivor curves for thermal inactivation which are non-linear (ie curves that present tailing, shoulder, or are concave downwards)

RATE COEFFICIENT

The rate of reduction in the number of viable cells, measured in $time^{-1}$ to give an expected survivor inactivation

STATIC / DYNAMIC

Static refers to data obtained in capillary tubes of small diameter. In contrast, dynamic refers to data determined in the pilot continuous steriliser

STERILISATION

The process of inactivating completely all forms of microbial life. A working definition is the survival level of a selected micro-organism at the point of minimum treatment. For vegetative bacteria the requirement for thermal sterilisation of the liquid is a survival level of 10^{-4} - 10^{-5}

STERILITY REQUIREMENT

A reduction in the number of viable cells to some specified number. This can be expressed as any of the following: N / N_0; $\log_{10} (N / N_0)$; $\ln (N / N_0)$

THERMAL INACTIVATION

A heating process that implies a loss of viability of the micro-organism, and hence, a reduction in the number of viable cells

VEGETATIVE BACTERIA

A non-spore forming bacterium

VIABLE COUNT

The number of survivor cells in a defined volume (eg cell ml^{-1}). The working definition equates viability "with the power to form a macroscopic colony on nutrient agar". Dilution is used to obtain separate colonies

APPENDIX B

REFEREED PUBLICATIONS FROM THIS THESIS

B.1 INTERNATIONAL REFEREED JOURNALS

Chiruta, J., Davey, K. R. and Thomas, C. J. (1999) A new non-linear kinetic model for survival of bacteria in liquid as affected by combined temperature - pH in continuous sterilisation, *Transactions of the Institution of Chemical Engineers, Part C, Bioproducts and Food Processing (submitted)*

Chiruta, J., Davey, K. R. and Thomas, C. J. (1997b) Thermal inactivation kinetics of three vegetative bacteria as influenced by combined temperature and pH in a liquid medium, *Transactions of the Institution of Chemical Engineers, Part C, Bioproducts and Food Processing*, **75**: 174-180

B.2 INTERNATIONAL REFEREED CONFERENCES

Chiruta, J., Davey, K. R. and Thomas, C. J. (1997a) Combined effect of temperature and pH on microbial death in continuous sterilisation of liquids, *7th International Congress on Engineering and Food*, April, Brighton, UK, A109-A112 (ISBN 85075 814 X)

Chiruta, J., Davey, K. R. and Thomas, C. J. (1997c) An *n*th order reaction model for thermal inactivation of *Listeria monocytogenes* in liquid with combined temperature and pH, *Proceedings of the 3rd International Conference on Modelling and Simulation*, October 29 - 31, Victoria University of Technology, Melbourne, Australia, 343-346 (ISBN 1 86 272 4903)

Chiruta, J., Davey, K. R. and Thomas, C. J. (1996a) Modelling the combined effect of temperature and pH on continuous thermal sterilisation of liquids, *The 2nd International Conference of Predictive Microbiology*, February 18 - 22, Hobart, Australia, Paper 6

B.3 AUSTRALASIAN REFEREED CONFERENCES

Davey, K. R., Thomas, C. J. and Chiruta, J. (1998a) Mathematical evaluation and design for a new time-dependent thermal inactivation of bacteria in liquid as influenced by combined temperature and pH, *14th Australasian Biotechnology Conference*, 19 - 23 April, Adelaide, Australia (*in press*)

Davey, K. R., Thomas, C. J. and Chiruta, J. (1998b) A new non-linear kinetic model for design of thermal inactivation of bacteria in liquid as effected by combined temperature and pH in a continuous steriliser, *26th Australasian Chemical Engineering Conference, CHEMECA '98*, 28 - 30 September, Port Douglas, Queensland, Australia, Paper 68 (ISBN 1 85825 683 5)

Chiruta, J., Davey, K. R. and Thomas, C. J. (1996b) Modelling the combined effect of process temperature and pH in continuous sterilisation of liquids, *24th Australian and New Zealand Chemical Engineering Conference, CHEMECA '96*, September 30 - October 2, Sydney, Australia, **3**: 129-134 (ISBN 0 85825 658 4)

APPENDIX C

SUMMARY OF RAW BENCH-SCALE EXPERIMENTAL DATA AS AFFECTED BY COMBINED *T*-pH

C.1 *P. FLUORESCENS*

Pages 131 to 134 are a summary of the raw bench-scale experimental data for *P. fluorescens* as affected by combined *T*-pH.

No.	T (°C)	pH	time (s)	N / N_0	log (N / N_0)
1	52	5.0	15	0.783	-0.106
2	52	5.0	30	0.744	-0.128
3	52	5.0	45	0.609	-0.215
4	52	5.0	60	0.523	-0.281
5	52	5.0	90	0.464	-0.333
6	52	5.0	120	0.460	-0.337
7	52	5.0	180	0.364	-0.439
8	52	5.0	240	0.338	-0.471
9	52	5.0	300	0.281	-0.551
10	52	5.4	15	0.479	-0.320
11	52	5.4	30	0.695	-0.158
12	52	5.4	45	0.749	-0.126
13	52	5.4	60	0.741	-0.130
14	52	5.4	90	0.681	-0.167
15	52	5.4	120	0.598	-0.223
16	52	5.4	180	0.450	-0.347
17	52	5.4	240	0.342	-0.466
18	52	5.4	300	0.254	-0.595
19	52	6.0	15	0.422	-0.375
20	52	6.0	30	0.453	-0.344
21	52	6.0	45	0.524	-0.281
22	52	6.0	60	0.541	-0.267
23	52	6.0	90	0.615	-0.211
24	52	6.0	120	0.464	-0.333
25	52	6.0	180	0.603	-0.220
26	52	6.0	240	0.570	-0.244
27	52	6.0	300	0.275	-0.561
28	52	6.5	15	0.473	-0.325
29	52	6.5	30	0.749	-0.126
30	52	6.5	45	0.855	-0.068
31	52	6.5	60	0.895	-0.048
32	52	6.5	90	0.895	-0.048
33	52	6.5	120	0.855	-0.068
34	52	6.5	180	0.744	-0.128
35	52	6.5	240	0.638	-0.195
36	52	6.5	300	0.547	-0.262
37	54	5.0	15	0.689	-0.162
38	54	5.0	30	0.666	-0.177

No.	T (°C)	pH	time (s)	N / N_0	log (N / N_0)
39	54	5.0	45	0.593	-0.227
40	54	5.0	60	0.584	-0.234
41	54	5.0	90	0.587	-0.231
42	54	5.0	120	0.479	-0.320
43	54	5.0	180	0.291	-0.536
44	54	5.0	240	0.251	-0.600
45	54	5.0	300	0.172	-0.764
46	54	5.4	15	0.725	-0.140
47	54	5.4	30	0.860	-0.066
48	54	5.4	45	0.845	-0.073
49	54	5.4	60	0.613	-0.213
50	54	5.4	90	0.618	-0.209
51	54	5.4	120	0.551	-0.259
52	54	5.4	180	0.536	-0.271
53	54	5.4	240	0.254	-0.595
54	54	5.4	300	0.188	-0.726
55	54	6.0	15	0.819	-0.087
56	54	6.0	30	0.735	-0.134
57	54	6.0	45	0.660	-0.180
58	54	6.0	60	0.689	-0.162
59	54	6.0	90	0.602	-0.220
60	54	6.0	120	0.552	-0.258
61	54	6.0	180	0.333	-0.478
62	54	6.0	240	0.198	-0.703
63	54	6.0	300	0.243	-0.614
64	54	6.5	15	0.905	-0.043
65	54	6.5	30	0.844	-0.074
66	54	6.5	45	0.782	-0.107
67	54	6.5	60	0.736	-0.133
68	54	6.5	90	0.691	-0.161
69	54	6.5	120	0.650	-0.187
70	54	6.5	180	0.535	-0.272
71	54	6.5	240	0.35	-0.456
72	54	6.5	300	0.243	-0.614
73	56	5.0	15	0.766	-0.116
74	56	5.0	30	0.602	-0.220
75	56	5.0	45	0.593	-0.227
76	56	5.0	60	0.352	-0.453
77	56	5.0	90	0.371	-0.431
78	56	5.0	120	0.308	-0.511
79	56	5.0	180	0.120	-0.921
80	56	5.0	240	0.007	-2.141
81	56	5.0	300	4.8E-06	-5.317
82	56	5.4	15	0.643	-0.192
83	56	5.4	30	0.628	-0.202

No.	T (°C)	pH	time (s)	N / N_0	log (N / N_0)
84	56	5.4	45	0.618	-0.209
85	56	5.4	60	0.589	-0.230
86	56	5.4	90	0.464	-0.333
87	56	5.4	120	0.626	-0.203
88	56	5.4	180	0.367	-0.435
89	56	5.4	240	0.014	-1.870
90	56	5.4	300	1.2E-05	-4.921
91	56	6.0	15	0.723	-0.141
92	56	6.0	30	0.670	-0.174
93	56	6.0	45	0.617	-0.210
94	56	6.0	60	0.607	-0.217
95	56	6.0	90	0.501	-0.300
96	56	6.0	120	0.386	-0.413
97	56	6.0	180	0.202	-0.695
98	56	6.0	240	0.027	-1.577
99	56	6.0	300	0.001	-2.889
100	56	6.5	15	0.844	-0.074
101	56	6.5	30	0.802	-0.096
102	56	6.5	45	0.708	-0.150
103	56	6.5	60	0.695	-0.158
104	56	6.5	90	0.514	-0.289
105	56	6.5	120	0.305	-0.516
106	56	6.5	180	0.050	-1.299
107	56	6.5	240	0.063	-1.203
108	56	6.5	300	0.038	-1.426
109	58	5.0	15	0.541	-0.267
110	58	5.0	30	0.442	-0.355
111	58	5.0	45	0.353	-0.452
112	58	5.0	60	0.296	-0.529
113	58	5.0	90	0.081	-1.094
114	58	5.0	120	8.6E-04	-3.068
115	58	5.0	180	9.6E-05	-4.016
116	58	5.0	240	7.1E-05	-4.148
117	58	5.0	300	2.4E-06	-5.625
118	58	5.4	15	0.575	-0.240
119	58	5.4	30	0.600	-0.222
120	58	5.4	45	0.365	-0.438
121	58	5.4	60	0.309	-0.510
122	58	5.4	90	0.166	-0.780
123	58	5.4	120	0.058	-1.237
124	58	5.4	180	0.002	-2.924
125	58	5.4	240	4.1E-05	-4.386
126	58	5.4	300	8.7E-05	-4.060

P. fluorescens continued ...

No.	T (°C)	pH	time (s)	N / N_0	log (N / N_0)
127	58	6.0	15	0.629	-0.201
128	58	6.0	30	0.501	-0.300
129	58	6.0	45	0.361	-0.442
130	58	6.0	60	0.304	-0.517
131	58	6.0	90	0.294	-0.532
132	58	6.0	120	0.002	-2.674
133	58	6.0	180	4.8E-05	-4.317
134	58	6.0	240	7.7E-07	-6.113
135	58	6.0	300	4.8E-07	-6.317
136	58	6.5	15	0.817	-0.088
137	58	6.5	30	0.65	-0.187
138	58	6.5	45	0.454	-0.343
139	58	6.5	60	0.382	-0.418
140	58	6.5	90	0.147	-0.833
141	58	6.5	120	0.144	-0.842
142	58	6.5	180	0.015	-1.824
143	58	6.5	240	4.2E-04	-3.379
144	58	6.5	300	2.6E-05	-4.583
145	60	5.0	15	0.518	-0.286
146	60	5.0	30	0.279	-0.554
147	60	5.0	45	0.115	-0.939
148	60	5.0	60	0.041	-1.385
149	60	5.0	90	0.004	-2.421
150	60	5.0	120	2.3E-04	-3.646
151	60	5.0	180	2.2E-07	-6.664
152	60	5.4	15	0.405	-0.393
153	60	5.4	30	0.373	-0.428
154	60	5.4	45	0.155	-0.810
155	60	5.4	60	0.068	-1.171
156	60	5.4	90	0.019	-1.733
157	60	5.4	120	9.8E-04	-3.011
158	60	5.4	180	1.2E-05	-4.939
159	60	6.0	15	0.696	-0.157
160	60	6.0	30	0.393	-0.406
161	60	6.0	45	0.170	-0.770
162	60	6.0	60	0.064	-1.194
163	60	6.0	90	6.2E-03	-2.205
164	60	6.0	120	3.9E-04	-3.413
165	60	6.0	180	3.5E-07	-6.460
166	60	6.5	15	0.583	-0.234
167	60	6.5	30	0.425	-0.372
168	60	6.5	45	0.225	-0.648
169	60	6.5	60	0.070	-1.155
170	60	6.5	90	9E-03	-2.022
171	60	6.5	120	9E-05	-4.046
172	60	6.5	180	8E-05	-4.097

C.2 *L. MONOCYTOGENES*

Pages 135 to 141 are a summary of the raw bench-scale experimental data for *L. monocytogenes* as affected by combined *T*-pH.

