

International Journal of Food Microbiology, Volume 155, Issues 1–2, 2 April 2012, Pages 29-35

**Modelling of Bacterial Growth with Shifts in Temperature Using
Automated Methods with *Listeria monocytogenes* and *Pseudomonas
aeruginosa* as examples**

Magdi Salih, Ioannis Mytilinaios, H K Schofield and RJW Lambert

Applied Microbiology Group,
Cranfield Health
Cranfield University
Cranfield MK43 0AL, U.K.

July 2011

37
38
39
40
41
42
43
44
45
46
47
48
49
50
51

Abstract

Time to detection (TTD) measurements using turbidometry allow a facile method for the measurement of bacterial growth rates under isothermal conditions. Growth rate measurements were carried out for *Listeria monocytogenes* at 25, 30 and 37°C and for *Pseudomonas aeruginosa* over the temperature range 25 to 45°C. No lags were observed with any of the isothermal studies. The classical 3 parameter logistic model was rearranged to provide the theoretical foundation for the observed TTD. A model was subsequently developed for the analysis of TTD data from non-isothermal studies based on the Malthusian approximation of the logistic model. The model was able to predict the TTD for cultures of *L. monocytogenes* or *P. aeruginosa* undergoing simple temperature shunts (e.g. 25 to 37°C and vice versa), and for a multiple temperature shunt for *L. monocytogenes* (25-37-25-37°C and 37-25-37-25°C) over a period of 24 hours. In no case did a temperature shunt induce a lag.

1 Introduction

The measurement of microbial growth rates, especially its temperature dependency, is of fundamental importance in food microbiology. For many food pathogens growth above 25 and below 45°C is usually rapid with an optimum around 37°C. Below 5 degrees only a few (often spoilage, e.g. *Pseudomonas aeruginosa*, but pathogens are also represented e.g. *Listeria monocytogenes*) have growth rates that would give rise to concern. This has been shown, for example, by [Thomas and O'Bierne \(2000\)](#) on the temperature abuse of vegetables with respect to spoilage (lactic acid bacteria) and risk (*L. monocytogenes*).

Within the literature several studies have looked at the effect of non-isothermal conditions on microbial growth using established modeling methods (e.g. [Baranyi et al., 1995](#); [Bovill et al., 2000](#); [Dalgaard et al., 2002](#); [Giannakourou et al., 2005](#); [Koutsoumanis, 2001](#); [Koutsoumanis et al., 2006](#); [Li and Torres, 1993](#); [Taoukis et al., 1999](#); [Zwietering et al., 1994](#)). The aim of many of these studies was to test the ability of using models based on growth data obtained isothermally to predict growth under non-isothermal conditions. [Zwietering et al., \(1994\)](#) concluded that, within the exponential phase, the hypothesis of no lag occurrence was accepted statistically in more than 70% of their experiments for *Lactobacillus plantarum*, however within the lag phase, the hypothesis of additional lag occurrence was accepted statistically in more than 90% of their experiments.

[Corradini and Peleg \(2005\)](#) have eloquently questioned the reasoning and conclusions being drawn from the use of the empirical standard primary and secondary models used to interpret and predict data from isothermal and fluctuating temperature studies. They suggest abandoning specific formats and using, instead, a generalized scheme for both primary and secondary modeling, "in the absence of a decisively superior theoretical model... [ad hoc empirical models] have the advantage of being simpler mathematically and free of assumptions that require independent verification".

79 Automated techniques such as turbidometry tend to come under fire from traditional
80 microbiologists since they cannot directly reproduce the standard microbial growth curve, which the
81 multitude of primary models are fitted to ([Augustin et al. 1999](#); [Dalgaard et al. 1994](#); [McClure et al.](#)
82 [1993](#)), yet their very persistence reflects their ease of use, the high quantity and quality of the data
83 obtained and the large savings in consumable costs over that of the traditional (plate-count)
84 methods. We have recently shown that the modified Gompertz and modified logistic models are at
85 odds with the observed time to detection data obtained using turbidometry ([Mytilinaios et al. 2011](#)).
86 The classic logistic model (and by default the Baranyi equation) were the only models used able to
87 reconstruct the observed TTD data. The three parameter model has a firm (if simple) theoretical
88 foundation. Its application to standard microbiological data results in mismatch due to
89 methodological inadequacies (plate counting) and the presence of lag and so is rarely used in its
90 original form. Herein we further examine the application of the basic logistic model to microbial
91 growth data (obtained as TTD) and use small temperature shifts (or shunts) to examine their effect
92 on the growth rates of *Listeria monocytogenes* and *Pseudomonas aeruginosa*.

93 2 Methods

94 2.1 CULTURE PREPARATION:

95 *Pseudomonas aeruginosa* (ATCC 15442) or *Listeria monocytogenes* (L-252, an industrial isolate
96 provided by Nestlé Research Centre, Lausanne– obtained from contaminated terrine) was grown
97 overnight in conical flasks containing 80 ml Tryptone Soya Broth, TSB (Oxoid CM 129) shaking
98 continuously at 30°C. The cells were harvested, centrifuged (510g, 10 minutes) and the resulting
99 cell pellets resuspended in 2 ml TSB. The inoculum was standardised by diluting to an
100 approximate OD=0.5 at 600 nm giving approximately 2×10^9 cfu/ml. This standardised culture was
101 subject to either ten decimal or ten half-fold dilutions in TSB.

102

103 2.2 PREPARATION OF MICRO-ARRAY PLATES

104 Each well in the Bioscreen micro-array plates was filled as follow: all wells except column 10
105 received 200µl of growth broth (TSB). The wells of column 10 were given 400µl of the appropriate
106 serial dilutions (decimal or half fold), with the highest inoculum (the zero dilution) in well 100. Using
107 a multi-pipette, 200µl were removed from each well of column 10 and transferred into the wells of
108 column 9, mixed by repeated syringing, and then 200µl were removed (using new tips) from the
109 wells of column 9 and transferred to column 8 etc. This was repeated across the plate discarding
110 200µl after the final mixing in column 1.

111

112 From the -5 and -6 decimal dilutions, 0.1 ml of each was transferred and spread onto previously
113 prepared tryptone soya agar (TSA) plates in triplicate and incubated at 30°C for 2 days. Plates with
114 <300 cfu were counted and the approximate log number of the initial (zero dilution) culture
115 calculated.

