

**PREDICTIVE MODELLING AND EXPERIMENTAL
STUDIES OF THERMAL INACTIVATION OF
BACTERIA AS AFFECTED BY COMBINED
TEMPERATURE AND PH IN LIQUID**

by

KHAR YEAN KHOO

School of Chemical Engineering
The University of Adelaide

A thesis submitted for examination for the degree of
Doctor of Philosophy
in
Biochemical Engineering

August 2006

DECLARATION

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 been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Ms Khar Yean Khoo:

Date:

NOTE

All notation used in this thesis is listed and defined in Nomenclature section. The definition of important terms used in this research is given in Appendix A.

NOMENCLATURE

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

a_w	water activity	
$a_0 - a_2$	coefficient of <i>Equivalent Lethality</i> [7.1]	
A	frequency factor in Arrhenius equation [2.2]	s^{-1}
A	cross-sectional area of the holding tube [6.1]	m^2
$A_{f,g}$	accuracy factor of a model f compared to g [4.1]	
$B_{f,g}$	bias factor of a model f compared to g [4.2]	
b	coefficient of Square-Root model [3.3]	
$c_0 - c_1$	coefficients of Classical Arrhenius model [3.1]	
$C_0 - C_1$	coefficients of D-LA model [3.2]	
C_i	liquid make-up concentration [K.1]	$kg\ m^{-3}$
C_O	outlet liquid concentration [K.1]	$kg\ m^{-3}$
C_p	heat capacity [K.2]	$kJ\ kg^{-1}\ K^{-1}$
$d_0 - d_3$	coefficients for expanded Square-Root model [3.4]	
D	decimal reduction time [2.3]	s or, min
E	activation energy for microbial inactivation [2.2]	$J\ mol^{-1}$
$E(t)$	RTD function or age distribution function [M.3]	
$E(\theta)$	normalised RTD function [M.6]	
k	thermal inactivation rate coefficient [2.1]	s^{-1}
k_T	thermal conductivity [N.2]	$W\ m^{-1}\ K^{-1}$
L	length of pasteuriser holding tube [2.5]	m
m_s	mass flow rate of steam [K.1]	$kg\ s^{-1}$
m_c	mass flow rate of Carbopol suspension [K.1]	$kg\ s^{-1}$
MSE	mean square error	
n	pseudoplastic index [2.7]	
n	number of data [3.6]	
n_T	total number of data	
N	concentration of viable micro-organisms at time, $t = t$ [2.1]	$CFU\ ml^{-1}$
N_0	initial bulk or average concentration of viable micro-organisms at time, $t = 0$ [2.1]	$CFU\ ml^{-1}$
$\langle N \rangle$	bulk or average concentration of viable micro-organisms at $t = t$ [2.4]	$CFU\ ml^{-1}$
N_T	number of terms in a model [3.6]	
P_s	steam pressure (absolute) [K.3]	kPa.a
pH	acidity of medium [3.2]	
pH*	“conceptual” pH [3.3]	