No.	T (°C)	pH	time (s)	N / N_0	log (N / N_0)
1	52	7.5	10	1.232	0.091
2	52	7.5	20	1.007	0.003
3	52	7.5	30	0.811	-0.091
4	52	7.5	60	0.591	-0.229
5	52	7.5	120	0.940	-0.027
6	52	7.5	180	0.979	-0.009
7	52	7.5	240	1.074	0.031
8	52	7.0	10	0.944	-0.025
9	52	7.0	20	0.951	-0.022
10	52	7.0	30	0.926	-0.034
11	52	7.0	60	1.017	0.007
12	52	7.0	120	0.757	-0.121
13	52	7.0	180	0.731	-0.136
14	52	7.0	240	0.718	-0.144
15	52	6.5	10	1.085	0.035
16	52	6.5	20	1.284	0.108
17	52	6.5	30	1.087	0.036
18	52	6.5	60	0.749	-0.126
19	52	6.5	120	1.079	0.033
20	52	6.5	180	0.942	-0.026
21	52	6.5	240	0.674	-0.171
22	52	6.0	10	1.078	0.032
23	52	6.0	20	1.095	0.039
24	52	6.0	30	1.107	0.044
25	52	6.0	60	0.972	-0.013
26	52	6.0	120	0.820	-0.086
27	52	6.0	180	0.822	-0.085
28	52	6.0	240	0.734	-0.134
29	52	5.5	10	0.940	-0.027
30	52	5.5	20	0.806	-0.094
31	52	5.5	30	0.801	-0.096
32	52	5.5	60	0.790	-0.103
33	52	5.5	120	0.772	-0.113
34	52	5.5	180	0.73	-0.137
35	52	5.5	240	0.452	-0.345
36	52	5.0	10	0.797	-0.099
37	52	5.0	20	0.748	-0.126
38	52	5.0	30	0.637	-0.196
39	52	5.0	60	0.682	-0.167
40	52	5.0	120	0.61	-0.215
41	52	5.0	180	0.573	-0.242
42	52	5.0	240	0.729	-0.137

No.	T (°C)	pH	time (s)	N / N_0	log (N / N_0)
43	52	4.5	10	0.900	-0.046
44	52	4.5	20	0.886	-0.053
45	52	4.5	30	0.714	-0.147
46	52	4.5	60	0.614	-0.212
47	52	4.5	120	0.414	-0.383
48	52	4.5	180	0.492	-0.308
49	52	4.5	240	0.343	-0.465
50	52	4.0	10	0.996	-0.002
51	52	4.0	20	0.659	-0.181
52	52	4.0	3C	0.508	-0.294
53	52	4.0	60	0.627	-0.203
54	52	4.0	120	0.217	-0.664
55	52	4.0	180	0.124	-0.908
56	52	4.0	240	0.097	-1.013
57	54	7.5	10	0.955	-0.020
58	54	7.5	20	0.857	-0.067
59	54	7.5	30	0.846	-0.073
60	54	7.5	60	0.788	-0.104
61	54	7.5	120	0.619	-0.209
62	54	7.5	180	0.539	-0.268
63	54	7.5	240	0.460	-0.338
64	54	7.0	10	0.962	-0.017
65	54	7.0	20	0.924	-0.034
66	54	7.0	30	0.848	-0.072
67	54	7.0	60	0.864	-0.064
68	54	7.0	120	0.831	-0.08
69	54	7.0	180	0.649	-0.188
70	54	7.0	240	0.639	-0.195
71	54	6.5	10	0.892	-0.05
72	54	6.5	20	0.899	-0.046
73	54	6.5	30	0.728	-0.138
74	54	6.5	60	0.606	-0.218
75	54	6.5	120	0.602	-0.220
76	54	6.5	180	0.270	-0.569
77	54	6.5	240	0.117	-0.934
78	54	6.0	10	0.696	-0.158
79	54	6.0	20	0.828	-0.082
80	54	6.0	30	0.82	-0.086
81	54	6.0	60	0.728	-0.138
82	54	6.0	120	0.771	-0.113
83	54	6.0	180	0.586	-0.232
84	54	6.0	240	0.354	-0.452
85	54	5.5	10	0.797	-0.099
86	54	5.5	20	0.764	-0.117
87	54	5.5	30	0.764	-0.117
88	54	5.5	60	0.679	-0.168
89	54	5.5	120	0.257	-0.591
90	54	5.5	180	0.067	-1.174
91	54	5.5	240	0.034	-1.475

No.	T (°C)	pH	time (s)	N / N_0	log (N / N_0)
92	54	5.0	10	0.624	-0.205
93	54	5.0	20	0.561	-0.251
94	54	5.0	30	0.526	-0.279
95	54	5.0	60	0.263	-0.581
96	54	5.0	120	0.120	-0.921
97	54	5.0	180	0.080	-1.096
98	54	5.0	240	0.009	-2.071
99	54	4.5	10	0.915	-0.039
100	54	4.5	20	0.770	-0.114
101	54	4.5	30	0.731	-0.136
102	54	4.5	60	0.419	-0.378
103	54	4.5	120	0.163	-0.788
104	54	4.5	180	0.003	-2.505
105	54	4.5	240	5E-04	-3.312
106	54	4.0	10	0.525	-0.28
107	54	4.0	20	0.187	-0.729
108	54	4.0	30	0.197	-0.707
109	54	4.0	60	0.146	-0.837
110	54	4.0	120	0.003	-2.519
111	54	4.0	180	7E-04	-3.137
112	54	4.0	240	1E-04	-3.837
113	56	7.5	10	0.737	-0.133
114	56	7.5	20	0.574	-0.241
115	56	7.5	30	0.435	-0.362
116	56	7.5	60	0.395	-0.403
117	56	7.5	120	0.122	-0.915
118	56	7.5	180	0.117	-0.931
119	56	7.5	240	0.099	-1.004
120	56	7.0	10	0.897	-0.047
121	56	7.0	20	0.711	-0.148
122	56	7.0	30	0.681	-0.167
123	56	7.0	60	0.565	-0.248
124	56	7.0	120	0.395	-0.403
125	56	7.0	180	0.355	-0.450
126	56	7.0	240	0.119	-0.924
127	56	6.5	10	0.849	-0.071
128	56	6.5	20	0.787	-0.104
129	56	6.5	30	0.863	-0.064
130	56	6.5	60	0.599	-0.223
131	56	6.5	120	0.350	-0.456
132	56	6.5	180	0.329	-0.483
133	56	6.5	240	0.286	-0.544
134	56	6.0	10	1.103	0.043
135	56	6.0	20	0.899	-0.046
136	56	6.0	30	0.847	-0.072
137	56	6.0	60	0.770	-0.114
138	56	6.0	120	0.704	-0.153
139	56	5.5	10	0.180	-0.745
140	56	5.5	20	0.086	-1.065
141	56	5.5	30	0.070	-1.154

No.	T (°C)	pH	time (s)	N / N_0	log (N / N_0)
142	56	5.5	60	0.050	-1.298
143	56	5.5	120	0.061	-1.212
144	56	5.5	180	0.027	-1.573
145	56	5.0	10	0.512	-0.291
146	56	5.0	20	0.898	-0.047
147	56	5.0	30	0.874	-0.059
148	56	5.0	60	0.656	-0.183
149	56	5.0	120	0.536	-0.271
150	56	5.0	180	0.306	-0.514
151	56	5.0	240	0.101	-0.996
152	56	4.5	10	0.492	-0.308
153	56	4.5	20	0.505	-0.297
154	56	4.5	30	0.184	-0.736
155	56	4.5	60	0.128	-0.893
156	56	4.5	120	0.006	-2.205
157	56	4.5	180	8E-04	-3.073
158	56	4.5	240	2E-04	-3.672
159	56	4.0	10	0.647	-0.189
160	56	4.0	20	0.431	-0.366
161	56	4.0	30	0.259	-0.587
162	56	4.0	60	0.097	-1.013
163	56	4.0	120	8E-05	-4.120
164	56	4.0	180	4E-05	-4.384
165	56	4.0	240	3E-06	-5.597
166	58	7.5	10	0.907	-0.042
167	58	7.5	20	0.614	-0.212
168	58	7.5	30	0.415	-0.382
169	58	7.5	60	0.272	-0.566
170	58	7.5	120	0.152	-0.818
171	58	7.5	180	0.070	-1.157
172	58	7.5	240	0.007	-2.172
173	58	7.0	10	0.912	-0.04
174	58	7.0	20	0.722	-0.141
175	58	7.0	30	0.666	-0.177
176	58	7.0	60	0.464	-0.334
177	58	7.0	120	0.201	-0.697
178	58	7.0	180	0.047	-1.326
179	58	7.0	240	0.016	-1.789
180	58	6.5	10	0.938	-0.028
181	58	6.5	20	0.820	-0.086
182	58	6.5	30	0.834	-0.079
183	58	6.5	60	0.725	-0.14
184	58	6.5	120	0.606	-0.218
185	58	6.5	180	0.149	-0.827
186	58	6.5	240	0.376	-0.425
187	58	6.0	10	0.784	-0.106
188	58	6.0	20	0.797	-0.099
189	58	6.0	30	0.679	-0.168
190	58	6.0	60	0.784	-0.106

No.	T (°C)	pH	time (s)	N / N_0	log (N / N_0)
191	58	6.0	120	0.499	-0.302
192	58	6.0	180	0.372	-0.430
193	58	6.0	240	0.034	-1.463
194	58	5.5	10	0.789	-0.103
195	58	5.5	20	0.732	-0.136
196	58	5.5	30	0.478	-0.321
197	58	5.5	60	0.384	-0.416
198	58	5.5	120	0.243	-0.615
199	58	5.5	180	0.010	-2.020
200	58	5.5	240	0.003	-2.592
201	58	5.0	10	1.071	0.030
202	58	5.0	20	0.873	-0.059
203	58	5.0	30	0.705	-0.152
204	58	5.0	60	0.624	-0.205
205	58	5.0	120	0.267	-0.573
206	58	5.0	180	0.008	-2.089
207	58	5.0	240	5E-05	-4.325
208	58	4.5	10	0.269	-0.571
209	58	4.5	20	0.213	-0.672
210	58	4.5	30	0.038	-1.421
211	58	4.5	60	0.069	-1.158
212	58	4.5	120	0.007	-2.144
213	58	4.5	180	3E-04	-3.530
214	58	4.5	240	3E-06	-5.500
215	58	4.0	10	0.236	-0.628
216	58	4.0	20	0.209	-0.680
217	58	4.0	30	0.209	-0.681
218	58	4.0	60	0.040	-1.399
219	58	4.0	120	9E-05	-4.052
220	58	4.0	180	2E-06	-5.727
221	58	4.0	240	4E-07	-6.427
222	60	7.5	10	0.684	-0.165
223	60	7.5	20	0.490	-0.310
224	60	7.5	30	0.392	-0.407
225	60	7.5	60	0.344	-0.464
226	60	7.5	120	0.010	-1.992
227	60	7.5	180	8E-05	-4.083
228	60	7.5	240	1E-06	-5.921
229	60	7.0	10	0.646	-0.190
230	60	7.0	20	0.531	-0.275
231	60	7.0	30	0.909	-0.041
232	60	7.0	60	0.563	-0.249
233	60	7.0	120	0.14	-0.854
234	60	7.0	180	0.022	-1.652
235	60	7.0	240	1E-03	-3.003
236	60	6.5	10	0.977	-0.010
237	60	6.5	20	0.617	-0.210
238	60	6.5	30	0.589	-0.230
239	60	6.5	60	0.269	-0.570

No.	T (°C)	pH	time (s)	N / N_0	log (N / N_0)
240	60	6.5	120	0.095	-1.020
241	60	6.5	180	0.006	-2.210
242	60	6.5	240	2E-05	-4.693
243	60	6.0	10	0.458	-0.339
244	60	6.0	20	0.077	-1.116
245	60	6.0	30	0.011	-1.971
246	60	6.0	60	0.007	-2.135
247	60	6.0	120	0.001	-2.983
248	60	6.0	180	3.7E-6	-5.433
249	60	5.5	10	0.731	-0.136
250	60	5.5	20	0.425	-0.372
251	60	5.5	30	0.331	-0.48
252	60	5.5	60	0.229	-0.64
253	60	5.5	120	0.006	-2.21
254	60	5.5	180	8E-05	-4.117
255	60	5.5	240	8E-08	-7.097
256	60	5.0	10	0.674	-0.171
257	60	5.0	20	0.667	-0.176
258	60	5.0	30	0.516	-0.287
259	60	5.0	60	0.180	-0.745
260	60	5.0	120	0.007	-2.152
261	60	5.0	180	2E-04	-3.807
262	60	4.5	10	0.458	-0.339
263	60	4.5	20	0.252	-0.599
264	60	4.5	30	0.137	-0.863
265	60	4.5	60	0.014	-1.840
266	60	4.5	120	5E-05	-4.314
267	60	4.5	180	8E-06	-5.107
268	60	4.0	10	0.458	-0.339
269	60	4.0	20	0.503	-0.298
270	60	4.0	30	0.026	-1.59
271	60	4.0	60	0.003	-2.583
272	60	4.0	120	7E-08	-7.187
273	62	7.5	10	0.667	-0.176
274	62	7.5	20	0.578	-0.238
275	62	7.5	30	0.336	-0.474
276	62	7.5	60	0.022	-1.660
277	62	7.5	120	1E-08	-7.886
278	62	7.0	10	0.421	-0.376
279	62	7.0	20	0.202	-0.696
280	62	7.0	30	0.004	-2.374
281	62	7.0	60	3E-05	-4.583
282	62	7.0	120	1E-06	-5.884
283	62	6.5	10	0.801	-0.096
284	62	6.5	20	0.572	-0.243
285	62	6.5	30	0.267	-0.573
286	62	6.5	60	0.031	-1.503
287	62	6.5	120	2E-07	-6.642

No.	T (°C)	pH	time (s)	N / N_0	log (N / N_0)
288	62	6.0	10	0.126	-0.899
289	62	6.0	20	0.123	-0.910
290	62	6.0	30	0.036	-1.438
291	62	6.0	60	8E-04	-3.104
292	62	5.5	10	0.529	-0.277
293	62	5.5	20	0.424	-0.373
294	62	5.5	30	0.141	-0.851
295	62	5.5	120	2E-06	-5.733
296	62	5.5	180	3E-08	-7.538
297	62	5.0	10	0.081	-1.090
298	62	5.0	20	0.023	-1.636
299	62	5.0	30	5E-04	-3.332
300	62	5.0	60	2E-06	-5.606
301	62	5.0	120	2E-06	-5.783
302	62	5.0	180	1E-06	-5.959
303	62	5.0	240	8E-07	-6.083
304	62	4.5	10	0.466	-0.332
305	62	4.5	20	0.174	-0.759
306	62	4.5	30	0.078	-1.110
307	62	4.5	120	3E-07	-6.561
308	62	4.0	10	0.073	-1.138
309	62	4.0	20	0.011	-1.965
310	62	4.0	30	0.001	-2.915

C.3 *E. COLI* (as reported by Davey, Hall and Thomas (1995). Davey K. R. *pers. comm.*)

Pages 142 to 146 are a summary of the raw bench-scale experimental data for *E. coli* as affected by combined *T*-pH.