116

117 For the study of the growth rates, identical plates were placed in different Bioscreens set at
118 particular temperatures. After a given time of incubation the plates were swapped between the
119 machines, without changing the running of the machines. Typical experiments lasted 1 to 2 days.
120 The optical density of the wells was read at 600nm every ten minutes.

121

122 One particular effect was noted with Bioscreen data when the plates were removed from a higher
123 incubation temperature to a lower (but not vice-versa); a kink in the OD/time plot due to the
124 temporary presence of condensation on the underneath of the lid of the Bioscreen plates. In
125 general the condensation took between 30 to 50 minutes to evaporate. Thus, TTD which met the
126 OD criterion were censored during the 30 to 50 minutes after the transfer.

127

128 **2.3 DATA ANALYSIS**

129 From the resulting Bioscreen OD/time data, the background OD due to the media was removed
130 from each. A time to detection (TTD) criterion of OD = 0.2 was then used on the background
131 corrected data: TTD were found using linear interpolation between OD/time values which
132 straddled the OD = 0.2 value.

133 **2.4 MODEL DEVELOPMENT**

134 Theoretical Background: From the classical logistic equation

135
$$N = \frac{M}{1 + \left(\frac{M}{N_0} - 1 \right) e^{-\mu t}} \quad (1)$$

136 where μ is the specific growth rate and M is the maximum population density (also known as the
137 carrying capacity, cfu/ml), the time taken (TTD_N) to reach a specific population level (N) from a
138 given initial value (N₀, cfu/ml) is given by

$$TTD_N = -\frac{1}{\mu} \ln N_0 + \frac{1}{\mu} \ln \left(\frac{N(M - N_0)}{M - N} \right) \quad (2)$$

The TTD_N is defined as the time to reach a given detection threshold (e.g. an optical density of 0.2) for which N_d is the equivalent microbial numbers per ml. If the assumption that $M \gg N_0$ is made then this can be approximated by

$$TTD = -\frac{1}{\mu} \ln N_0 + \frac{1}{\mu} \left\{ \frac{N_d M}{M - N_d} \right\} \quad (3)$$

When $N_0 = 1$, the TTD is given by the right hand expression of eq.3, if $M \gg N_d$ then this can be approximated by N_d/μ . Hence, a plot of the initial inoculum against the TTD will give a gradient equal to the negative reciprocal of the growth rate, the TTD intercept at $N_0 = 1$, is the time taken for one organism to reach the TTD criterion. This expression can be considered as the basis of the methodology of [Cuppers and Smelt \(1993\)](#) described below. In the presence of a lag equation 2 can be supplemented with a lag term, else the Baranyi equation can be used, but the required parameters have to be found using a more involved numerical technique.

Geometric or Malthusian model

For a given set of environmental conditions a plot of the log of the initial inoculum size against the time to detection (TTD) gives a straight line relationship with gradient equal to the reciprocal of the specific growth rate ([Cuppers and Smelt 1993](#)). In the absence of a lag the line will intersect the log initial inoculum axis at the detection value for the given OD criterion used.

If two microtitre plates each containing identically prepared multiple inocula are incubated at different temperatures then each inoculum will grow at a rate dictated by the temperature and media conditions. The difference between the two plates will be governed only by the differential effect of the temperature. For each initial inoculum the time to detection can be given by

163
$$TTD = m (\log I - \log I_D) \quad (4)$$

164 Where $\log I$ is the log of the initial inoculum used, $\log I_D$ is the log of the detection number and m is
 165 the gradient of the TTD, $\log I$ plot.

166 We hypothesise that if at time t_i the incubation temperature is changed then, whether in the
 167 absence or not of an induced lag, the growth rate will increase or decrease to meet the demands of
 168 the new incubation temperature. In the absence of any induced lag Figure 1 shows the expected
 169 result on the time to detection/log initial inoculum plot. Incubation at temperature T_0 gives an
 170 observed gradient of M_0 ; at time t_i , the temperature is changed to T_1 (where, in this case, T_0 is
 171 more optimal for growth than T_1), the gradient changes to M_1 , commensurate with the new
 172 incubation temperature.

173

174 From simple geometrical arguments it can be shown that the TTD for multiple changes of
 175 temperature is given by equation 5.

176

177 For time: $t_i < t < t_{i+1}$, for $i = 0, 1, 2, \dots$, where $t_0 = 0$,

178

179
$$\text{---} \quad (5)$$

180 For example,

181 For $i = 0$

182

183 For $i = 1$, ---

184

185 For $i = 2$, $\text{---} \quad \text{---}$

186

187 If lags are induced at t_i , then the expression for t_i can be replaced by $t_i = t_i + \lambda_i$. For a given
188 experiment the values of t_i are fixed.
189 Since the models are simple linear models, the majority of the modelling was carried out using
190 Excel (Microsoft) and the data analysis add-in package.

191 **3 Results**

192 **3.1 ISO-THERMAL STUDIES**

193 A plot of the optical density/time curves for multiple inocula of *Listeria monocytogenes* 252 (from
194 1.2×10^9 cfu ml⁻¹ to 1×10^4 cfu ml⁻¹) incubated at 25°C is shown in [Figure 2](#) (without background
195 correction carried out). From analyses of the curves for inocula which had initial OD greater than
196 the background it was evident that there was no lag present. A plot of the time to detection defined
197 as the time to reach an OD = 0.2 against the log of the initial inoculum gave a straight line plot
198 ([Figure 3a](#)). The gradient of the fitted line is the reciprocal of the growth rate, and the intercept on
199 the TTD axis is the time taken for one organism to reach the detection criterion. For zero lag the
200 line cuts the log initial inoculum axis at the log inoculum size equivalent to the optical density of the
201 TTD criterion. The microbial density obtained from direct plating of an OD = 0.2 at 600nm in the
202 Bioscreen was equivalent to $9.09 \log_{10}$ cfu ml⁻¹ (95% CI: 8.97 – 9.21). Similar findings with respect
203 to lag and the fit of the TTD data were found with data obtained at 30 and 37°C ([Table 1](#)).