pH_{max}	value of pH at which k is a maximum	
Q	volumetric flow in holding tube [6.1]	$\text{m}^3 \text{s}^{-1}$
r	radius of glass capillary (hematocrit) tube	mm
R	universal gas constant [2.2]	$\text{kg m}^2 \text{s}^{-2} \text{K}^{-1} \text{mol}^{-1}$
R	a temperature interval $[T_2 - T_1]$ [4.1]	$^{\circ}\text{C}$
R^2	correlation coefficient [3.6]	
s	number of finite sections in holding tube [6.3]	
t	exposure time or mean residence time in holding tube [2.1]	s
T	exposure temperature [2.2]	K
T_1	immersion temperature [N.1]	$^{\circ}\text{C}$
T_i	inlet liquid temperature [K.2]	$^{\circ}\text{C}$
T_0	exposure temperature immediate to outlet of steam injector (Chapter 7)	$^{\circ}\text{C}$
T_s	saturated steam temperature at (absolute) steam pressure [K.2]	$^{\circ}\text{C}$
T_{amb}	ambient temperature [N.1]	$^{\circ}\text{C}$
T^*	“conceptual” temperature [3.3]	$^{\circ}\text{C}$
T_{max}	value of T at which k is a maximum	$^{\circ}\text{C}$
\bar{T}	mean temperature [6.2]	$^{\circ}\text{C}$
u	liquid velocity in holding tube [2.4]	m s^{-1}
$\langle u \rangle$	bulk or average liquid velocity in holding tube [2.4]	m s^{-1}
$V(R)$	volume of R region such that $V(R) = T_2 - T_1$ [4.1]	$^{\circ}\text{C}$
z	temperature increase required to reduce the decimal reduction time by one-log unit	$^{\circ}\text{C}$ or K
$\%B_{f,g}$	per cent bias between models f and g [4.4]	
$\%D_{f,g}$	per cent discrepancy between models f and g [4.3]	
$\%V$	per cent variance accounted for [3.6]	

Subscripts

b	batch (<i>static</i>) pasteurisation [4.5]
c	continuous (<i>dynamic</i>) pasteurisation [4.6]

Greek

α_T	thermal diffusivity [N.2]	$\text{m}^2 \text{s}^{-1}$
α	coefficient of Weibull model [8.2]	
$\alpha_0 - \alpha_6$	coefficients of n^{th} Order Polynomial model [3.5]	
β	coefficient of Weibull model [8.2]	
$\beta_1 - \beta_2$	coefficients of KDT and MKDT models [5.1]	
$\gamma_0 - \gamma_1$	coefficients of Modified Weibull models [8.3]	
λ	latent heat [K.2]	$\text{kg m}^2 \text{s}^{-2}$

τ	mean residence time [M.3]	s
ρ	density [N.2]	kg m ⁻³
θ	t/τ [M.6]	
θ	$\frac{\alpha t}{r^2}$ [N.1]	s

Mathematical

log base 10 i.e. log₁₀

ln base e i.e. log_e

SUMMARY

Continuous thermal pasteurisation of various bulk liquid media is an important step in the food and allied industries. The design of a continuous flow pasteuriser is typically predicated on mathematical models developed from experimental data – usually batch, bench-scale, methods. Of particular interest is the effect of combined pasteuriser temperature (T) and liquid pH on inactivation and survivor of contaminants. However, bench-scale thermal survivor data may not adequately mirror those in a continuous flow pasteuriser. This research presents the development and experimental validation of rigorous models for thermal pasteurisation of bacteria as affected by combined process T -pH in both batch, bench-scale capillary studies (*static*) and in a pilot continuous flow pasteuriser (*dynamic*), within a defined liquid and range of exposure time, temperature and pH (t - T -pH). Five integrated stages in synthesis and model analysis were undertaken using stringent criteria for goodness of fit of an adequate model established.

First, four published predictive models were assessed against published static data ($n_T = 248$) for the thermal inactivation of *Escherichia coli* (ATCC 25922) in a Carbopol® 941 liquid food simulant in batch capillaries over a range of t - T -pH. The models tested were the Classical Arrhenius, Davey Linear-Arrhenius (D-LA), Square-Root (Belehradek) and a third-order Polynomial model (nOP). Analysis showed the D-LA model best satisfied the criteria for model selection and explained 96.0 %V in the thermal inactivation rate coefficient.

Second, the D-LA model was assessed against limited, published dynamic data ($n_T = 109$) for the same *E. coli* strain in identical food simulant. The model explained 60 %V in the thermal inactivation rate coefficient. On average, model predictions of survivor numbers from the *dynamic* data were less than that predicted from the *static* data, i.e. for a given (t - T -pH) more bacterial cells were apparently inactivated in the continuous flow pasteuriser than in bench-scale, batch capillary studies. Overall, however it was not clear from extensive analyses of available data whether there is a statistically significant difference in survivor numbers of viable *E. coli* between batch *static* and continuous flow *dynamic* data.