No.	T (°C)	pH	time (s)	N / N_0	log (N / N_0)
1	54	4.0	12	0.262	-0.581
2	54	4.0	24	0.146	-0.837
3	54	4.0	36	0.037	-1.427
4	54	4.0	40	0.003	-2.573
5	54	4.0	49	5E-04	-3.345
6	54	4.0	60	0.004	-2.441
7	54	4.5	12	0.850	-0.071
8	54	4.5	24	0.163	-0.789
9	54	4.5	36	0.085	-1.068
10	54	4.5	40	0.043	-1.363
11	54	4.5	49	0.012	-1.923
12	54	4.5	60	0.019	-1.724
13	54	5.0	12	0.842	-0.075
14	54	5.0	24	0.302	-0.520
15	54	5.0	36	0.246	-0.609
16	54	5.0	40	0.057	-1.241
17	54	5.0	49	0.035	-1.461
18	54	5.0	60	0.082	-1.088
19	54	5.5	12	0.889	-0.051
20	54	5.5	24	0.370	-0.432
21	54	5.5	36	0.412	-0.385
22	54	5.5	40	0.223	-0.651
23	54	5.5	49	0.119	-0.923
24	54	5.5	60	0.227	-0.644
25	54	6.0	12	0.911	-0.041
26	54	6.0	24	0.508	-0.294
27	54	6.0	36	0.666	-0.177
28	54	6.0	40	0.230	-0.638
29	54	6.0	49	0.268	-0.572
30	54	6.0	60	0.259	-0.587
31	54	6.5	12	0.953	-0.021
32	54	6.5	24	0.545	-0.263
33	54	6.5	36	0.717	-0.145
34	54	6.5	40	0.353	-0.452
35	54	6.5	49	0.258	-0.588
36	54	6.5	60	0.583	-0.234
37	54	7.0	12	0.687	-0.163
38	54	7.0	24	0.503	-0.298
39	54	7.0	36	0.508	-0.294
40	54	7.0	40	0.166	-0.78
41	54	7.0	49	0.258	-0.589
42	54	7.0	60	0.258	-0.589

E. coli continued ...

No.	T (°C)	pH	time (s)	N / N_0	log (N / N_0)
43	54	7.5	12	0.621	-0.207
44	54	7.5	24	0.264	-0.578
45	54	7.5	36	0.159	-0.799
46	54	7.5	40	0.126	-0.899
47	54	7.5	49	0.106	-0.974
48	54	7.5	60	0.077	-1.116
49	56	4.0	10	0.004	-2.434
50	56	4.0	20	0.005	-2.28
51	56	4.0	30	0.006	-2.249
52	56	4.0	40	0.002	-2.736
53	56	4.0	50	9E-05	-4.038
54	56	4.5	10	0.042	-1.378
55	56	4.5	20	0.121	-0.919
56	56	4.5	30	9E-04	-3.055
57	56	4.5	40	0.002	-2.811
58	56	4.5	50	3E-04	-3.561
59	56	5.0	10	0.064	-1.194
60	56	5.0	20	0.188	-0.725
61	56	5.0	30	0.001	-2.878
62	56	5.0	40	0.007	-2.134
63	56	5.0	50	6E-04	-3.260
64	56	5.5	10	0.131	-0.881
65	56	5.5	20	0.214	-0.670
66	56	5.5	30	9E-04	-3.031
67	56	5.5	40	0.030	-1.522
68	56	5.5	50	0.003	-2.575
69	56	6.0	10	0.208	-0.682
70	56	6.0	20	0.083	-1.079
71	56	6.0	30	0.002	-2.708
72	56	6.0	40	0.035	-1.457
73	56	6.0	50	0.008	-2.088
74	56	6.5	10	0.166	-0.78
75	56	6.5	20	0.072	-1.145
76	56	6.5	30	0.002	-2.611
77	56	6.5	40	0.031	-1.513
78	56	6.5	50	0.006	-2.221
79	56	7.0	10	0.185	-0.733
80	56	7.0	20	0.021	-1.670
81	56	7.0	30	0.003	-2.585
82	56	7.0	40	0.010	-2.003
83	56	7.0	50	0.004	-2.452
84	56	7.5	10	0.121	-0.918
85	56	7.5	20	0.006	-2.249
86	56	7.5	30	0.003	-2.484
87	56	7.5	40	0.002	-2.615
88	56	7.5	50	2E-04	-3.686
89	58	4.0	8	0.004	-2.436
90	58	4.0	16	4E-05	-4.410
91	58	4.0	24	9E-09	-8.049

No.	T (°C)	pH	time (s)	N / N_0	log (N / N_0)
92	58	4.0	32	2E-08	-7.812
93	58	4.0	40	2E-08	-7.812
94	58	4.5	8	0.014	-1.858
95	58	4.5	16	0.002	-2.807
96	58	4.5	24	9E-07	-6.049
97	58	4.5	32	2E-06	-5.636
98	58	4.5	40	8E-07	-6.110
99	58	5.0	8	0.013	-1.899
100	58	5.0	16	0.002	-2.701
101	58	5.0	24	9E-07	-6.047
102	58	5.0	32	8E-06	-5.114
103	58	5.0	40	4E-06	-5.415
104	58	5.5	8	0.006	-2.254
105	58	5.5	16	0.006	-2.213
106	58	5.5	24	3E-04	-3.553
107	58	5.5	32	1E-05	-5.000
108	58	5.5	40	3E-06	-5.511
109	58	6.0	8	0.005	-2.312
110	58	6.0	16	0.003	-2.533
111	58	6.0	24	5E-06	-5.308
112	58	6.0	32	5E-05	-4.329
113	58	6.0	40	8E-06	-5.073
114	58	6.5	8	0.002	-2.632
115	58	6.5	16	0.001	-2.934
116	58	6.5	24	1E-04	-3.879
117	58	6.5	32	3E-05	-4.480
118	58	6.5	40	2E-08	-7.812
119	58	7.0	8	0.002	-2.618
120	58	7.0	16	4E-04	-3.369
121	58	7.0	24	3E-06	-5.572
122	58	7.0	32	1E-05	-4.967
123	58	7.0	40	7E-06	-5.160
124	58	7.5	8	3E-04	-3.517
125	58	7.5	16	5E-05	-4.277
126	58	7.5	24	2E-05	-4.783
127	58	7.5	32	2E-06	-5.812
128	58	7.5	40	2E-08	-7.812
129	60	4.0	6	8E-04	-3.099
130	60	4.0	12	1E-08	-7.870
131	60	4.0	18	1E-08	-7.870
132	60	4.0	24	1E-08	-7.967
133	60	4.0	30	5E-07	-6.265
134	60	4.5	6	9E-04	-3.031
135	60	4.5	12	2E-06	-5.693
136	60	4.5	18	7E-07	-6.166
137	60	4.5	24	2E-06	-5.793
138	60	4.5	30	5E-07	-6.265
139	60	5.0	6	0.002	-2.618
140	60	5.0	12	3E-06	-5.569

E. coli continued ...

No.	T (°C)	pH	time (s)	N / N_0	log (N / N_0)
141	60	5.0	18	1E-08	-7.870
142	60	5.0	24	1E-08	-7.967
143	60	5.0	30	1E-08	-7.967
144	60	5.5	6	0.007	-2.175
145	60	5.5	12	5E-05	-4.325
146	60	5.5	18	5E-06	-5.267
147	60	5.5	24	1E-08	-7.967
148	60	5.5	30	5E-07	-6.265
149	60	6.0	6	0.003	-2.555
150	60	6.0	12	3E-04	-3.599
151	60	6.0	18	2E-05	-4.754
152	60	6.0	24	2E-06	-5.793
153	60	6.0	30	1E-08	-7.967
154	60	6.5	6	7E-04	-3.161
155	60	6.5	12	2E-05	-4.666
156	60	6.5	18	5E-06	-5.267
157	60	6.5	24	1E-08	-7.967
158	60	6.5	30	1E-08	-7.967
159	60	7.0	6	6E-04	-3.199
160	60	7.0	12	5E-06	-5.325
161	60	7.0	18	9E-06	-5.057
162	60	7.0	24	2E-06	-5.793
163	60	7.0	30	1E-08	-7.967
164	60	7.5	6	2E-05	-4.668
165	60	7.5	12	7E-07	-6.166
166	60	7.5	18	1E-08	-7.870
167	60	7.5	24	5E-07	-6.265
168	60	7.5	30	1E-08	-7.967
169	62	4.0	4	4E-04	-3.367
170	62	4.0	8	3E-06	-5.504
171	62	4.0	12	1E-08	-7.903
172	62	4.0	16	1E-08	-7.903
173	62	4.0	20	8E-09	-8.111
174	62	4.5	4	0.002	-2.646
175	62	4.5	8	4E-06	-5.359
176	62	4.5	12	1E-08	-7.903
177	62	4.5	16	1E-08	-7.903
178	62	4.5	20	8E-09	-8.111
179	62	5.0	4	0.011	-1.975
180	62	5.0	8	3E-06	-5.504
181	62	5.0	12	1E-08	-7.903
182	62	5.0	16	1E-08	-7.903
183	62	5.0	20	8E-09	-8.111
184	62	5.5	4	0.022	-1.667
185	62	5.5	8	2E-06	-5.726
186	62	5.5	12	1E-08	-7.903
187	62	5.5	16	1E-08	-7.903
188	62	5.5	20	8E-09	-8.111

E. coli continued ...

No.	T (°C)	pH	time (s)	N / N_0	log (N / N_0)
189	62	6.0	4	0.006	-2.232
190	62	6.0	8	1E-05	-4.975
191	62	6.0	12	1E-08	-7.903
192	62	6.0	16	1E-08	-7.903
193	62	6.0	20	8E-09	-8.111
194	62	6.5	4	2E-04	-3.726
195	62	6.5	8	7E-06	-5.125
196	62	6.5	12	1E-08	-7.903
197	62	6.5	16	1E-08	-7.903
198	62	6.5	20	8E-09	-8.111
199	62	7.0	4	1E-08	-7.967
200	62	7.0	8	4E-06	-5.426
201	62	7.0	12	6E-07	-6.200
202	62	7.0	16	1E-08	-7.903
203	62	7.0	20	8E-09	-8.111
204	62	7.5	4	5E-05	-4.269
205	62	7.5	8	1E-08	-7.903
206	62	7.5	12	1E-08	-7.903
207	62	7.5	16	2E-06	-5.726
208	62	7.5	20	8E-09	-8.111

APPENDIX D

SUMMARY OF LINEAR REGRESSION ANALYSIS FOR LINEAR-ARRHENIUS-DAVEY EQUATION TO BENCH-SCALE DATA AS AFFECTED BY COMBINED *T*-pH

D.1 INTRODUCTION

In this Appendix linear regression analyses are presented for all three bacteria studied as affected by combined *T*-pH.

D.2 LINEAR REGRESSION ANALYSES FOR *P. FLUORESCENS*

Regression Statistics for Pseudomonas fluorescens (eq. 4.1)	
Multiple R	0.975624
R Square	0.951843
Adjusted R Square	**0.943**
Standard Error	0.278142
Observations	20

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	3	24.46573	8.155243	105.4153	9.45E-11
Residual	16	1.237808	0.077363		
Total	19	25.70354			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	116.1818	11.2822	10.2978	1.82E-08	92.26465	140.099	92.26465	140.099
pH	2.557121	3.03445	0.842697	0.411819	-3.87563	8.989867	-3.87563	8.989867
pH2	-0.24988	0.263599	-0.94796	0.357251	-0.80869	0.308925	-0.80869	0.308925
1/T	-41684.9	2379.757	-17.5164	7.31E-12	-46729.7	-36640	-46729.7	-36640

D.3 LINEAR REGRESSION ANALYSES FOR *L.MONOCYTOGENES*

Regression Statistics for Listeria monocytogenes (eq. 4.2)	
Multiple R	0.931046
R Square	0.866846
Adjusted R Square	**0.858**
Standard Error	0.585495
Observations	48

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	3	98.19449	32.7315	95.48148	2.74E-19
Residual	44	15.0834	0.342805		
Total	47	113.2779			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	129.7457	8.504108	15.25683	3.49E-19	112.6068	146.8846	112.6068	146.8846
1/T	-41160.2	2693.764	-15.2798	3.3E-19	-46589.1	-35731.2	-46589.1	-35731.2
pH	-2.72894	0.851504	-3.20485	0.002516	-4.44503	-1.01285	-4.44503	-1.01285
pH2	0.19376	0.073765	2.626704	0.01182	0.045096	0.342425	0.045096	0.342425

D.4 LINEAR REGRESSION ANALYSES FOR *E. COLI*

Regression Statistics for Escherichia coli (eq. 4.3)	
Multiple R	0.963663
R Square	0.928646
Adjusted R Square	**0.923**
Standard Error	0.342435
Observations	40

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	3	54.93988	18.31329	156.1748	1.08E-20
Residual	36	4.221415	0.117262		
Total	39	59.1613			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	138.781	6.516899	21.29557	5.42E-22	125.5642	151.9979	125.5642	151.9979
1/T	-44866.4	2096.977	-21.3958	4.64E-22	-49119.3	-40613.6	-49119.3	-40613.6
pH	-1.52329	0.545546	-2.79222	0.008328	-2.62971	-0.41687	-2.62971	-0.41687
pH2	0.124435	0.04726	2.632951	0.012393	0.028586	0.220283	0.028586	0.220283

APPENDIX E

SUMMARY OF LINEAR REGRESSION ANALYSES FOR LOG-LINEAR, *n*OE AND CDT MODELS TO BENCH-SCALE DATA AS AFFECTED BY *T* AND COMBINED *T*-pH

E.1 INTRODUCTION

This Appendix presents linear regression analyses for log-linear forms, CDT model and *n*OE model, for data *T* and *T*-pH dependent, for all three bacteria studied.