204

205 *Pseudomonas aeruginosa* was analysed in a similar manner but over a wider range of
206 temperatures ([Table 1](#)). An optimum growth temperature of approximately 39°C was apparent;
207 with a specific growth rate of 1.51 ln cfu/ml/hr. None of the studies conducted showed the
208 presence of a lag. [Figure 3b](#) shows data obtained at 37 and 30°C, the fitted lines cut the log
209 inoculum axis at the detection number threshold value. The data shown for growth at 37°C has
210 approximately constant variance until the initial inoculum level is less than 10^2 cfu/ml. Below this
211 level the variance increases. To preclude the need for weighted regression data below this
212 threshold were censored in the regression fits.

213

214 **3.2 NON-ISOTHERMAL STUDIES**

215 Two temperature shunt studies

216 *Listeria monocytogenes*: [Figure 4](#) shows the observed TTD data from two plates incubated initially
217 for 400 mins at 25 and 37°C and then transferred to the Bioscreens incubating at 37 and 25°C
218 respectively; [Figure 5](#) shows a similar experiment done with incubation at 30 and 37°C. The
219 gradients of the lines are given in [Table 2](#) and in each case the gradients obtained follow
220 approximately those found from the isothermal data ([Table 1](#)). Further, there were no indications of
221 induced lags after the plates were exchanged. A lag would result in a vertical gap between the two
222 rates.

223 *Pseudomonas aeruginosa*: [Table 4](#) gives the observed gradients and intercepts for a temperature
224 shunt of 25 to 39°C and vice versa and also for 34 and 39°C. [Figure 6](#) shows the data obtained
225 from the Bioscreen incubating at 37°C for the 37 to 30°C shunt. The initial incubation at 37°C gave
226 a gradient of -95.2 (-99.90 to -90.50 : 95% CI) mins/log₁₀ cfu/ml; the gradient of the plate initially
227 incubated at 30°C but placed into the 37°C incubator after 300mins was -92.96 (-96.9 to -89.02 :
228 95% CI) mins/log₁₀ cfu/ml. The horizontal separation of the two was calculated as 0.887
229 log₁₀cfu/ml., i.e. the plate incubated at 30°C was growth retarded relative to growth at 37°C by just
230 less than 1 log cfu/ml after 300 minutes of incubation.

231

232 Multiple Temperature Shunts: [Figure 7](#) shows the observed times to detection for multiple initial
233 inocula of *L.monocytogenes* undergoing either a 37-25-37-25°C or a 25-37-25-37°C temperature
234 incubation sequence, changing temperatures after 360, 500 and 900 minutes. The observed
235 gradients were -107.9, -179.3,-105.2,-NA for the 37-25-37-25°C sequence and -193.5,-104.7,-
236 172.6,-110.0 for the 25-37-25-37°C sequences respectively. Superimposed on [Figure 7](#) are the
237 predicted values from the Geometric model, the TTD predictions of which are based on the growth
238 rate data given in [Table 1](#).

239

240 Modelling

241 The Geometric model (Eq. 5) can be either used to predict the outcome of hypothetical experiments
242 –as was done for the multiple temperature shunt with *Listeria* shown in [Figure 7](#), or can be used to

243 fit the observed data by minimising the sum of squares of the errors. Another method of using the
244 predictive capacity of the model is to predict the TTD observed from a single Bioscreen incubating
245 at a given temperature, when identical plates are moved in or out of the machine. [Figure 8](#) shows a
246 prediction of the pattern of TTD/log initial inocula from the single Bioscreen incubating at 37°C.
247 Using the growth rates described in Table 1, in 360 minutes the model predicts that 3.27 logs of
248 growth will occur in this plate, whereas the other plate incubating in the other machine at 25°C will
249 increase by only 1.97 logs. When the latter plate is placed in the machine at 37°C, if there are no
250 lags then over the next 240 minutes there will be further increase of 2.18 logs in this plate. By
251 calculating the log increase in the numbers of *L. monocytogenes* at 37°C and that incubated at
252 25°C and then subsequently placed at 37°C, the pattern shown in [Figure 8](#) was obtained. The
253 observed data are overlain on the predicted lines. No lags were observed.
254

255 4 Discussion

256 The simple, classical 3-parameter logistic model can model the time to detection data obtained
257 from turbidometric experiments using multiple initial inocula incubated iso-thermally. In all cases
258 studied no lags were observed either from the OD/incubation time plots or from the plotted or
259 modelled data. Plots of the log initial inoculum against the TTD cut the log I_0 axis at $9.22 \log_{10}$
260 cfu/ml (95% CI $9.05 - 9.4 \log_{10}$ cfu/ml) and at $9.15 \log_{10}$ cfu/ml (95% CI $8.9 - 9.4$) for *L.*
261 *monocytogenes* and *P. aeruginosa* respectively. The detection number (N_d) for each species was
262 confirmed by plate counting and from calibration curves of OD against microbial numbers. In the
263 presence of a lag the plot would fail to cross the axis at the N_d , and a vertical separation equal to
264 the lag between the x-axis and the TTD of the N_d would be present. This was not observed in any
265 of the isothermal studies performed.

266

267 The linear approximation (eq.4) to the logistic expression (eq.2) assumes that $M \gg N_0$ and also
268 that $M \gg N_d$; when $N_0 = N_d$, TTD = 0. If $M < 10N_d$ then curvature of the observed TTD occurs, if M
269 $< 3 N_d$ then this curvature is substantial and the mismatch between eq.4 and eq.2 becomes
270 significant. In the cases studied here, $M > 10N_d$ in all cases and this curvature was not observed. If
271 the detection threshold is increased, e.g. use of a higher OD threshold, then curvature is observed.
272 Conversely, lowering the threshold would reduce any observed curvature. Equation 4 does not
273 require the estimation of the maximum population density (MPD) and in the absence of a lag is a
274 two parameter model. Rearranging eq. 4 results in the Malthusian approximation of biological
275 growth – i.e. growth without limit. The value of M is, however, used in the full form of the logistic
276 model. M can be obtained through plate count, from dilutions of the MPD culture to produce a
277 calibration curve using OD or from using the phenomenon of curvature discussed above.