Third, although the D-LA model best satisfied the criteria for goodness of fit of a model, it failed to accurately predict the observed tails in the *static* survivor data. New models (KDT and a modified KDT) were synthesised to predict tails and shoulders in survivor data. The modified KDT (MKDT) form gave improved predictive capability over the KDT model when assessed against published *static* survivor data for *E. coli* and *L. monocytogenes* ($n_T = 355$) in the Carbopol food simulant. This model, however, could not be readily integrated with equations describing the performance of a continuous flow pasteuriser. Analyses indicated that a greater density of *dynamic* survivor data for *E. coli* was needed.

Fourth, a pilot continuous flow pasteuriser was constructed and used to generate a greater density of *dynamic* survivor data of *E. coli* (ATCC 25922) in a Carbopol® 941 carrier liquid for rigorous comparison with predictions from the Lin (1976) isothermal continuous

laminar flow process model. Direct steam injection heating was used. Extensive dye and digital-video studies, in a section of glass holding tube confirmed the practical implementation of the assumptions of laminar flow and rapid condensation of steam. Extensive practical experiments highlighted a non-isothermal condition along the holding tube. A highly linear dependence ($R^2 > 0.90$) of exposure temperature with holding tube length, i.e. exposure time, was demonstrated. This was accounted for using mathematical approaches and quantitatively incorporated into a D-LA model for the rate coefficient in an extended Lin process model. A block experimental design of 4 T (54, 56, 58, 60 °C) x 4 pH (4.5, 5.5, 6.5, 7.5) x 3 replicates with a total of $n_T = 834$ exposure times (16 – 198 s) was carried out in the pilot continuous flow pasteuriser. Findings highlighted that greater numbers of *E. coli* were thermally inactivated in the flow pasteuriser than predicted. From a practical operating view, the predictions from the extended Lin model were therefore conservative - with reduced risk to public health. Highly significant differences in the rates of heat-up of bacteria in the pilot pasteuriser (*dynamic*) (0.0104 s) compared with that in the batch (*static*) capillary tubes (1.6 s) and, mode of heat transfer, together with partial effects of dispersion with increasing length of pasteuriser holding tube, are postulated to be the controlling process influences for the difference between the experimental survivor data and the extended Lin model predictions. The lack of agreement between the continuous pasteuriser data and predictions from the extended Lin model indicated that this model cannot be practically applied. A direct comparison of the experimentally derived *dynamic* survivor data from the pilot pasteuriser (as $\ln N/N_0$) was also made with both the published *static* and *dynamic* data at a number of defined t - T -pH. This comparison revealed that overall, more *E. coli* were inactivated in the pilot continuous flow pasteuriser than described by published batch *static* capillary and *dynamic* data. Importantly, these comparisons showed that batch thermal survivor data for *E. coli* do not adequately mirror those obtained in continuous flow systems.

Fifth, in a search for an improved model for the inactivation data, the newly derived MKDT model was assessed against the experimental pilot pasteuriser data. This model was rejected, however, because it could not account satisfactorily for all tails in survivor curves. A Weibull form model with two coefficients (a scale factor (α) and a shape factor (β)) also did not adequately predict tailing and could not be reliably extrapolated with holding time. However, a modified Weibull form, also with two model coefficients (β_0, β_1), did give an improved fit to available experimental data.

This research highlighted statistically significant differences between the *dynamic* thermal survivor data for *E. coli* and standard bench-scale *static* capillary data for a defined liquid and range of t - T -pH. It is likely that findings from this study can be generalised. However, validation should be carried out for a range of common indicator micro-organisms in a range of liquid foods.