E.2 LINEAR REGRESSION ANALYSES FOR LOG-LINEAR AND CDT MODELS TO CONTINUOUS SURVIVOR DATA FOR *E. COLI* AS AFFECTED BY *T* (as reported by Davey and Wood (1984) K R Davey ***pers. comm.***)

Regression Statistics for Log-linear 1 model, T dependent	
Multiple R	0.9460862
R Square	0.895079
Adjusted R Square	**0.881**
Standard Error	0.3806512
Observations	18

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	2	18.541498	9.2707488	63.982379	4.534E-08
Residual	15	2.1734302	0.1448953		
Total	17	20.714928			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	24.996288	2.6688495	9.3659412	1.175E-07	19.307766	30.684809	19.307766	30.684809
1/T	-1698.5285	161.84344	-10.494886	2.637E-08	-2043.4898	-1353.5671	-2043.4898	-1353.5671
lnt	2.0659674	0.2333009	8.8553774	2.412E-07	1.568698	2.5632368	1.568698	2.5632368

Regression Statistics for Log-linear 2 model, T dependent	
Multiple R	0.954348
R Square	0.9107801
Adjusted R Square	**0.890**
Standard Error	0.3595308
Observations	17

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	3	17.154064	5.7180213	44.235776	4.398E-07
Residual	13	1.6804108	0.1292624		
Total	16	18.834475			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	1120.5115	1020.5395	1.09796	0.2921422	-1084.2296	3325.2526	-1084.2296	3325.2526
1/T	-13891.101	11264.166	-1.2332117	0.2393311	-38225.847	10443.645	-38225.847	10443.645
lnt	2.1314687	0.2362403	9.0224622	5.854E-07	1.6211028	2.6418347	1.6211028	2.6418347
ln1/T	218.06578	203.50184	1.0715667	0.3034056	-221.57312	657.70468	-221.57312	657.70468

Regression Statistics for CDT 1 model, T dependent	
Multiple R	0.9764083
R Square	0.9533732
Adjusted R Square	**0.943**
Standard Error	0.1190062
Observations	18

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	3	4.054098	1.351366	95.418828	1.474E-09
Residual	14	0.1982745	0.0141625		
Total	17	4.2523725			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-40.092616	32.528183	-1.2325501	0.2380419	-109.85869	29.67346	-109.85869	29.67346
1/T	5039.2611	3611.1633	1.3954675	0.1846162	-2705.9207	12784.443	-2705.9207	12784.443
1/T2	-161390.86	100026.84	-1.6134756	0.1289474	-375927.29	53145.562	-375927.29	53145.562
lnt	0.9559735	0.07352	13.00291	3.316E-09	0.7982887	1.1136583	0.7982887	1.1136583

Regression Statistics for CDT 2 model, T dependent	
Multiple R	0.9782236
R Square	0.9569215
Adjusted R Square	**0.944**
Standard Error	0.1187065
Observations	18

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	4	4.0691866	1.0172967	72.19364	9.577E-09
Residual	13	0.1831859	0.0140912		
Total	17	4.2523725			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	2.3185103	1.5817379	1.4657993	0.1664694	-1.098626	5.7356467	-1.098626	5.7356467
lnt	-3.7480742	11.223853	-0.3339383	0.7437516	-27.995729	20.499581	-27.995729	20.499581
lnt/T	480.62838	1222.8813	0.3930294	0.700667	-2161.2456	3122.5024	-2161.2456	3122.5024
lnt/T2	-20866.327	33790.339	-0.6175235	0.5475545	-93865.902	52133.248	-93865.902	52133.248
(lnt)2	0.4835537	0.1787495	2.705203	0.0180143	0.0973889	0.8697185	0.0973889	0.8697185

Regression Statistics for CDT model, T dependent	
Multiple R	0.9790413
R Square	0.9585219
Adjusted R Square	**0.946**
Standard Error	0.1164805
Observations	18

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	4	4.0759924	1.0189981	75.104694	7.499E-09
Residual	13	0.1763801	0.0135677		
Total	17	4.2523725			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	1.4554446	1.7301821	0.8412089	0.4154368	-2.2823859	5.1932752	-2.2823859	5.1932752
lnt	5.8655971	3.0863078	1.9005224	0.0797679	-0.8019642	12.533158	-0.8019642	12.533158
lnt/T	-396.02788	129.54953	-3.0569611	0.0091762	-675.90258	-116.15319	-675.90258	-116.15319
(lnt)2	-0.3749088	0.9118415	-0.4111557	0.6876556	-2.3448222	1.5950046	-2.3448222	1.5950046
(lnt)2/T	41.860578	44.182278	0.9474518	0.3606887	-53.589411	137.31057	-53.589411	137.31057

E.3 LINEAR REGRESSION ANALYSES FOR *n*OE MODEL TO BENCH-SCALE SURVIVOR DATA FOR *L. MONOCYTOGENES* AS AFFECTED BY COMBINED *T*-pH

Regression Statistics for nOE, T-pH dependent (without 52°C)	
Multiple R	0.946554
R Square	0.895964
Adjusted R Square	**0.895**
Standard Error	0.985024
Observations	236

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	3	1938.598	646.1993	665.9978	1.1E-113
Residual	232	225.1032	0.970272		
Total	235	2163.701			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	69.72363	7.956745	8.762834	4.09E-16	54.04691	85.40035	54.04691	85.40035
1/T	-24294.4	2656.321	-9.14588	3.1E-17	-29528	-19060.8	-29528	-19060.8
pH	-0.3377	0.058206	-5.80186	2.14E-08	-0.45238	-0.22302	-0.45238	-0.22302
n	1.074025	0.024467	43.8965	2.3E-114	1.025818	1.122231	1.025818	1.122231

E.4 LINEAR REGRESSION ANALYSES FOR LOG-LINEAR AND CDT MODELS TO BENCH-SCALE SURVIVOR DATA FOR *P. FLUORESCENS* AS AFFECTED BY COMBINED *T*-pH

Regression Statistics for Log-linear model, T-pH dependent	
Multiple R	0.87409
R Square	0.764034
Adjusted R Square	**0.758**
Standard Error	0.59069
Observations	172

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	4	188.6685	47.16713	135.1822	2.78E-51
Residual	167	58.26883	0.348915		
Total	171	246.9374			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	5.481538	6.337308	0.864963	0.3883	-7.03003	17.99311	-7.03003	17.99311
1/T	-838.21	50.99861	-16.4359	9.95E-37	-938.895	-737.525	-938.895	-737.525
pH	2.119928	2.197476	0.964711	0.336085	-2.21849	6.458343	-2.21849	6.458343
pH2	-0.21171	0.190892	-1.10907	0.268996	-0.58859	0.165161	-0.58859	0.165161

Regression Statistics for CDT1 model, T-pH dependent	
Multiple R	0.804715
R Square	0.647566
Adjusted R Square	**0.639**
Standard Error	0.313413
Observations	172

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	4	30.14098	7.535245	76.712	8.35E-37
Residual	167	16.40403	0.098228		
Total	171	46.54501			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	4.688779	3.362497	1.394434	0.165039	-1.9497	11.32726	-1.9497	11.32726
1/T	-341.819	27.05923	-12.6323	4.19E-26	-395.242	-288.397	-395.242	-288.397
pH	0.265667	1.165953	0.227854	0.820038	-2.03624	2.567576	-2.03624	2.567576
pH2	-0.03044	0.101285	-0.30057	0.764119	-0.23041	0.169521	-0.23041	0.169521
lnt	0.337213	0.025913	13.01349	3.52E-27	0.286055	0.388372	0.286055	0.388372

Regression Statistics for CDT2 model, T-pH dependent	
Multiple R	0.875998
R Square	0.767373
Adjusted R Square	**0.756**
Standard Error	0.254629
Observations	172

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	5	35.71739	7.143477	110.1775	7.28E-51
Residual	167	10.82762	0.064836		
Total	172	46.54501			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	0	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A
lnt	1.356301	0.265977	5.099314	9.17E-07	0.83119	1.881412	0.83119	1.881412
lnt/T	-89.1242	5.028908	-17.7224	3.26E-40	-99.0526	-79.1958	-99.0526	-79.1958
pHlnt	0.016101	0.043636	0.368978	0.712611	-0.07005	0.102249	-0.07005	0.102249
(pHlnt)2	-0.00067	0.000791	-0.8525	0.395158	-0.00224	0.000888	-0.00224	0.000888
lnt2	0.082754	0.026733	3.095611	0.002304	0.029976	0.135531	0.029976	0.135531

Regression Statistics for CDT model, T-pH dependent	
Multiple R	0.965832
R Square	0.932831
Adjusted R Square	**0.930**
Standard Error	0.137651
Observations	172

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	6	43.41864	7.23644	381.9168	5.36E-94
Residual	165	3.126368	0.018948		
Total	171	46.54501			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	1.8432	0.222774	8.27384	4.15E-14	1.403344	2.283056	1.403344	2.283056
lnt	9.774723	1.153358	8.47501	1.25E-14	7.497481	12.05197	7.497481	12.05197
lnt/T	-1388.73	128.8412	-10.7786	7.62E-21	-1643.12	-1134.34	-1643.12	-1134.34
lnt/T2	43597.77	3632.022	12.00372	2.92E-24	36426.55	50769	36426.55	50769
(lnt)2	1.250137	0.06463	19.3431	2.8E-44	1.12253	1.377745	1.12253	1.377745
(lnt)2/T	-58.7135	3.429668	-17.1193	2.01E-38	-65.4851	-51.9418	-65.4851	-51.9418
pH(lnt)2	-0.00443	0.000861	-5.14912	7.38E-07	-0.00613	-0.00273	-0.00613	-0.00273

E.5 LINEAR REGRESSION ANALYSES FOR LOG-LINEAR AND CDT MODELS TO BENCH-SCALE SURVIVOR DATA FOR *L. MONOCYTOGENES* AS AFFECTED BY COMBINED *T*-pH

Regression Statistics for Log-linear model, T-pH dependent	
Multiple R	0.861783
R Square	0.74267
Adjusted R Square	**0.739**
Standard Error	0.773553
Observations	310

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	4	504.2768	126.0692	210.6828	9.35E-85
Residual	305	174.7281	0.598384		
Total	309	679.0048			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	20.88214	1.535262	13.60168	5.67E-33	17.86056	23.90372	17.86056	23.90372
1/T	-932.38	45.04359	-20.6995	3.27E-59	-1021.03	-843.729	-1021.03	-843.729
pH	-2.64883	0.458246	-5.78036	1.91E-08	-3.55071	-1.74694	-3.55071	-1.74694
pH2	0.189987	0.03962	4.79524	2.59E-06	0.11201	0.267964	0.11201	0.267964

Regression Statistics for CDT1 model, T-pH dependent	
Multiple R	0.818194
R Square	0.669442
Adjusted R Square	**0.665**
Standard Error	0.329196
Observations	310

ANOVA

	df	SS	MS	F	Significance F
Regression	4	66.93801	16.7345	154.4202	5E-72
Residual	305	33.05281	0.10837		
Total	309	99.99082			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	7.984088	0.632842	12.61625	1.19E-29	6.738799	9.229377	6.738799	9.229377
1/T	-311.319	18.23295	-17.0745	2.42E-46	-347.197	-275.441	-347.197	-275.441
pH	-0.95854	0.190691	-5.02667	8.52E-07	-1.33377	-0.5833	-1.33377	-0.5833
pH2	0.070038	0.016468	4.252883	2.81E-05	0.037632	0.102444	0.037632	0.102444
lnt	0.298312	0.017319	17.22454	6.51E-47	0.264232	0.332391	0.264232	0.332391

Regression Statistics for CDT2 model, T-pH dependent	
Multiple R	0.880616
R Square	0.775485
Adjusted R Square	**0.772**
Standard Error	0.271748
Observations	310

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	5	77.54138	15.50828	210.006	2.4E-96
Residual	304	22.44943	0.073847		
Total	309	99.99082			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	0.325934	0.238555	1.366285	0.172859	-0.14349	0.795363	-0.14349	0.795363
lnt	2.213027	0.180844	12.2372	2.88E-28	1.857162	2.568891	1.857162	2.568891
lnt/T	-87.5294	3.764108	-23.2537	1.96E-69	-94.9364	-80.1224	-94.9364	-80.1224
pHlnt	-0.28044	0.038778	-7.232	3.88E-12	-0.35675	-0.20413	-0.35675	-0.20413
pH2lnt	0.020751	0.003344	6.205471	1.78E-09	0.01417	0.027331	0.01417	0.027331
lnt2	0.06937	0.016453	4.216208	3.28E-05	0.036994	0.101747	0.036994	0.101747

Regression Statistics for CDT model, T-pH dependent	
Multiple R	0.912368
R Square	0.832416
Adjusted R Square	**0.829**
Standard Error	0.235555
Observations	310

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	7	83.23397	11.89057	214.2975	4E-113
Residual	302	16.75685	0.055486		
Total	309	99.99082			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	0.506176	0.207672	2.437389	0.015371	0.09751	0.91484	0.09751	0.914843
lnt	7.323082	1.138279	6.433471	4.88E-10	5.083119	9.56304	5.083119	9.563045
lnt/T	-891.765	128.1866	-6.95677	2.16E-11	-1144.02	-639.513	-1144.02	-639.513
lnt/T2	25824.23	3654.046	7.067297	1.1E-11	18633.61	33014.84	18633.61	33014.84
(lnt)2	0.763473	0.068472	11.15011	2.07E-24	0.62873	0.89822	0.62873	0.898216
(lnt)2/T	-25.8543	3.461394	-7.46934	8.72E-13	-32.6658	-19.0428	-32.6658	-19.0428
pH(lnt)2	-0.06972	0.00738	-9.44719	9.96E-19	-0.08424	-0.05520	-0.08424	-0.0552
(pH)2(lnt)2	0.005234	0.000636	8.232792	5.57E-15	0.003983	0.00648	0.003983	0.006484

E.6 LINEAR REGRESSION ANALYSES FOR LOG-LINEAR AND CDT MODELS TO BENCH-SCALE SURVIVOR DATA FOR *E. COLI* AS AFFECTED BY COMBINED *T*-pH (as reported by Davey, Hall and Thomas (1995) K R Davey *pers. comm.)*

Regression Statistics for Log-linear model, T-pH dependent	
Multiple R	0.903933
R Square	0.817095
Adjusted R Square	**0.813**
Standard Error	0.502684
Observations	208

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	4	229.1559	57.28897	226.7157	1.1E-73
Residual	203	51.29622	0.252691		
Total	207	280.4521			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	29.36035	1.245642	23.57046	5.08E-60	26.9043	31.81641	26.9043	31.81641
1/T	-1444.71	49.48759	-29.1934	1.36E-74	-1542.29	-1347.14	-1542.29	-1347.14
pH	-2.06438	0.351194	-5.87817	1.68E-08	-2.75683	-1.37192	-2.75683	-1.37192
pH2	0.172587	0.030424	5.672768	4.79E-08	0.1126	0.232574	0.1126	0.232574

Regression Statistics for CDT1 model, T-pH dependent	
Multiple R	0.946386
R Square	0.895646
Adjusted R Square	**0.894**
Standard Error	0.213395
Observations	208