278

279 When a temperature shunt was applied to growing bacteria, the cultures reduced or increased
280 their growth rate commensurate with the incubation temperature. When cultures were shunted

281 from a lower temperature to a higher temperature there was no evidence of an induced lag and
282 growth continued at the rate dictated by the new temperature. When cultures were shunted from a
283 higher to a lower temperature condensation on the inside of the plate lid occurred and this led to
284 unusable data for a period after the shunt (the period depended on the temperature difference).
285 From the observed, fitted and predicted data it can be concluded that no induction of lag occurred
286 when moving from the higher to the lower temperatures used: the intercept of the regression lines
287 for each temperature coincide at the time of the temperature shunt, if lags were present this would
288 not occur (e.g. Figure 7).

289

290

291

292

293

5 References

- Augustin, J.-C., Rosso, L., and Carlier, V., 1999. Estimation of temperature dependent growth rate and lag time of *Listeria monocytogenes* by optical density measurements, *Journal of Microbiological Methods* 38, 137–146.
- Baranyi, J., Robinson, T.P., Kaloti, A., and Mackey, B.M., 1995. Predicting growth of *Brochothrix thermosphacta* at changing temperature, *International Journal of Food Microbiology* 27, 61-75.
- Bovill, R., Bew, J., Cook, N., D'Agostino, M., Wilkinson, N., and Baranyi, J., 2000. Predictions of growth for *Listeria monocytogenes* and *Salmonella* during fluctuating temperature, *International Journal of Food Microbiology* 59, 157–165.
- Corradini, M.G. and Peleg, M. 2005. Estimating non-isothermal bacterial growth in foods from isothermal experimental data. *Journal of Applied Microbiology* 99, 187-200.
- Cuppers, H.G.A.M and Smelt, J.P.P.M., 1993. Time to turbidity measurement as a tool for modelling spoilage by *Lactobacillus*. *Journal of Industrial Microbiology* 12, 168-171.
- Dalgaard, P., Buch, P., and Silberg, S., 2002. Seafood Spoilage Predictor—development and distribution of a product specific application software, *International Journal of Food Microbiology* 73, 343– 349.
- Dalgaard, P., Ross T., Kamperman, L., Neumeyer, K., and McMeekin, T.A., 1994. Estimation of bacterial growth rates from turbidimetric and viable count data. *International Journal of Food Microbiology* 23, 391–404.
- Giannakourou, M.C., Koutsoumanis, K., Nychas, G.J.E. and Taoukis, P.S., 2005. Field evaluation of the application of time temperature integrators for monitoring fish quality in the chill chain, *International Journal of Food Microbiology* 102 323– 336.
- Koutsoumanis, K., 2001. Predictive modelling of the shelf life of fish under nonisothermal conditions. *Applied and Environmental Microbiology* 67, 1821–1829.

327 Koutsoumanis, K., Stamatiou, A., Skandamis, P. and Nychas, G.-J.E., 2006. Development of a
328 microbial model for the combined effect of temperature and pH on spoilage of ground meat, and
329 validation of the model under dynamic temperature conditions. *Applied and Environmental*
330 *Microbiology* 72,124-134.
331
332 Li, K.-Y. and Torres, A. J., 1993. Microbial growth estimation in liquid media exposed to
333 temperature fluctuations. *Journal of Food Science* 58, 644-648.
334
335 McClure, P.J., Cole, M.B., Davies, K.W., and Anderson, W.A., 1993. The use of automated
336 turbidimetric data for the construction of kinetic models. *Journal of Industrial Microbiology* 12, 277–
337 285.
338
339 Mytilinaios, I., Salih, M., Schofield, H.K. and Lambert, R.J.W. 2011. Growth curve prediction from
340 optical density data. *International Journal of Food Microbiology* *submitted*.
341
342 Taoukis, P.S., Koutsoumanis, K., Nychas, G.-J.E., 1999. Use of time temperature integrators and
343 predictive modelling for shelf life control of chilled fish under dynamic storage conditions.
344 *International Journal of Food Microbiology* 53, 21– 31.
345
346 Thomas, C. and O’Beirne, D., 2000. Evaluation of the impact of short-term temperature abuse on
347 the microbiology and shelf life of a model ready-to-use vegetable combination product,
348 *International Journal of Food Microbiology* 59, 47–57.
349
350 Zwietering, M. H., De Wit, J. C., Cuppers, H. G. A. M., and Van 'T Riet, K., 1994. Modeling of
351 Bacterial Growth with Shifts in Temperature, *Applied and Environmental Microbiology* 60, 204-213.
352

Table 1. Isothermal Rate data

Organism	Temp/°C	gradient	LCI	UCI	Intercept	LCI	UCI	r ²	Obs
<i>Listeria monocytogenes</i>	25	-183.40	-185.94	-180.86	1687.6	1671.7	1703.6	0.993	153
	30	-130.30	-131.90	-128.70	1221.4	1211.0	1231.7	0.994	153
	37	-109.98	-110.85	-109.10	999.22	993.90	1004.6	0.997	160
<i>Pseudomonas aeruginosa</i>	25	-202.24	-205.33	-199.15	1908.7	1889.2	1928.1	0.991	161
	27	-140.73	-143.54	-137.92	1295.1	1279.1	1311.1	0.984	157
	30	-123.84	-125.24	-122.43	1122.4	1114.3	1130.5	0.994	154
	34	-99.17	-100.80	-97.54	883.05	873.74	892.34	0.989	157
	35	-96.04	-97.52	-94.57	889.61	880.60	898.62	0.991	149
	37	-91.80	-92.86	-90.75	846.13	840.00	852.27	0.995	157
	39	-91.38	-93.26	-89.49	826.70	818.78	834.78	0.987	122
	42	-100.84	-102.67	-99.01	923.14	911.90	934.37	0.988	147
	45	-125.81	-128.41	-123.21	1137.8	1121.7	1153.9	0.983	160