ACKNOWLEDGMENTS

I would like to express my sincere appreciation to numerous people to whom I am deeply indebted for their assistance during the course of this research study. In particular, I am grateful to the following:

Dr K R (Ken) Davey, School of Chemical Engineering, University of Adelaide, my principal supervisor, for valuable supervision, encouragement and fruitful discussions

Dr Connor Thomas, School of Molecular and Biomedical Science, University of Adelaide, my co-supervisor, for support and helpful guidance in microbiological aspects

Ben Daughtry, Senior Research Scientist, Food Safety Research Group, South Australian Research and Development Institute, for help with R-programming and regression analyses in Appendix D; Don Creighton, Visiting Research Fellow, School of Chemistry and Physics, for advice in the calibration of thermocouples in Appendix I; A/Prof Brian O'Neill, School of Chemical Engineering, University of Adelaide, for guidance in calculations of the re-parameterised model of Appendix M; Andrew Wright, Peter Kay, Brian Mulchay, Jason Peak, Bob Jarrad and Chris Mansell, Technical Officers, School of Chemical Engineering, for help with construction and modification of the pilot continuous flow pasteuriser; Garry Penney, Rosslyn Hammond and Shirley Coad, Central Services Unit, School of Molecular and Biomedical Science, for support in the preparation of sterile media and glassware.

I would like to dedicate this thesis to my parents.

I hope that the results of my efforts justify the expectations and confidence of the people concerned, and the interest, support and encouragement of my family, friends and colleagues.

TABLE OF CONTENTS

SUMMARY	ii
ACKNOWLEDGMENTS	vi
REFEREED PUBLICATIONS FROM THIS THESIS	xii
CHAPTER 1 INTRODUCTION	1
1.1 Background	1
1.2 Aims	3
1.3 Thesis Outline	3
CHAPTER 2 LITERATURE REVIEW	5
2.1 Introduction	5
2.2 Thermal Processes to Achieve Sterilisation and Pasteurisation	5
2.3 Principles Involved in Designing a Continuous Pasteuriser	6
2.4 Development of Process Models	7
2.4.1 Factors Affecting Thermal Sterilisation/Pasteurisation	16
2.5 Performance Equations for Continuous Flow Steriliser/Pasteuriser with Laminar Flow	18
2.5.1 The Lin Process Model	19
2.6 Research Hypothesis	20
2.7 Summary and Concluding Remarks	21
CHAPTER 3 EVALUATION OF FOUR PREDICTIVE MODELS FOR THERMAL INACTIVATION KINETICS OF BACTERIA FROM BATCH DATA	23
3.1 Introduction	23
3.2 Thermal Inactivation Data	24
3.3 The Four Kinetic Models	24
3.4 Establishment of Criteria for Fit of an Adequate Model	26
3.5 Fitting of Models and Data Analyses	28
3.6 Results and Discussion	30
3.7 Conclusions	40
CHAPTER 4 A COMPARISON OF THERMAL SURVIVOR FOR <i>ESCHERICHIA COLI</i> BETWEEN PUBLISHED BATCH AND CONTINUOUS DATA	41
4.1 Introduction	41
4.2 Materials and Methods	42
4.2.1 The Davey Linear-Arrhenius Model	42

4.2.2	Accuracy and Bias Indices	42
4.2.3	Thermal Inactivation Data	43
4.2.4	Analyses of Survivor Data	44
4.3	Results and Discussion	44
4.3.1	Comparison of Rate Coefficient of Thermal Inactivation between Batch and Continuous Data	44
4.3.2	Comparison of Survivor Ratio for Thermal Inactivation of Batch and Continuous Data	52
4.3.3	Accuracy and Bias Indices	55
4.3.4	Differences between Batch and Continuous Data	56
4.4	Conclusions	58