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	4	79.33992	19.83498	435.5755	2.2E-98
Residual	203	9.244093	0.045537		
Total	207	88.58401			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	17.80628	0.528789	33.67367	4.95E-85	16.76366	18.8489	16.76366	18.8489
1/T	-850.237	21.00805	-40.4719	3.8E-99	-891.659	-808.815	-891.659	-808.815
pH	-1.07567	0.149086	-7.21508	1.05E-11	-1.36962	-0.78171	-1.36962	-0.78171
pH2	0.090254	0.012915	6.98816	3.92E-11	0.064789	0.115719	0.064789	0.115719
lnt	0.465294	0.026242	17.73062	4.11E-43	0.413551	0.517036	0.413551	0.517036

Regression Statistics for CDT2 model, T-pH dependent	
Multiple R	0.94378
R Square	0.89072
Adjusted R Square	**0.883641**
Standard Error	0.218373
Observations	208

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	5	78.90357	15.78071	330.9235	3.28E-95
Residual	203	9.68044	0.047687		
Total	208	88.58401			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	0	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A
lnt	6.379356	0.178233	35.79214	1.22E-89	6.027929	6.730783	6.027929	6.730783
lnt/T	-278.858	7.318847	-38.1014	1.93E-94	-293.289	-264.427	-293.289	-264.427
pHlnt	-0.35187	0.049638	-7.08866	2.19E-11	-0.44974	-0.254	-0.44974	-0.254
(pHlnt)2	0.029176	0.0043	6.784895	1.25E-10	0.020697	0.037655	0.020697	0.037655
lnt2	-0.01101	0.010519	-1.0468	0.296435	-0.03175	0.009729	-0.03175	0.009729

Regression Statistics for CDT model, T-pH dependent	
Multiple R	0.962145
R Square	0.925723
Adjusted R Square	**0.923**
Standard Error	0.18138
Observations	208

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	7	82.00426	11.71489	356.0893	3.2E-109
Residual	200	6.57975	0.032899		
Total	207	88.58401			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	0.056523	0.288094	0.196196	0.008447	-0.51157	0.624614	-0.51157	0.624614
lnt	-6.49015	2.369268	-2.73931	0.006713	-11.1621	-1.81821	-11.1621	-1.81821
lnt/T	1393.876	301.7843	4.618783	6.9E-06	798.7896	1988.963	798.7896	1988.963
lnt/T2	-56934.6	9514.35	-5.98408	9.9E-09	-75695.9	-38173.3	-75695.9	-38173.3
(lnt)2	-1.44412	0.313273	-4.60979	7.18E-06	-2.06186	-0.82638	-2.06186	-0.82638
(lnt)2/T	99.40218	16.17446	6.145624	4.24E-09	67.50786	131.2965	67.50786	131.2965
pH(lnt)2	-0.10426	0.012376	-8.42487	7.01E-15	-0.12867	-0.07986	-0.12867	-0.07986
(lnt)2(pH)2	0.008578	0.001072	8.00107	9.81E-14	0.006464	0.010692	0.006464	0.010692

APPENDIX F

SIZING THE CONTINUOUS STERILISER LENGTH FROM BENCH-SCALE DATA USING THE CDT MODEL FOR *E. COLI* INACTIVATION AS AFFECTED BY COMBINED *T*-pH

F.1 INTRODUCTION

In this Appendix the critical coefficients are first calculated for *E. coli* data, and the performance equations for a continuous steriliser used to determine a value of the length of a continuous steriliser for a 10^{-5} reduction in number of viable cells for a range of temperature and pH. A summary is given in Table F.3.

F.2 CALCULATION OF THE CRITICAL COEFFICIENTS FOR THE CDT MODEL FOR *E. COLI* FOR COMBINED *T*-pH BASED ON BENCH-SCALE DATA

Calculation in the Tables below are based on the experimental data of Davey, Hall and Thomas 1985).

Table F.1 Values of the critical coefficients of the CDT model for bench-scale data of *E. coli* for combined effect *T*-pH

Temp	pH 4.0		pH 4.5		pH 5.0		pH 5.5	
(°C)	ω	Ω	ω	Ω	ω	Ω	ω	Ω
54	-0.203	0.117	-0.203	0.101	-0.203	0.090	-0.203	0.083
56	0.245	0.051	0.245	0.035	0.245	0.024	0.245	0.017
58	0.618	-0.010	0.618	-0.026	0.618	-0.037	0.618	-0.044
60	0.926	-0.067	0.926	-0.083	0.926	-0.094	0.926	-0.101
62	1.180	-0.121	1.180	-0.136	1.180	-0.148	1.180	-0.155
70	1.803	-0.304	1.803	-0.320	1.803	-0.331	1.803	-0.338

(a) *T* range 54°C to 70°C; pH range 4.0 to 5.5

Temp	pH 6.0		pH 6.5		pH 7.0		pH 7.5	
(°C)	ω	Ω	ω	Ω	ω	Ω	ω	Ω
54	-0.203	0.080	-0.203	0.081	-0.203	0.087	-0.203	0.097
56	0.245	0.014	0.245	0.016	0.245	0.021	0.245	0.031
58	0.618	-0.047	0.618	-0.046	0.618	-0.040	0.618	-0.030
60	0.926	-0.104	0.926	-0.103	0.926	-0.097	0.926	-0.087
62	1.180	-0.158	1.180	-0.156	1.180	-0.150	1.180	-0.140
70	1.803	-0.341	1.803	-0.339	1.803	-0.334	1.803	-0.324

(b) *T* range 54°C to 70°C; pH range 4.0 to 5.5

F.3 PREDICTED LENGTH OF PILOT CONTINUOUS STERILISER USING THE CDT-DYNAMIC MODEL FOR COMBINED *T*-pH

Calculations are based on a flow velocity of 10 cm s^{-1} in the holding tube, to achieve a survivor reduction of 10^{-5}.

Table F.2 Predicted lengths for the pilot steriliser calculated using the CDT-Dynamic model for bench-scale data of *E. coli* for combined effect *T*-pH

pH 4.0

T (°C)	ln *t*	*t* (*s*)	L (cm)	L (m)
54	4.8	123.5	1234.9	12.3
56	3.9	49.5	494.7	4.9
58	3.0	19.1	191.5	1.9
60	2.2	9.4	93.7	0.9
62	1.8	6.1	60.6	0.6
70	1.2	3.3	33.5	0.3

pH 4.5

T (°C)	ln *t*	*t* (*s*)	L (cm)	L (m)
54	5.3	192.7	1927.1	19.3
56	4.3	77.1	771.1	7.7
58	3.3	25.8	258.1	2.6
60	2.4	10.8	108.2	1.1
62	1.9	6.5	65.3	0.7
70	1.2	3.4	34.2	0.3

pH 5.0

T (°C)	ln *t*	*t* (*s*)	L (cm)	L (m)
54	5.7	288.9	2888.7	28.9
56	4.8	122.4	1223.8	12.2
58	3.6	35.9	359.3	3.6
60	2.5	12.4	124.4	1.2
62	1.9	7.0	69.7	0.7
70	1.2	3.5	34.8	0.3

pH 5.5

T (°C)	ln *t*	*t* (*s*)	L (cm)	L (m)
54	6.0	390.0	3899.6	39.0
56	5.2	181.7	1817.0	18.2
58	3.9	49.4	493.8	4.9
60	2.6	13.9	139.1	1.4
62	2.0	7.3	73.0	0.7
70	1.3	3.5	35.2	0.4

Table F.2 continued ...

pH 6.0				
T (°C)	ln t	t (s)	L (cm)	L (m)
54	6.1	444.7	4446.9	44.5
56	5.4	220.2	2202.3	22.0
58	4.1	58.9	589.0	5.9
60	2.7	14.7	146.6	1.5
62	2.0	7.5	74.5	0.7
70	1.3	3.5	35.4	0.4

pH 6.5				
T (°C)	ln t	t (s)	L (cm)	L (m)
54	6.0	414.5	4145.4	41.5
56	5.3	198.4	1983.8	19.8
58	4.0	53.4	533.9	5.3
60	2.7	14.2	142.5	1.4
62	2.0	7.4	73.7	0.7
70	1.3	3.5	35.3	0.4

pH 7.0				
T (°C)	ln t	t (s)	L (cm)	L (m)
54	5.8	321.6	3216.1	32.2
56	4.9	140.1	1401.0	14.0
58	3.7	39.9	398.6	4.0
60	2.6	12.9	129.3	1.3
62	2.0	7.1	70.9	0.7
70	1.3	3.5	35.0	0.3

pH 7.5				
T (°C)	ln t	t (s)	L (cm)	L (m)
54	5.4	220.0	2199.9	22.0
56	4.5	89.0	890.3	8.9
58	3.4	28.5	285.2	2.9
60	2.4	11.3	113.1	1.1
62	1.9	6.7	66.7	0.7
70	1.2	3.4	34.4	0.3

A summary of the predicted steriliser holding tube length (L) for a range of combined *T*-pH based on the CDT model derived from bench-scale inactivations of *E. coli* in the selected carrier liquid is given in Table F.3.

Table F.3 Predicted holding tube length for a range of combined *T*-pH for the inactivation of *E. coli* in Carbopol carrier liquid from the newly synthesised CDT model based on the bench-scale data

T (°C)	L (m)							
	pH 4.0	**pH 4.5**	**pH 5.0**	**pH 5.5**	**pH 6.0**	**pH 6.5**	**pH 7.0**	**pH 7.5**
54	12.3	19.3	28.9	39.0	44.5	41.5	32.2	22.0
56	4.9	7.7	12.2	18.2	22.0	19.8	14.0	8.9
58	1.9	2.6	3.6	4.9	5.9	5.3	4.0	2.9
60	0.9	1.1	1.2	1.4	1.5	1.4	1.3	1.1
62	0.6	0.7	0.7	0.7	0.7	0.7	0.7	0.7
70	0.3	0.3	0.3	0.4	0.4	0.4	0.3	0.3
Average L (m)	4	6	9	13	**15**	14	10	7

APPENDIX G

SUMMARY OF CONTINUOUS STERILISER RAW DATA AND CDT MODEL PREDICTIONS

G.1 INTRODUCTION

This Appendix presents the continuous steriliser experimental data for *E. coli* as affected by combined effect *T*-pH, and the survivors predictions calculated using the CDT-Hydrodynamic model.

G.2 CONTINUOUS STERILISER EXPERIMENTAL DATA FOR COMBINED *T*-pH

L	***T*=54°C**	**pH 4.5**	**9.18 cm/*s***	***T*=56°C**	**pH 4.5**	**8.43 cm/*s***	***T*=58°C**	**pH 4.5**	**11.46 cm/*s***
(m)	*t* (*s*)	*N*	log (*N*/*N*$_0$)	*t* (*s*)	*N*	log (*N*/*N*$_0$)	*t* (*s*)	*N*	log (*N*/*N*$_0$)
0	0.0	9.41E+06	0.000	0.0	9.65E+07	0.000	0.0	4.22E+07	0.000
1	10.9	1.30E+06	-0.860	11.9	5.74E+07	-0.226	8.7	3.60E+06	-1.069
2	21.8	9.15E+05	-1.012	23.7	3.76E+07	-0.409	17.5	6.90E+05	-1.786
3	32.7	1.78E+05	-1.723	35.6	1.79E+06	-1.732	26.2	4.70E+05	-1.953
4	43.6	5.50E+04	-2.233	47.5	5.15E+05	-2.273	34.9	1.79E+04	-3.372
5	54.5	4.50E+04	-2.320	59.3	1.82E+05	-2.724	43.6	3.90E+03	-4.034
6	65.4	2.00E+04	-2.673	71.2	1.71E+05	-2.752	52.4	1.00E+02	-5.625
7	76.3	6.51E+03	-3.160	83.0	6.80E+03	-4.152	61.1	0.00E+00	-
8	87.2	2.00E+03	-3.673	94.9	3.90E+03	-4.393			
9	98.0	8.10E+02	-4.065	106.8	1.40E+02	-5.838			
10	108.9	6.00E+01	-5.195	118.6	9.60E+02	-5.002			
11	119.8	2.00E+01	-5.673	130.5	1.60E+01	-6.780			
12	130.7	0.00E+00	-	142.4	0.00E+00	-			

L	***T*=54°C**	**pH 6.0**	**9.24 cm/*s***	***T*=56°C**	**pH 6.0**	**9.23 cm/*s***	***T*=58°C**	**pH 6.0**	**9.68 cm/*s***
(m)	*t* (*s*)	*N*	log (*N*/*N*$_0$)	*t* (*s*)	*N*	log (*N*/*N*$_0$)	*t* (*s*)	*N*	log (*N*/*N*$_0$)
0	0.0	1.43E+07	0.000	0.00	1.90E+07	0.000	0.0	1.32E+09	0.000
1	10.8	8.70E+06	-0.216	10.8	4.40E+06	-6.35	10.3	8.44E+08	-0.194
2	21.7	6.10E+06	-0.370	21.7	4.00E+06	-0.677	20.7	2.45E+08	-0.731
3	32.5	1.75E+06	-0.912	32.5	2.32E+06	-0.913	31.0	6.72E+06	-2.293
4	43.3	1.67E+06	-0.933	43.3	1.37E+06	-1.142	41.3	4.28E+04	-4.489
5	54.1	9.10E+05	-1.196	54.2	1.90E+06	-1.000	51.7	2.73E+02	-6.684
6	64.9	8.30E+05	-1.236	65.0	1.70E+06	-1.048	62.0	1.66E+01	-7.900
7	75.8	3.63E+05	-1.595	75.8	1.20E+06	-1.200	72.3	0.00E+00	-
8	86.6	3.50E+05	-1.611	86.7	1.60E+06	-1.075			
9	97.4	5.75E+04	-2.396	97.5	1.85E+06	-1.012			
10	108.2	3.85E+04	-2.570	108.3	1.63E+06	-1.067			
11	119.1	5.00E+03	-3.456	119.2	1.70E+05	-2.048			
12	129.9	1.38E+03	-4.015	130.0	5.20E+04	-2.563			
13	140.7	2.55E+02	-4.749	140.9	1.27E+03	-4.175			
14	151.5	1.20E+02	-5.076	151.7	1.50E+02	-5.103			
15	162.3	4.00E+01	-5.553	162.5	1.70E+01	-6.048			
16	173.2	0.00E+00	-	173.4	0.00E+00	-			

continuos steriliser experimental data continued ...