Table 2. Temperature shifts: *Listeria monocytogenes*

25 to 37°C						37 to 25°C				
		Coefficients	LCL	UCL	r ² (obs)		Coefficients	LCL	UCL	r ² (obs)
t < 400 mins	Intercept	1758.9	1645.7	1872.0	0.943	Intercept	972.97	954.82	991.12	0.984
	m0	-194.75	-209.15	-180.35	(47)	m0	-107.88	-110.46	-105.30	(112)
t > 400 mins	Intercept	1121.9	1110.1	1133.7	0.988	Intercept	1441.0	1418.5	1463.4	0.992
	m1	-104.25	-106.47	-102.02	(103)	m1	-198.8	-204.40	-193.21	(45)

30 to 37°C						37 to 30°C				
		Coefficients	LCL	UCL	r ² (obs)		Coefficients	LCL	UCL	r ² (obs)
t < 410 mins	Intercept	1333.6	1302.5	1364.6	0.985	Intercept	983.14	975.06	991.23	0.997
	m0	-147.31	-151.55	-143.07	(72)	m0	-109.30	-110.48	-108.12	(108)
t > 410 mins	Intercept	1068.5	1057.7	1079.4	0.991	Intercept	1166.1	1144.5	1187.7	0.986
	m1	-106.60	-108.82	-104.38	(84)	m1	-145.56	-151.02	-140.10	(44)

Table 3. Temperature shifts: *Pseudomonas aeruginosa*

25 to 39°C						39 to 25°C				
		Coefficients	LCL	UCL	r ² (obs)		Coefficients	LCL	UCL	r ² (obs)
t < 300 mins	Intercept	2081.9	1783.8	2380.0	0.91	Intercept	854.35	820.60	888.1	0.951
	m0	-228.26	-264.20	-192.33	(19)	m0	-91.89	-96.58	-87.21	(80)
t > 300 mins	Intercept	1035.8	1025.4	1046.1	0.987	Intercept	1639.5	1600.7	1678.2	0.972
	m1	-91.79	-93.57	-90.00	(140)	m1	-208.55	-216.8	-200.31	(75)

34 to 39°C						39 to 34°C				
		Coefficients	LCL	UCL	r ² (obs)		Coefficients	LCL	UCL	r ² (obs)
t < 300 mins	Intercept	907.07	866.26	947.89	0.954	Intercept	811.66	832.49	854.58	0.980
	m0	-99.12	-104.81	-93.43	(61)	m0	-89.00	-91.96	-86.03	(75)
t > 300 mins	Intercept	868.04	855.48	880.59	0.982	Intercept	872.48	854.58	890.38	0.973
	m1	-92.64	-95.24	-90.02	(93)	m1	-100.47	-104.55	-93.39	(69)

Table 4. Observed parameters for the 37/25/37/25 and 25/37/25/37 temperature shunts of *Listeria monocytogenes*

time	Temp	Parameter	Estimate	LCI	UCI	r2 (obs)
		log Id	9.48	9.26	9.71	
<360	37	m0	-107.92	-108.36	-106.47	0.998(38)
<600	25	m1	-179.29	-188.04	-170.54	0.992 (17)
<900	37	m2	-104.62	-106	-103.25	0.998(52)
>900	25	m3	-	-	-	no obs

time	Temp	Parameter	Estimate	LCI	UCI	r2 (obs)
		log Id	9.58	7.98	11.52	
<360	25	m0	-193.5	-212.59	-174.4	0.981 (12)
<600	37	m1	-104.73	-106.54	-102.93	0.997(42)
<900	25	m2	-174.69	-189.12	-160.26	0.971 (21)
>900	37	m3	-109.52	-114.69	-104.35	0.984 (33)

Caption to Figures

Figure 1. The geometrical basis for equation 5: for a given rate (M_0), over the time period T_x initial inocula between $\log I_D - \log I_x$ will reach the detection threshold of $\log I_d$. At T_x the rate is changed (M_1) and the TTD now follow the new rate. If a lag is induced a vertical separation at T_x equal to the time of lag will be present before growth recommences.

Figure 2. Optical density/incubation time curves for *Listeria monocytogenes* incubated at 25°C.

Figure 3a. Observed TTD data for multiple initial inocula of *L. monocytogenes* (L252) at 25 (triangles), 30 (squares) and 37°C (circles). The solid lines are the fitted regression lines (Table 1)

Figure 3b. Observed TTD data for multiple initial inocula of *P. aeruginosa* ATCC 15442) at 30 (squares) and 37°C (circles). The solid lines are the fitted regression lines (Table 1)

Figure 4. Observed TTD data for *L. monocytogenes* incubating at 37 or 25°C with a temperature shunt to 25°C or 37°C (open circles and open squares respectively) after 400 minutes incubation at the initial temperature. Solid lines are the fitted regression lines (Table 2).

Figure 5. Observed TTD data for *L. monocytogenes* incubating at 37 or 30°C with a temperature shunt to 30°C or 37°C (open circles and open squares respectively) after 410 minutes incubation at the initial temperature. Solid lines are the fitted regression lines (Table 2).

Figure 6. Observed TTD data (symbols) for an initial plate of multiple inocula of *P. aeruginosa* incubating at 37°C for 300 minutes, replaced by an identical filled plate after incubation at 30°C for 300 minutes. The solid lines are the predicted TTD based on the data of Table 1 and the use of the logistic model to calculate the expected increase in numbers in both plates.

Figure 7. Observed TTD data for *L. monocytogenes* incubating at 37-25-37-25°C (open circles) or 25-37-25-37°C (open squares) with temperature shunts occurring at 360, 600 and 900 minutes. The solid lines are the predicted values based on the data of Table 1, with $N_d = 9.4$.

Figure 8. Observed TTD data (symbols) and predicted data (solid line) from a single Bioscreen incubating at 37°C for multiple inocula of *L. monocytogenes* undergoing plate changes to and from another machine incubating at 25°C (see figure 7). The solid lines are the predicted TTD based on the data of Table 1 and the use of the logistic model to calculate the expected increase in numbers in both plates during the periods of incubations at 37 and 30°C.