CHAPTER 5 DEVELOPMENT OF A NEW QUANTITATIVE MODEL FOR PREDICTING TAILING IN SURVIVOR CURVES FROM PUBLISHED BATCH DATA

59

5.1	Introduction	59
5.2	Material and Methods	59
5.2.1	Development of a New (KDT) Model for Thermal Inactivation Kinetics	59
5.2.2	Thermal Inactivation Data and KDT Model Fit	60
5.3	Results and Discussion	61
5.3.1	Validation of the KDT Model with Batch Data for <i>E. coli</i>	61
5.3.2	Modified KDT (MKDT) Model and Validation with Batch Data for <i>E. coli</i> and <i>L. monocytogenes</i>	66
5.4	Conclusions	71

CHAPTER 6 CONSTRUCTION AND COMMISSIONING OF AN EXPERIMENTAL PILOT CONTINUOUS FLOW PASTEURISER

72

6.1	Introduction	72
6.2	Pilot Continuous Flow Pasteuriser	72
6.2.1	Sizing of the Pilot Continuous Flow Pasteuriser	73
6.2.2	Pasteuriser Flow Diagram	75
6.2.3	Direct Steam Injector	77
6.2.4	Operational Nomogram for the Pilot Pasteuriser	78
6.2.5	Practical Implementation of Inherent Assumptions of the Lin (1976) Continuous Flow Process Model to the Pilot Pasteuriser	78
6.2.6	Dye and Digital-video Studies of Continuous Operation of the Pilot Pasteuriser	80
6.2.7	Temperature Profiling of the Holding Tube in Continuous Operation of the Pilot Pasteuriser	82
6.3	Extension to the Lin Process Model for Non-isothermal Holding Tube Flow	84
6.3.1	Mean Temperature	84
6.3.2	Accumulative Temporal Distribution of Exposure Temperature	84

6.3.3	Substitution of a Linear Temperature-time Profile	85
6.3.4	Summary	85
6.4	Concluding Remarks	86
CHAPTER 7 EXPERIMENTAL PASTEURISER RESULTS AND COMPARISON WITH LIN MODEL PREDICTIONS AND PUBLISHED DATA		87
7.1	Introduction	87
7.2	Methodology	87
7.2.1	Experimental Design	87
7.2.2	Preparation and Handling of Bacterium and Carrier Liquid	88
7.2.3	A Typical Pasteuriser Trial	91
7.3	Experimental Results and Comparison with Extended Lin Model Predictions	92
7.3.1	Summary of Pasteuriser Data	92
7.3.2	Comparison of Pasteuriser Data with Predictions from the Extended Lin Model for Non-isothermal Holding Tube Flow	95
7.3.3	Apparent High Rates of Thermal Inactivation in the Pilot Pasteuriser	101
7.3.4	Review of Experimental Trials from the Pilot Pasteuriser - Error Analyses	103
7.3.5	Indices for <i>Equivalent Lethality (EL)</i>	108
7.3.6	Summary	109
7.4	Comparison of Pasteuriser Data with Independent Published Continuous and Batch Data	109
7.5	Summary and Concluding Remarks	112
CHAPTER 8 ASSESSMENT OF THREE PREDICTIVE MODELS FOR EXPERIMENTAL DATA FROM A PILOT CONTINUOUS FLOW PASTEURISER		114
8.1	Introduction	114
8.2	Three Predictive Model Forms	114
8.3	Fitting of Models and Data Analyses	115
8.4	Results	116
8.4.1	Accuracy of Model Predictions	116
8.4.2	Model Coefficients as a Function of Combined Exposure Temperature and pH	122
8.4.3	Accuracy and Bias Indices	122
8.5	Selection of an Appropriate Model	123
8.6	Concluding Remarks	124
CHAPTER 9 CONCLUSIONS AND RECOMMENDATIONS		125
9.1	Conclusions	125