L	*T*=54°C	pH 7.5	8.82 cm/*s*	*T*=56°C	pH 7.5	9.68 cm/*s*	*T*=58°C	pH 7.5	10.06 cm/*s*
(m)	*t* (*s*)	*N*	log (N/N_0)	*t* (*s*)	*N*	log (N/N_0)	*t* (*s*)	*N*	log (N/N_0)
0	0.0	2.26E+07	0.000	0.0	1.23E+07	0.000	0.0	1.20E+07	0.000
1	11.3	1.65E+07	-0.137	10.3	7.62E+06	-0.208	9.9	4.80E+06	-0.398
2	22.7	1.00E+06	-1.354	20.7	2.78E+06	-0.646	19.9	1.28E+06	-0.972
3	34.0	7.50E+05	-1.479	31.0	1.56E+06	-0.897	29.8	6.32E+05	-1.279
4	45.4	5.14E+05	-1.643	41.3	1.62E+06	-0.880	39.8	1.26E+05	-1.979
5	56.7	1.45E+05	-2.193	51.7	1.24E+06	-0.996	49.7	1.80E+04	-2.824
6	68.0	1.00E+05	-2.354	62.0	4.28E+05	-1.458	59.6	3.56E+03	-3.528
7	79.4	3.00E+04	-2.877	72.3	1.65E+05	-1.872	69.6	2.20E+01	-5.737
8	90.7	1.60E+04	-3.150	82.6	3.24E+04	-2.579	79.5	0.00E+00	-
9	102.0	1.15E+04	-3.293	93.0	1.12E+04	-3.041			
10	113.4	6.00E+03	-3.576	103.3	2.51E+03	-3.690			
11	124.7	5.00E+03	-3.655	113.6	1.00E+03	-4.090			
12	136.1	5.00E+03	-3.655	124.0	1.50E+02	-4.914			
13	147.4	1.00E+03	-4.354	134.3	1.00E+01	-6.090			
14	158.7	4.50E+01	-5.701	144.6	2.00E+00	-6.789			
15	170.1	1.00E+01	-6.354	155.0	0.00E+00	-			
16	181.4	0.00E+00	-						

G.3 PREDICTIONS OF THE CDT-HYDRODYNAMIC MODEL FOR COMBINED *T*-pH

In the tables below log (N/N_0) are calculated (pred log(dyn)) using the CDT-Hydrodynamic model for combined *T*-pH at the corresponding velocities.

54/4.5pH velocity 9.18 cm/s

L (m)	*t* (*s*)	log(dyn)	pred log(dyn)	*t* (*s*)	log(static)
0	0.0	0.000	0.000	12	-0.071
1	10.9	-0.860	-1.258	24	-0.789
2	21.8	-1.012	-1.578	36	-1.068
3	32.7	-1.723	-1.883	40	-1.363
4	43.6	-2.233	-2.179	49	-1.923
5	54.5	-2.320	-2.469	60	-1.724
6	65.4	-2.673	-2.755		
7	76.3	-3.160	-3.039		
8	87.2	-3.673	-3.322		
9	98.0	-4.065	-3.605		
10	108.9	-5.195	-3.888		
11	119.8	-5.673	-4.171		
12	130.7	-	-		

t (*s*)	*k*dyn
10.9	0.182
21.8	0.107
32.7	0.121
43.6	0.118
54.5	0.098
65.4	0.094
76.3	0.095
87.2	0.097
98.0	0.095
108.9	0.110
119.8	0.109

54/6.0pH velocity 9.24 cm/s

L (m)	*t* (*s*)	log(dyn)	pred log(dyn)	*t* (*s*)	log(static)
0	0.0	0.000	0.000	12	-0.041
1	10.8	-0.216	-1.124	24	-0.294
2	21.7	-0.370	-1.305	36	-0.177
3	32.5	-0.912	-1.473	40	-0.638
4	43.3	-0.933	-1.630	49	-0.572
5	54.1	-1.196	-1.780	60	-0.587
6	64.9	-1.236	-1.923		
7	75.8	-1.595	-2.063		
8	86.6	-1.611	-2.198		
9	97.4	-2.396	-2.331		
10	108.2	-2.570	-2.461		
11	119.1	-3.456	-2.589		
12	129.9	-4.015	-2.716		
13	140.7	-4.749	-2.840		
14	151.5	-5.076	-2.964		
15	162.3	-5.553	-3.086		
16	173.2	-	-		

t (*s*)	*k*dyn
10.8	0.046
21.7	0.039
32.5	0.065
43.3	0.050
54.1	0.051
64.9	0.044
75.8	0.048
86.6	0.043
97.4	0.057
108.2	0.055
119.1	0.067
129.9	0.071
140.7	0.078
151.5	0.077
162.3	0.079

54/7.5pH **velocity 8.82 cm/*s***

L (m)	*t* (*s*)	log(dyn)	pred log(dyn)	*t* (*s*)	log(static)
0	0.0	0.000	0.000	12	-0.207
1	11.3	-0.137	-1.244	24	-0.578
2	22.7	-1.354	-1.546	36	-0.799
3	34.0	-1.479	-1.832	40	-0.899
4	45.4	-1.643	-2.106	49	-0.974
5	56.7	-2.193	-2.374	60	-1.116
6	68.0	-2.354	-2.638		
7	79.4	-2.877	-2.898		
8	90.7	-3.150	-3.156		
9	102.0	-3.293	-3.414		
10	113.4	-3.576	-3.670		
11	124.7	-3.655	-3.926		
12	136.1	-3.655	-4.182		
13	147.4	-4.354	-4.439		
14	158.7	-5.701	-4.695		
15	170.1	-6.354	-4.953		
16	181.4	-	-		

t (*s*)	*k*dyn
11.3	0.028
22.7	0.137
34.0	0.100
45.4	0.083
56.7	0.089
68.0	0.080
79.4	0.083
90.7	0.080
102.0	0.074
113.4	0.073
124.7	0.067
136.1	0.062
147.4	0.068
158.7	0.083
170.1	0.086

56/4.5pH **velocity 8.43 cm/*s***

L (m)	*t* (*s*)	log(dyn)	pred log(dyn)	*t* (*s*)	log(static)
0	0.0	0.000	0.000	10	-1.378
1	11.9	-0.226	-2.508	20	-0.919
2	23.7	-0.409	-3.379	30	-3.055
3	35.6	-1.732	-4.091	40	-2.811
4	47.5	-2.273	-4.720	50	-3.561
5	59.3	-2.724	-5.297		
6	71.2	-2.752	-5.836		
7	83.0	-4.152	-6.347		
8	94.9	-4.393	-6.836		
9	106.8	-5.838	-7.305		
10	118.6	-5.002	-7.760		
11	130.5	-6.780	-8.201		
12	142.4	-	-		

t (*s*)	*k*dyn
11.9	0.044
23.7	0.040
35.6	0.112
47.5	0.110
59.3	0.106
71.2	0.089
83.0	0.115
94.9	0.106
106.8	0.126
118.6	0.097
130.5	0.120

56/6.0pH **velocity 9.23 cm/*s***

L (m)	*t* (*s*)	log(dyn)	Pred log(dyn)	*t* (*s*)	log(static)
0	0.00	0.000	0.000	10	-0.682
1	10.8	-0.635	-0.635	20	-1.079
2	21.7	-0.677	-0.677	30	-2.708
3	32.5	-0.913	-0.913	40	-1.457
4	43.3	-1.142	-1.142	50	-2.088
5	54.2	-1.000	-1.000		
6	65.0	-1.048	-1.048		
7	75.8	-1.200	-1.200		
8	86.7	-1.075	-1.075		
9	97.5	-1.012	-1.012		
10	108.3	-1.067	-1.067		
11	119.2	-2.048	-2.048		
12	130.0	-2.563	-2.563		
13	140.9	-4.175	-4.175		
14	151.7	-5.103	-5.103		
15	162.5	-6.048	-6.048		
16	173.4	-	-		

t (*s*)	*k*dyn
10.8	0.135
21.7	0.072
32.5	0.065
43.3	0.061
54.2	0.042
65.0	0.037
75.8	0.036
86.7	0.029
97.5	0.024
108.3	0.023
119.2	0.040
130.0	0.045
140.9	0.068
151.7	0.077
162.5	0.086

56/7.5pH **velocity 9.68 cm/*s***

L (m)	*t* (*s*)	log(dyn)	pred log(dyn)	*t* (*s*)	log(static)
0	0.0	0.000	0.000	10	-0.918
1	10.3	-0.208	-2.324	20	-2.249
2	20.7	-0.646	-3.064	30	-2.484
3	31.0	-0.897	-3.656	40	-2.6154
4	41.3	-0.880	-4.172	50	-3.6864
5	51.7	-0.996	-4.639		
6	62.0	-1.458	-5.072		
7	72.3	-1.872	-5.479		
8	82.6	-2.579	-5.865		
9	93.0	-3.041	-6.234		
10	103.3	-3.690	-6.589		
11	113.6	-4.090	-6.932		
12	124.0	-4.914	-7.265		
13	134.3	-6.090	-7.588		
14	144.6	-6.789	-7.903		
15	155.0	-	-		

t (*s*)	*k*dyn
10.3	0.046
20.7	0.072
31.0	0.067
41.3	0.049
51.7	0.044
62.0	0.054
72.3	0.060
82.6	0.072
93.0	0.075
103.3	0.082
113.6	0.083
124.0	0.091
134.3	0.104
144.6	0.108

58/4.5pH **velocity 11.46 cm/*s***

L (m)	*t* (*s*)	log(dyn)	Pred log(dyn)	*t* (*s*)	log(static)
0	0.0	0.000	0.000	8	-1.858
1	8.7	-1.069	-3.669	16	-2.807
2	17.5	-1.786	-5.107	24	-6.049
3	26.2	-1.953	-6.134	32	-5.636
4	34.9	-3.372	-6.952	40	-6.110
5	43.6	-4.034	-7.640		
6	52.4	-5.625	-8.238		
7	61.1	-	-		

t (*s*)	*k*dyn
8.7	0.282
17.5	0.235
26.2	0.172
34.9	0.222
43.6	0.213
52.4	0.247

58/6.0pH **velocity 9.68 cm/*s***

L (m)	*t* (*s*)	log(dyn)	Pred log(dyn)	*t* (*s*)	log(static)
0	0.0	0.000	0.000	8	-2.312
1	10.3	-0.194	-3.559	16	-2.533
2	20.7	-0.731	-4.555	24	-5.308
3	31.0	-2.293	-5.160	32	-4.329
4	41.3	-4.489	-5.585	40	-5.073
5	51.7	-6.684	-5.909		
6	62.0	-7.900	-6.166		
7	72.3	-	-		

t (*s*)	*k*dyn
10.3	0.043
20.7	0.081
31.0	0.170
41.3	0.250
51.7	0.298
62.0	0.293

58/7.5pH **velocity 10.06 cm/*s***

L (m)	*t* (*s*)	log(dyn)	pred log(dyn)	*t* (*s*)	log(static)
0	0.0	0.000	0.000	8	-3.517
1	9.9	-0.398	-3.832	16	-4.277
2	19.9	-0.972	-5.235	24	-4.783
3	29.8	-1.278	-6.207	32	-5.812
4	39.8	-1.979	-6.966	40	-7.812
5	49.7	-2.824	-7.594		
6	59.6	-3.528	-8.131		
7	69.6	-5.737	-8.602		
8	79.5	-	-		

t (*s*)	*k*dyn
9.9	0.092
19.9	0.112
29.8	0.099
39.8	0.114
49.7	0.131
59.6	0.136
69.6	0.190

APPENDIX H

SIZING THE STEAM INJECTOR FOR THE PILOT CONTINUOUS STERILISER

H.1 NOTATION USED IN THIS APPENDIX

Numbers in parentheses after the description refer to equations in which symbols are first used or defined

A	area of liquid-side flow (H.4), cm^2
C_i	liquid inlet concentration (H.2), g $litre^{-1}$
C_0	liquid outlet concentration (H.2), g $litre^{-1}$
C_p	heat capacity at constant pressure (H.2), $m^2\ s^{-2}$ / °C
d	diameter of the inlet steam port (H.1), mm
D	inside diameter of the holding tube (H.13), mm
D_i	liquid diameter in the steam injector (H.3), mm
f	friction factor (H.7)
g_c	standard acceleration (H.1), cm s^{-2}
k	consistency index (H.3), kg m^{-1} s^{-n}
k	rate coefficient for thermal inactivation (2.3, H.6), s^{-1}
L	length of steriliser holding tube (2.1, H.13), m
L_s	length of steam injector (subchapter H.3.2), m
M	mean molecular weight (H.1), kg $mole^{-1}$
n	pseudoplastic index (2.2, H.3)
N	steam inlet ports (H.1)
pH	acidity of the medium (H.6)
P_l	liquid pressure (absolute) in injector (H.1), kg m^{-2}
P_s	steam pressure (absolute) (H.1), kg m^{-2}
ΔP	pressure loss in holding tube (H.13), kg m^{-2}
Q	volumetric flow rate (H.4), l min^{-1}, $cm^3\ s^{-1}$
Q_i	mass flow rate entering injector (H.14), kg s^{-1}
r	radius (H.9), cm
R	gas constant (H.1), kg $m^2\ s^{-2}\ K^{-1}$ $mole^{-1}$
Re	Reynolds number in the holding tube of the continuous steriliser (H.3)
t	time or residence time in the holding tube (2.1, H.6), *s*

T	temperature, Kelvin and ^{o}C (H.1)
T_i	inlet liquid temperature, Kelvin and ^{o}C (H.2)
T_0	liquid temperature at injector discharge (H.2), Kelvin and ^{o}C
T_S	steam saturation temperature at P_s (H.2), Kelvin and ^{o}C
$<u>$	bulk velocity of the liquid in holding tube (2.1, H.3), cm s^{-1}
W	mass flow rate of steam (H.1), kg s^{-1}

Greek Letters

λ	enthalpy of reaction (latent heat) (H.2), kg m^2 s^{-2}
π	3.14159 (H.1)
ρ	density (H.3), kg m^3
τ	shear stress (H.5), kg m^{-1} s^{-2}
τ_w	wall shear stress inside injector (H.7), kg m^{-1} s^{-2}

H.2 ANALYTICAL DEVELOPMENT

The steam-side and liquid-side flows are treated separately in this thesis. The design constraints for the steam injector are:

- steam velocity in the inlet ports must be less than the sonic velocity;
- diameter of the port must not be so small as to foul easily.

For the liquid stream the flow diameter is estimated from a simultaneous solution of two equations, respectively:

- Reynolds number in a tube
- total liquid (liquid and condensed steam) flow rate.