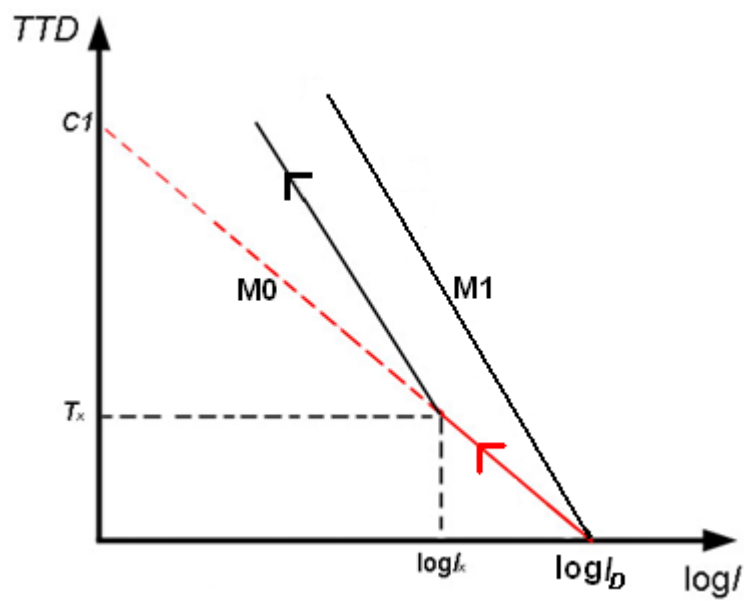


Figure 1.

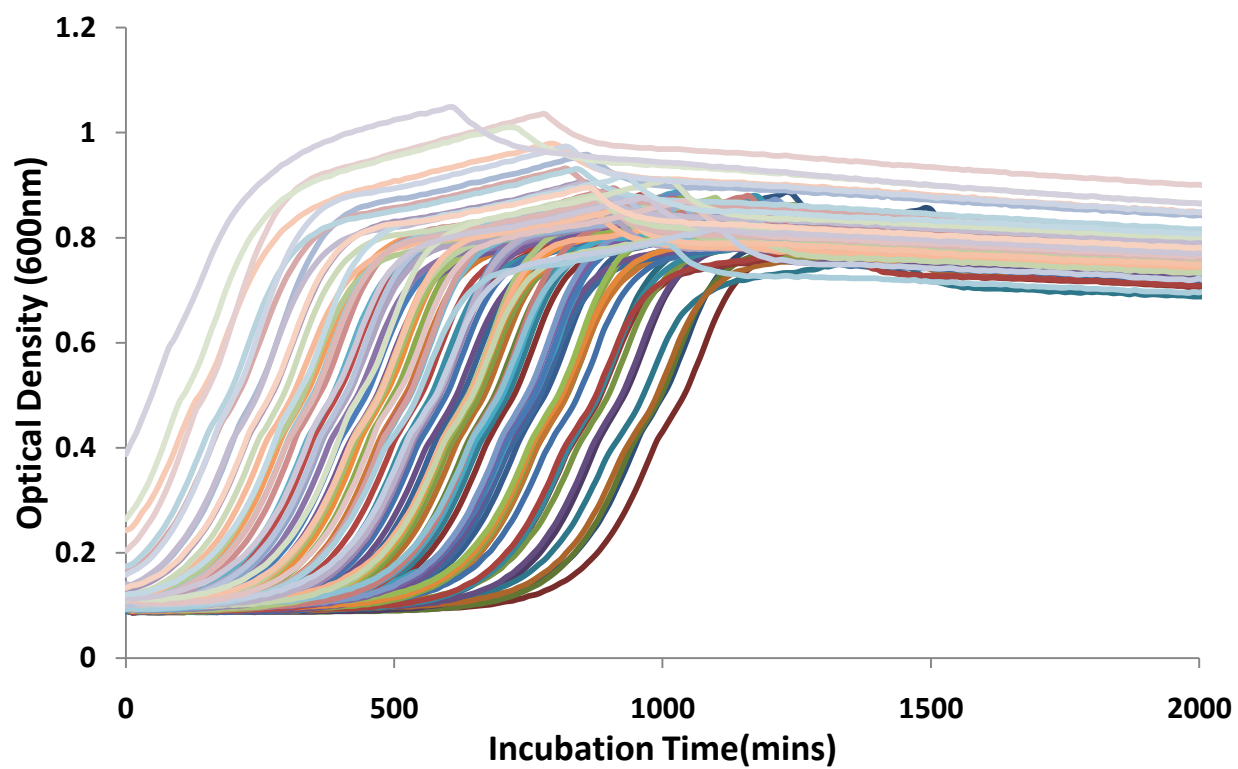
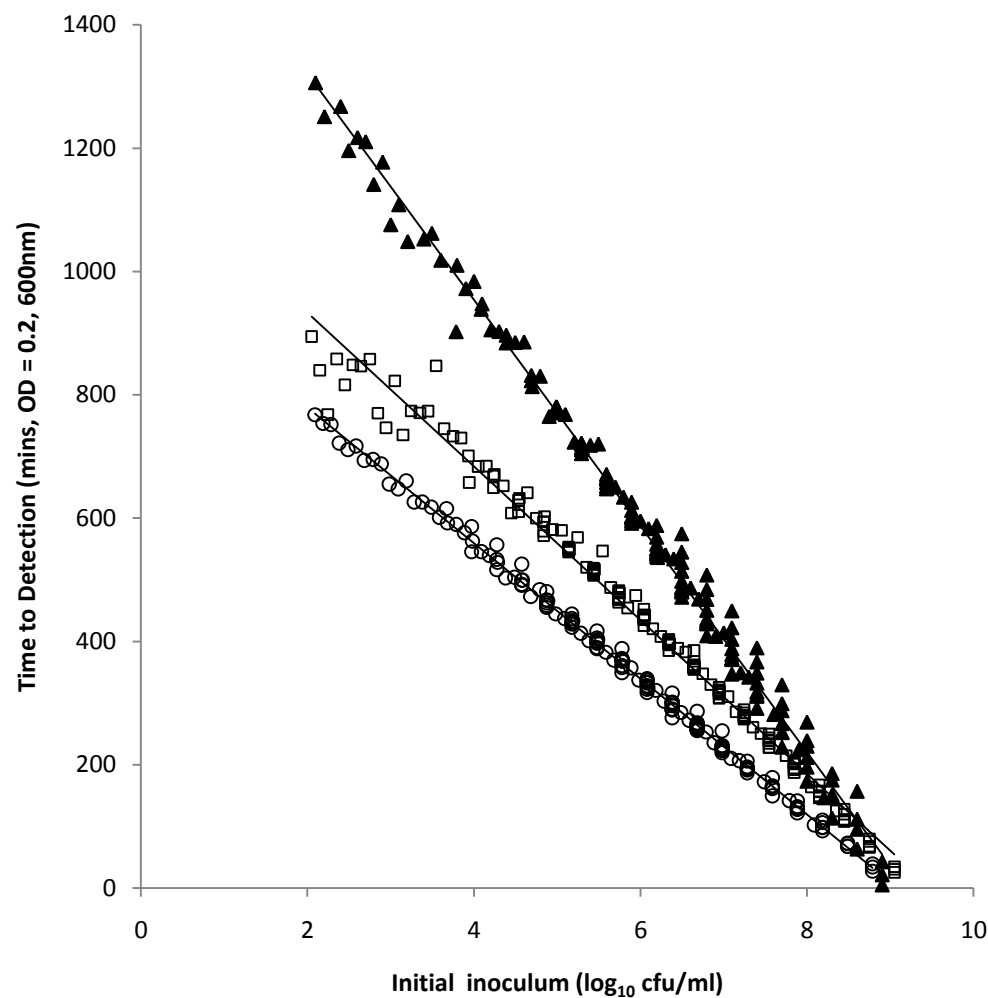