9.2	Recommendations for Relevant Future Research	126
APPENDIX A	DEFINITION OF SOME IMPORTANT TERMS USED IN THIS RESEARCH	127
APPENDIX B	SUMMARY OF REGRESSION ANALYSES OF FOUR PREDICTIVE MODELS FOR PUBLISHED BATCH DATA OF <i>E. COLI</i>	129
APPENDIX C	SUMMARY OF REGRESSION ANALYSES OF THE DAVEY LINEAR-ARRHENIUS MODEL FOR PUBLISHED CONTINUOUS SURVIVOR DATA OF <i>E. COLI</i>	131
APPENDIX D	SUMMARY OF REGRESSION ANALYSES FOR NEW KDT AND MKDT MODELS AGAINST PUBLISHED BATCH DATA OF THERMAL INACTIVATION	132
D.1	Summary of Regression Analyses for the KDT Model against <i>E. coli</i> data	132
D.2	Summary of Regression Analyses for the MKDT Model against <i>E. coli</i> data	135
D.3	Summary of Regression Analyses for the MKDT Model against <i>L. monocytogenes</i> data	137
APPENDIX E	SUMMARY OF EXPERIMENTAL PASTEURISER DATA OF <i>E. COLI</i> AND LIN MODEL PREDICTIONS	141
E.1	Experimental Pasteuriser Data	141
E.2	Comparison of Experimental Pasteuriser Data with Extended Lin Process Model Predictions	152
APPENDIX F	NON-LINEAR REGRESSION ANALYSES OF THREE PREDICTIVE MODELS FOR EXPERIMENTAL PASTEURISER DATA OF <i>E. COLI</i>	163
F.1	R Program Output for the MKDT Model	163
F.2	R Program Output for the Weibull Model	174
F.3	R Program Output for the Modified Weibull Model	183
APPENDIX G	SAFE OPERATING PROCEDURES (SOPs) AND OPERATIONAL NOMOGRAM FOR PILOT CONTINUOUS PASTEURISER	195
G.1	Safe Operating Procedures (SOPs)	195

REFEREED PUBLICATIONS FROM THIS THESIS

Refereed International Journals

Khoo K Y, Davey K R and Thomas C J 2003 Assessment of four models for predicting thermal inactivation kinetics of *Escherichia coli* in liquid as affected by combined exposure time, liquid temperature and pH. *Transactions of Institution of Chemical Engineering, Part C, Food and Bioproducts Processing* **81**: 129-137.

Davey K R, Khoo K Y and Thomas C J 2006 A comparison of thermal survival of *Escherichia coli* between standard capillary tubes and continuous flow pasteurisation. *Transactions of Institution of Chemical Engineering, Part C, Food and Bioproducts Processing* - submitted.

Khoo K Y, Davey K R and Thomas C J 2006 Evaluation of three models for predicting inactivation of *Escherichia coli* in liquid as affected by combined exposure time, liquid temperature and pH in a pilot continuous pasteuriser. *International Journal of Food Research* – in preparation.

Refereed International Conferences

Davey K R, Thomas C J and Khoo K Y 2003 Comparison of thermal inactivation kinetics for *Escherichia coli* in bench-scale capillary tests and in a continuous steriliser as affected by combined liquid temperature and pH. *4th International Conference, Predictive Modelling in Foods*, Quimper, France, June 15th – 19th, Predictive Modelling Methodologies: Inactivation (Session 5), pp 170-173. (ISBN 9 05682 400 7).

Refereed Australasian Conferences

Khoo K Y, Davey K R and Thomas C J 2003 Comparison of four thermal inactivation models for the combined effect of temperature and pH on *Escherichia coli* in liquid. *31st Australasian Chemical Engineering Conference (Products and Processes for the 21st Century)*, CHEMECA 2003, Adelaide, SA, Australia, September 29th – October 1st, Food and Wine 1 (Session 3C), paper 062. (ISBN 0 86396 829 5).

Khoo K Y, Davey K R and Thomas C J 2004 Assessment of a new quantitative model for predicting tailing in batch survivor data as affected by combined temperature and pH. *32nd Australasian Chemical Engineering Conference (Sustainable Processes)*, CHEMECA 2004, Australia Technology Park, Sydney, NSW, Australia, September 26th – 29th, Food 2 (Session H2), paper 152. (ISBN 1 87704 012 6).