H.2.1 Steam-Side

Extended Bernoulli Equation

The injection of steam through a number of inlet ports, N, into the liquid causes turbulent flow. The extended Bernoulli equation can be used as it provides a relationship between pressure and friction. For the direct steam injector, this equation can be written in terms of steam inlet ports as following (Davey 1980):

$$N = (4\,W / \pi\, d^2)\,[(2\,R\,T / g_c\,M)\ln(P_l / P_s) / (P_l^2 - P_s^2)]^{1/2} \qquad (H.1)$$

Steam Flow and Liquid Dilution

A term for dilution of the liquid flow by condensing steam can be obtained from a heat and mass balance around the injector. Following the work of Davey (1980) a final equation is obtained of the form:

$$C_i = C_0\,[C_p\,(T_0 - T_i) / (\lambda + C_p\,(78 + 0.22\,P_s) + C_p\,T_i - 2\,C_p\,T_0) + 1] \qquad (H.2)$$

The diameter of the inlet steam port, d, must be selected such that the steam velocity in the port holes is less than the sonic velocity, u_s, (Perry 1973). In addition, d must not be so small as to foul easily; a minimum value based on experience is d = 0.8 mm (Davey 1980).

H.2.2 Liquid-Side

Turbulent Flow

The Reynolds number for a power law liquid in a tube is given by the relation (Wilkinson 1960, Skelland 1967):

$$Re = D_i^n \langle u \rangle^{2-n} \rho / \{k [(6 n + 2) / n]^n / 8\} \qquad (H.3)$$

The volumetric flow rate is given by:

$$Q = A \langle u \rangle \rho \qquad (H.4)$$

For a successful operation, the liquid flow inside the injector body should be turbulent with Re > 3000 and the liquid cross section D_i be as small as possible. Even in smooth tubes there will be turbulence at Re < 3000 caused by the injected steam (Davey 1980). Equations H.3 and H.4 will, however, provide a good estimate of the flow dimensions of an injector.

Shear Degradation of Liquid

For highly viscous liquids, physical degradation may occur because of high shear if D_i is too small. A small diameter may also be subject to fouling.

Pressure Loss

The macroscopic method of Bird, Stewart and Lightfoot (1962) may be used to estimate the pressure loss due to contraction and expansion of the liquid on entering and discharging from the injector. For highly viscous liquids at low velocities and in long holding tubes, the pressure loss through the injector will be small compared with that along the holding tube of the steriliser.

H.2.3 Design Equations

Equations H.3 and H.4 are the design equations, subject to the constraint of equation H.1 and H.2.

H.3 SOLUTION OF EQUATIONS AT THE CONDITIONS OF EXPERIMENTAL STERILISER

H.3.1 Liquid-Side

Dimensions of Experimental Steriliser

The inside diameter of the holding tube is D = 0.95 cm. Bulk liquid velocities of $\langle u \rangle$ = 5 cm s^{-1} to 15 cm s^{-1} match those used in commercial sterilisers (Pfiefer and Vojnovich 1952). Corresponding liquid volumetric flow rates are in the range Q = 0.214 l min^{-1} to 0.642 l min^{-1} (3.57 cm^3 s^{-1} to 10.7 cm^3 s^{-1}).

The Liquid

The power law equation for the test liquid for n = 0.37 is:

$$\tau = k (- du / dr)^{0.37} \qquad \text{(H.5)}$$

where k is given by equation (5.15). The liquid density is assumed to be ρ = 1 g cm^{-3}.

The Micro-organism

The test bacterium used for the calculations of the steriliser dimensions is *E. coli*. The thermal inactivation kinetics in the test liquid to obtain a reduction of $- \log_{10} [\langle N \rangle / \langle N_0 \rangle] < 5$ are given by CDT model (equation 5.13, Table 5.2). The micro-organism has a heat sensitivity in the range of 57°C to 64°C. Substitution of coefficients of equation (5.13) gives:

$$k = 2.3[5.6x10^{-2}-(6.5-1.4x10^{3}/T+5.7x10^{4}/T^{2})(\ln t)+(1.4+99/T-0.1pH+8.6x10^{-3}pH^{2})(\ln t)^{2}]/t \quad (H.6)$$

Flow Diameter of Steam Injector

A temperature of 60°C is assumed for the liquid inside the injector. The liquid diameter in the injector, D_i, can be determined from equations H.3 and H.4 by assuming a medium or upper value of Q = 0.642 l min^{-1} (10.7 cm^3 s^{-1}).

H.3.1.1 First approximation

Simultaneous solution of equations H.3 and H.4 for 60°C with Re = 3000, n = 0.37 and Q = 0.642 l min^{-1} gives (Davey 1980):

$$D_i = 0.124 \text{ cm}$$
$$\langle u \rangle = 880.238 \text{ cm } s^{-1}$$

The resulting wall shear stress inside the injector can be estimated from the friction factor (Wilkinson 1960, Skelland 1967):

$$f = 2\,\tau_w / \langle u \rangle^2 = f(Re) \quad (H.7)$$

At n = 0.37 and Re = 3000, $f = 4 \times 10^{-3}$ (Wilkinson 1960, p.67, Fig 38a).

$$\tau_w = 1.55 \times 10^3 \text{ dyne cm-}^2 \quad (H.8)$$

The corresponding wall shear rate is obtained from equation H.5:

$$(-du / dr)_w = 3.708 \times 10^4 \, s^{-1} \quad (H.9)$$

This shear rate is about 68 times the average from which the constants in the rheological equation were determined (Davey 1980). At these shear rate conditions the liquid might be susceptible to physical degradation, and the desirable friction reducing property of the high molecular weight test liquid will be lost (Pallerson, Zakin and Rodriguez 1969, Davey 1980).

H.3.1.2 Second approximation

The values of the wall shear stress $(- du / dr)_w$ were obtained from the relation (Wilkinson 1960):

$$(- du / dr)_w = [(3\ n + 1) / 4\ n]\ (8 \langle u \rangle / D_i) \qquad \text{(H.10)}$$

Davey (1980) showed that a value of $D_i = 0.15$ cm is seen to be the best compromise as shear rates in the injector and the holding tube will match those from which the rheological constants were determined. The value of the liquid-side flow diameter is therefore fixed at $D_i = 0.15$ cm.

Test Liquid Make-up and Dilution

The concentration of the test liquid which gives a 2 kg m^{-3} solution Carbopol when diluted with condensed steam, is obtained from equation F.2, with the following substitutions:

$C_p = 4.219$ kJ kg^{-1} / °C

$\lambda = 2258$ kJ kg^{-1}

$T_i = 20$°C

that is,

$$C_i = 0.2\ [4.219\ (T_0 - 20) / (2671 + 0.9282\ P_s - 8.438\ T_0) + 1] \qquad \text{(H.11)}$$

Solution of equation H.11 requires that T_0 and P_s be specified. The holding tube temperature at the injector discharge T_0 can be readily specified, but P_s must be specified from a consideration of the steam-side flow.

H.3.2 Steam-Side

Diameter of Steam Inlet Holes

The value of the diameter of the steam inlet ports is selected at d = 0.12 cm. After substitution, the number of steam inlet ports is given by (Davey 1980):

$$N = 1.88x10^3 [(0.11P_s+342) \ln(P_l/P_s)/(P_l^2/P_s^2)]^{1/2} Q(T_0-20)/(2258+0.9282P_s+4.219(78-T_0) \quad (H.12)$$

where R = 8.539 x 10^4 g cm mol^{-1} K^{-1}, M = 18 and g_c = 980 cm s^{-2}.

Liquid Pressure Inside Injector

The liquid pressure inside the injector, P, will be about equal to the static pressure loss along the holding tube, plus 100 kPa, ie atmospheric pressure. The pressure loss for laminar flow of a power law liquid in a tube is given by equation (Wilkinson 1960):

$$\Delta P = k [(3 n + 1) / 4 n]^n (8 \langle u \rangle / D)^n 4 L / D \quad (H.13)$$

The length of the steriliser holding tube L was obtained from the CDT hydrostatic model (Chapter 6, Appendix F). Calculations for the pressure loss were made using equation H.13. For the range of velocities and temperatures used in the experimental trials, it was found that for values of $-\log_{10} [\langle N \rangle / \langle N_0 \rangle] \leq 5$, the length of the holding tube could go up to 44 m (54°C, pH 6.0). However at the mid-range temperature of 56°C, the length is ≤ 20 m.

Steam Pressure

Experience with direct steam injectors suggests that the operating steam pressure should be about 20 kPa to 50 kPa above the liquid pressure inside the injector (Davey 1980):

$$P_s = 130 + 50 = 180 \text{ kPa.a}$$

Number of Steam Inlets

Equation H.12 gives N = 1 for the following substitutions:

$Q = 0.428$ l min^{-1} (a midrange value)

$T_0 = 60°C$

$P_l = 130$ kPa.a

$P_s = 180$ kPa.a

Based on equation H.12, C_i is determined to complete the design and specification of the dependent variables, P_s, for a range of values of the independent variables, Q and T_0.

Residence Time in the Steam Injector

At the lower flow rate of Q = 0.236 l min^{-1} (3.925 cm^3 s^{-1}), the residence time in the steam injector of the liquid containing the bacteria is a maximum at:

$t = 0.0183$ s

$L_s = 3.7$ cm

$\langle u \rangle = 201.78$ cm s^{-1}

H.4 MASS AND ENERGY BALANCE FOR STEAM INJECTOR

Figure 6.2 represents the injector with inlet and outlet streams.

Energy balance

If T_i is used as the datum temperature, the inlet liquid stream has zero energy.

$$W + W\,(T_s - T_0)\,C_p = (W + Q_i)\,C_p\,(T_0 - T_i) \qquad \text{(H.14)}$$

Hence

$$W = Q_i\,C_p\,(T_0 - T_i) / (\lambda + C_p\,T_s + C_p\,T_i - 2\,C_p\,T_0) \qquad \text{(H.15)}$$

Mass balance

An expression for the liquid make up concentration, C_i, can be obtained in terms of the outlet liquid concentration, C_0, to allow for dilution with condensed steam:

$$C_i\,Q_i = C_0\,(W + Q_i) \qquad \text{(H.16)}$$

Hence

$$C_i = C_0\,(W + Q_i) / Q_i \qquad \text{(H.17)}$$

Table H.1 Calculation for the Carbopol dilution

P_s	Concentration (%)									
(kPa.a)	54°C	56°C	58°C	60°C	62°C	64°C	66°C	68°C	70°C	72°C
120	5.5	5.8	6.2	6.5	6.8	7.2	7.5	7.8	8.2	8.5
130	5.5	5.8	6.1	6.5	6.8	7.1	7.5	7.8	8.2	8.5
140	5.5	5.8	6.1	6.4	6.8	7.1	7.4	7.8	8.1	8.5
150	5.4	5.8	6.1	6.4	6.8	7.1	7.4	7.8	8.1	8.4
160	5.4	5.7	6.1	6.4	6.7	7.1	7.4	7.7	8.1	8.4
170	5.4	5.7	6.1	6.4	6.7	7.0	7.4	7.7	8.0	8.4
180	5.4	5.7	6.0	6.4	6.7	7.0	7.3	7.7	8.0	8.3
190	5.4	5.7	6.0	6.3	6.7	7.0	7.3	7.6	8.0	8.3
200	5.3	5.7	6.0	6.3	6.6	7.0	7.3	7.6	8.0	8.3
Ave. Conc.	5.4	5.7	6.1	6.4	6.7	7.1	7.4	7.7	8.1	8.4

The average value for the Carbopol dilution over T range 54°C to 72°C is 6.9% ± 1.5, that was averaged for a practical propose to 7%.

APPENDIX I

THERMOCOUPLES

I.1 MEASUREMENT ERROR

Type T copper-constantan thermocouples were used to measure and monitor the temperature of the liquid in pilot continuous steriliser.

Measurement errors are most difficult to be predicted. An error translates in that there may be a difference between the temperature of the product and the temperature of the probe making the measurement. For this kind of experimental work, an accurate temperature reading is requested.

I.2 CALIBRATION OF THERMOCOUPLES

The thermocouples were calibrated by immersing them in a controlled temperature water bath of known temperature. The temperature of water bath was measured with a fully immersed reference mercury thermometer.

The value of temperature for each thermocouple was displayed on a panel mount digital controller with a ten-way switch box.

Figure I.1 Calibration curve for copper-constantan thermocouples

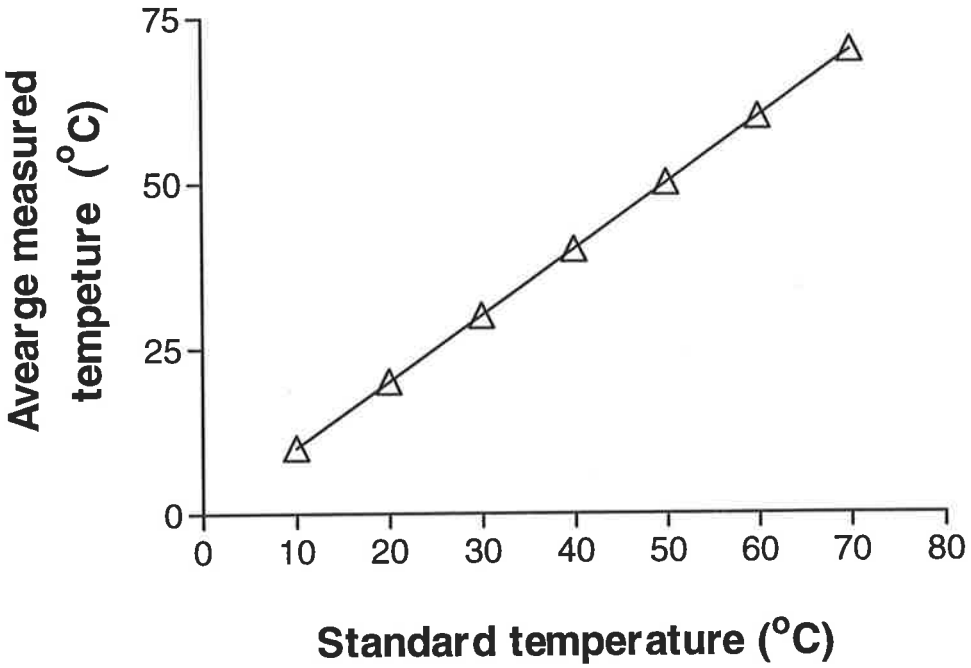


Table I.1 Calibration data for copper-constantan thermocouples

	Temperature (°C)						
Standard	10.0	20.0	30.0	40.0	50.0	60.0	70.0
th/couple1	10.2	20.0	30.0	40.1	50.1	60.0	70.0
th/couple2	10.1	20.1	30.0	40.0	50.0	60.0	70.0
th/couple3	10.1	20.0	30.0	40.0	50.1	60.0	70.2
th/couple4	10.0	20.4	30.0	40.0	50.2	60.0	70.0
th/couple5	10.3	20.1	30.0	40.0	50.2	60.0	70.1
th/couple6	10.1	20.3	30.1	40.0	50.1	60.0	70.1
th/couple7	10.0	20.0	30.1	40.1	50.0	60.0	70.0
th/couple8	10.0	20.0	30.0	40.0	50.0	60.0	70.0
th/couple9	10.1	20.1	30.0	40.0	50.0	60.0	70.1
th/couple10	10.0	20.1	30.0	40.1	50.0	60.0	70.0
th/couple11	9.9	20.0	29.9	40.0	50.1	60.0	70.0
th/couple12	10.2	20.0	30.1	40.2	50.0	60.1	70.0
th/couple13	10.0	20.0	30.1	40.0	50.0	60.1	70.0
th/couple14	10.1	20.0	30.0	40.0	50.1	60.1	70.0
th/couple15	10.1	20.1	30.0	40.0	50.1	60.0	70.1

APPENDIX J

EQUATIONS FOR CONCENTRATION OF THE TEST LIQUID IN CONTINUOUS STERILISER

J.1 INTRODUCTION

The equations for the concentration of test liquid used in the experimental trials are derived. A 2.14 kg m^{-3} (0.214% w/v) solution of the test liquid is required when diluted with the sodium hydroxide used for neutralisation of the Carbopol and the 400 ml of bacterial suspension. This is further diluted to 2 kg m^{-3} by condensing steam in the steriliser.