Figure 2.

Figure 3a



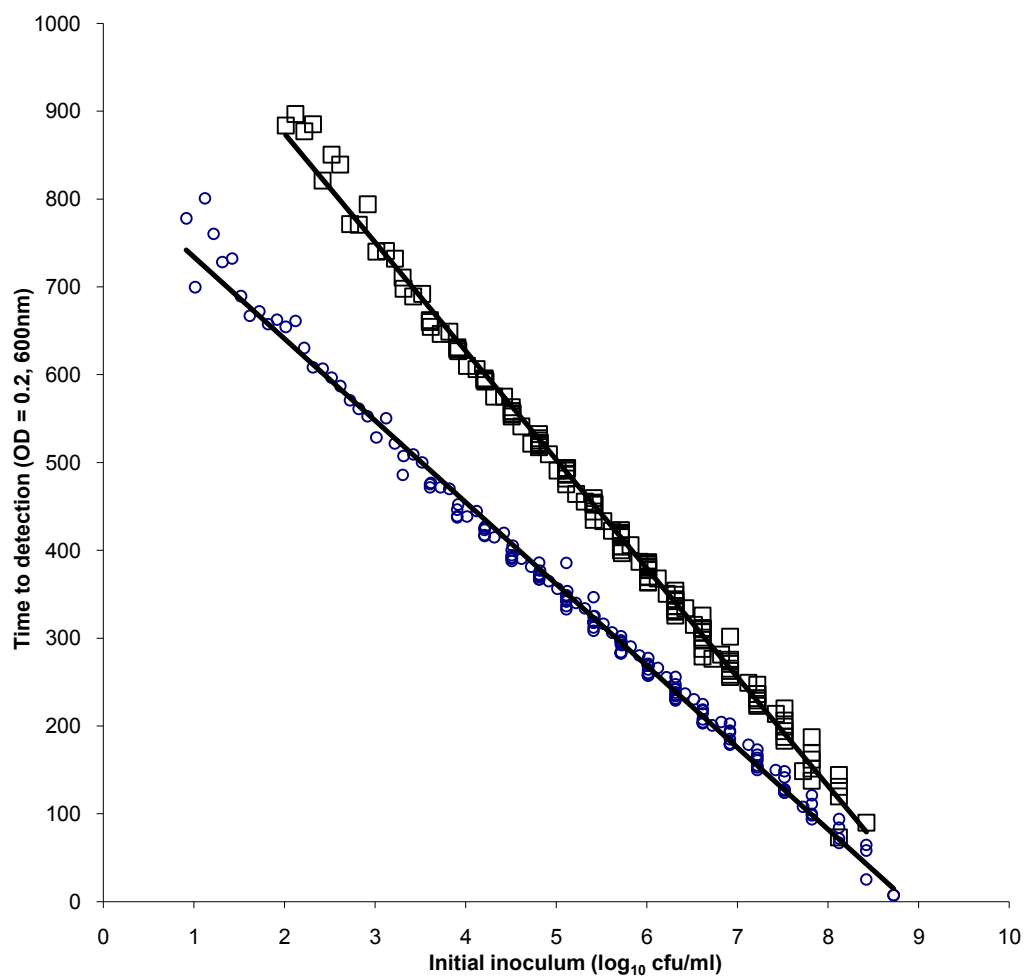


Figure 3b.

Figure 4.