J.2 EQUATIONS

The diameter of the suspension vessel is 20 cm. The volume of water transferred is therefore 3.14 H litre, where H is the measured height of water in the vessel in cm.

Let X be the mass of Carbopol 934 resin and Y the volume of sodium hydroxide. For neutralisation, the ratio by weight of the 100 kg m^{-3} solution of sodium hydroxide to Carbopol is 5:1.

The liquid make-up concentration was calculated as a function of P_s and T (Table J.1).

$$Y = 5\ X \text{ ml} \tag{J.1}$$

$$X / (X + (3.14\ H + 0.4)) = 0.214 / 100 \tag{J.2}$$

$$X = 2.17\ (3.14\ H + 0.4) \text{ g} \tag{J.3}$$

Table J.1 Test liquid make-up concentration (C_i (g l^{-1})) as a function of P_s and T

P_s	Concentration (%)									
(kPa.a)	54°C	56°C	58°C	60°C	62°C	64°C	66°C	68°C	70°C	72°C
120	2.11	2.12	2.12	2.13	2.14	2.14	2.15	2.16	2.16	2.17
130	2.11	2.12	2.12	2.13	2.14	2.14	2.15	2.16	2.16	2.17
140	2.11	2.12	2.12	2.13	2.14	2.14	2.15	2.16	2.16	2.17
150	2.11	2.12	2.12	2.13	2.14	2.14	2.15	2.16	2.16	2.17
160	2.11	2.11	2.12	2.13	2.13	2.14	2.15	2.15	2.16	2.17
170	2.11	2.11	2.12	2.13	2.13	2.14	2.15	2.15	2.16	2.17
180	2.11	2.11	2.12	2.13	2.13	2.14	2.15	2.15	2.16	2.17
190	2.11	2.11	2.12	2.13	2.13	2.14	2.15	2.15	2.16	2.17
200	2.11	211	2.12	2.13	2.13	2.14	2.15	2.15	2.16	2.17
Ave.	2.11	2.11	2.12	2.13	2.13	2.14	2.15	2.15	2.16	2.17
Conc.										**2.14**

APPENDIX K

A PROCEDURE TO ESTIMATE THE EFFECTS OF DISPERSION

K.1 INTRODUCTION

This appendix presents a procedure to estimate the effects of dispersion on continuous sterilisation. This subject is new and not enough data resources were found in the literature.

K.2 DERIVED CALCULATIONS

The effect of axial dispersion in the steriliser holding tube was considered on intuitive grounds. The hypotheses were based on the correlation of the Peclet number (Pe) in terms of Reynolds (Re) and Damkohler (Da) numbers for fluid flow in a tubular steriliser.

It was appreciated that a very rough extrapolation of the available data in the literature (Lee 1992, pg. 244, Fig. 8.5), for Pe $\approx$ 10, corresponded to survivors of 10^{-7}. These values were compared with experimental data obtained in a pilot continuous steriliser (see Table K.1).

Table K.1 Effect of dispersion on the thermal inactivation in a continuous steriliser

T	pH	L	u	Re	1/Pe	k(ln)	t	kL/u	N / N_0	$\log(N/N_0)$		
°C		m	cms^{-1}			s^{-1}	s			cont. data	CDTdyn.	dispers.
54	4.5	11	9.18	872	1000	0.109	120	13	1.0E-07	-5.7	-4.2	-7.0
54	6.0	15	9.24	878	1000	0.079	162	13	1.0E-07	-5.6	-3.1	-7.0
54	7.5	15	8.82	838	1000	0.086	170	15	1.0E-07	-6.4	-5.0	-7.0
56	4.5	11	8.43	801	1000	0.120	131	16	1.0E-07	-6.8	-8.2	-7.0
56	6.0	15	9.23	877	1000	0.086	163	14	1.0E-07	-6.0	-5.4	-7.0
56	7.5	14	9.68	920	1000	0.708	145	16	1.0E-07	-6.8	-7.9	-7.0
58	4.5	6	11.46	1089	1000	0.247	52	13	1.0E-07	-5.6	-8.2	-7.0
58	6.0	6	9.68	920	1000	0.293	62	18	1.0E-07	-7.9	-6.2	-7.0
58	7.5	7	10.06	956	1000	0.190	70	13	1.0E-07	-5.7	-8.6	-7.0

APPENDIX L

SAFETY OPERATIONAL PROCEDURES (SOP's) FOR PILOT CONTINUOUS STERILISER

UNIVERSITY OF ADELAIDE

DEPARTMENT OF CHEMICAL ENGINEERING

CHEMICAL ENGINEERING LABORATORIES

SAFETY OPERATING PROCEDURE

FOR PILOT CONTINUOUS STERILISER

Project Designer: **Juliana Chiruta**

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Date: **15 May 1998**

This section has been developed according to Australian Standards For Sterilisers (AS 1410) to assist in achieving uniform levels of safety and efficiency for materials, design, construction, testing and installation of an experimental installation for sterilisation of liquids.

Part One – Start-Up Procedure

WARNING – SERIOUS BURN HAZARD

- Always wear safety glasses when open up the steam valve
- Wear protective gloves when handling anything near to autoclave
- Wear safety boots to avoid slipping
- Wear an overall coat for laboratory

EXPERIMENTAL PROCEDURE

- Inspect rig: see that the rig is clean, free of obvious mechanical defects or signs of disrepair, and properly set up
- Open the feed ball valve from the boiler and allow temperature to rise to the matrix temperature. The liquid should rich this matrix temperature in less than 25 min
- Ensure there is sufficient pressure (min 400 kPa) by opening the rear door and inspecting the gauge
- Switch on magnetic pump
- Open needle-valve
- Slowly open pressure regulator to the assumed value of 240 kPa
- Check temperature to be in the required range using the TC read-on monitor
- Use the needle valve for another adjustment of temperature if required
- To stop the rig, close up the regulator, the stop valve, and the magnetic pump

The person who operates the steriliser has to ware protective clothes, that will protect him/her to any skin exposure while operate. Protective clothes consist of: protective gloves designed for high temperatures, glasses, safety shoes, and an overall coat for laboratory.

Part Two – Shutdown Procedure

- Turn off the pressure regulator to the steam inlet
- Turn off the needle-valve
- Switch off magnetic pump
- Switch off boiler

Part Three – Maintenance Procedure

- Clean the tank, magnetic pump and pipes in situ using the steam only (see start-up procedure)
- Clean all the moisture from the apparatus and surrounding floor

Part Four – Safety and Points to Note

WARNING – SERIOUS BURN HAZARD

- Always wear safety glasses when open up the steam valve
- Wear protective gloves when handling anything near to autoclave
- Wear safety boots to avoid slipping
- Wear an overall coat for laboratory
- Take great care when operating the rig

WARNING – ALWAYS CHECK FOR

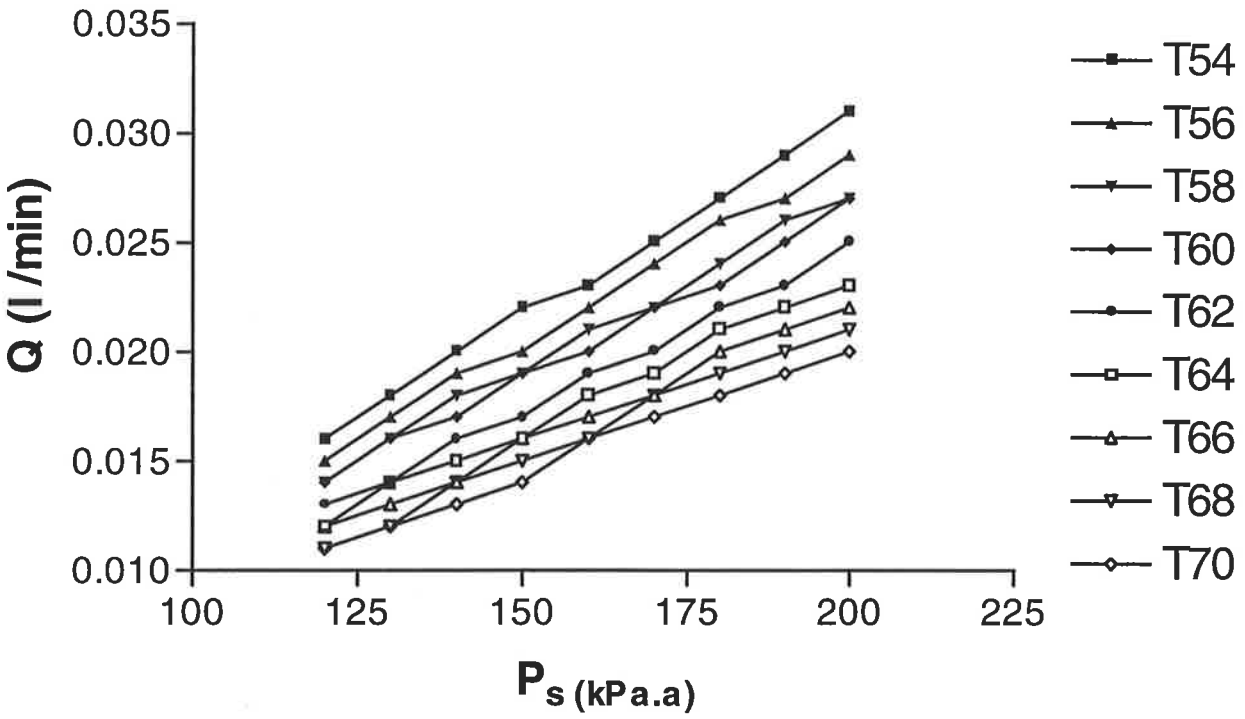
- The main switchboard for thermocouples together with the temperature monitor have to be placed into a box specially designed for this purpose, to avoid exposure to a 240 V
- A pressure-relieve device has to be placed in the case of too much pressure in the system, just after the pressure regulator
- All tubing system have to be insulated with polystyrene insulation for protection against high temperatures

NOTE

- Steriliser operates in a low range of temperatures and is not subject to high pressures or pressurised vessels
- For an initial adjustment of the pressure a nomogram has been drawn that allows an easy calculation of the initial pressures as temperature and flow rate are changed

NOMOGRAM FOR OPERATION OF THE STERILISER

Figure below is a nomogram of the flow rate (litre/min) versus pressure (kPa) for nine values of temperature. The nomogram is a guide that allows adjustment of the steam pressure in order to obtain the required sterilisation temperature.



NOTATION

Numbers in parentheses after description refer to equations in which symbols are first used or defined.

a_w	water activity (Table 2.1)
A	frequency factor in Arrhenius equation (2.5), s^{-1}
C_0-C_4 ;C_i	coefficients for a particular liquid-bacterial system (2.7, 5.19, 5.22, 5.23)
C_0'-C_3'; C_0"-C_4"	
D	decimal reduction time (2.6), min
e_t, $e^{(k)}$, $e_n{}^{(k)}$	statistically independent error at each time point and distributed as normal with zero mean and $e_t \sim N(0, \sigma^2)$ (5.4, 5.7, 5.9, Table 5.4)
E	activation energy for microbial inactivation (2.5), J mol^{-1}
k	rate coefficient for thermal inactivation (2.3), s^{-1}
L	length of steriliser holding tube (2.1), m
MSE	Mean Square Error (5.2)
n	pseudoplastic index (2.2)
n	order of reaction (5.4)
n_T	number of experimental (survival) data (5.1)
N, N_0 N_t	number of viable cells present at time t (5.4, 5.5, 5.6), cells ml^{-1}
N_T	number of terms in a model (5.1)
$<N>$, $<N_0>$	bulk number of viable cells present at time t (2.3), cells ml^{-1}
pH	acidity of the medium (2.7)
r^2	multiple regression coefficient (5.1)
R	holding tube radius (2.2), cm
R	gas constant (2.5), kg m^2 s^{-2} K^{-1} $mole^{-1}$
t	time or residence time in the holding tube (2.1), s
T	temperature, Kelvin and °C (2.5, 2.7)
u	velocity of the liquid in holding tube (2.1), cm s^{-1}
$<u>$	bulk velocity of the liquid in holding tube (2.2), cm s^{-1}
$\%V$	*per cent variance accounted for* (5.1), %

Greek Letters

α	coefficient (Table 5.3)
$\varepsilon, \varepsilon_1, \varepsilon_2$	critical coefficients (5.20, Table 5.4)
σ^2	standard error (Table 5.3)
$\omega, \omega_1, \omega_2$	critical coefficients (5.20, Table 5.4)
$\Omega, \Omega_1, \Omega_2$	critical coefficients (5.20, Table 5.4)

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