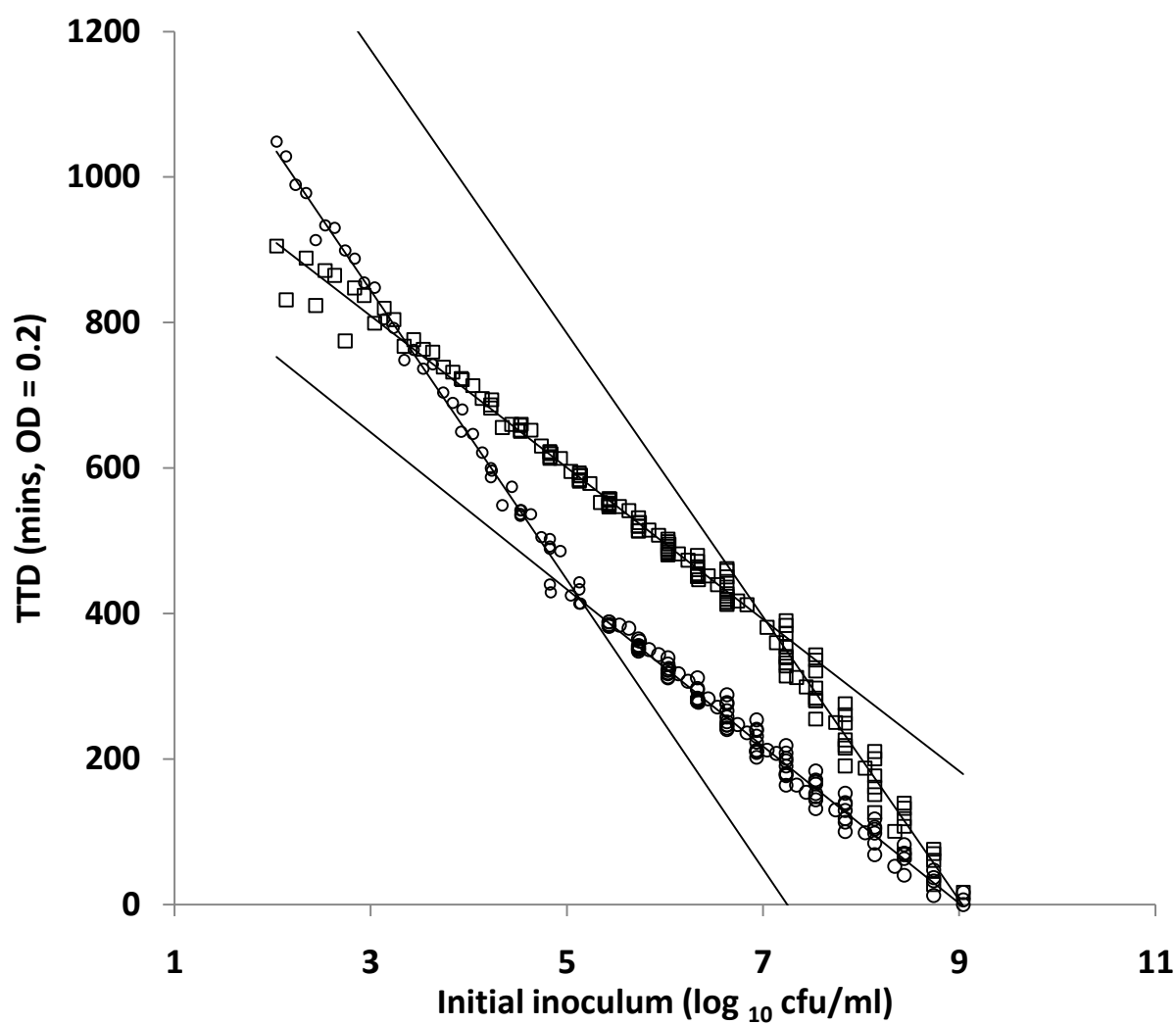


Figure 5.

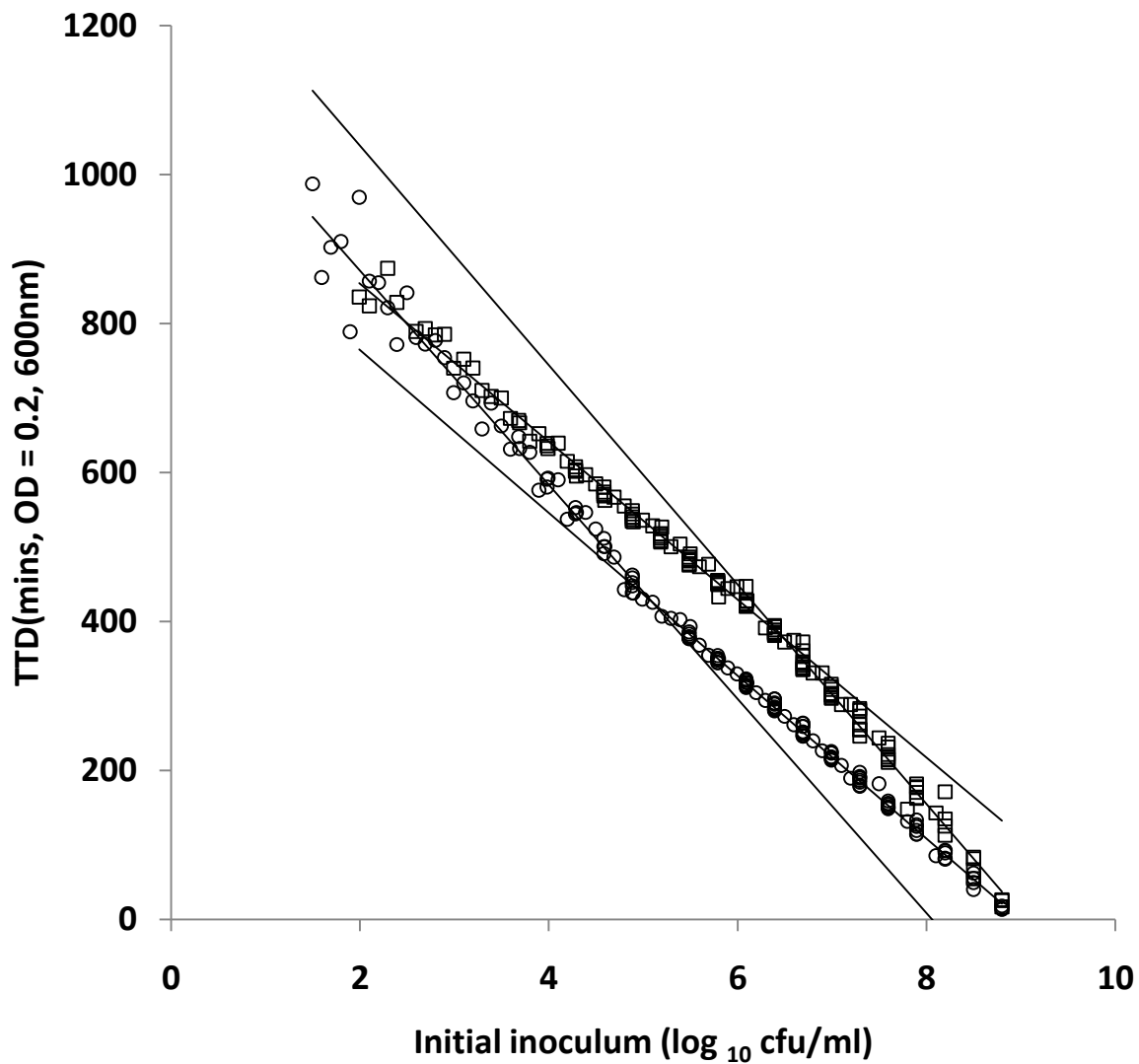


Figure 6

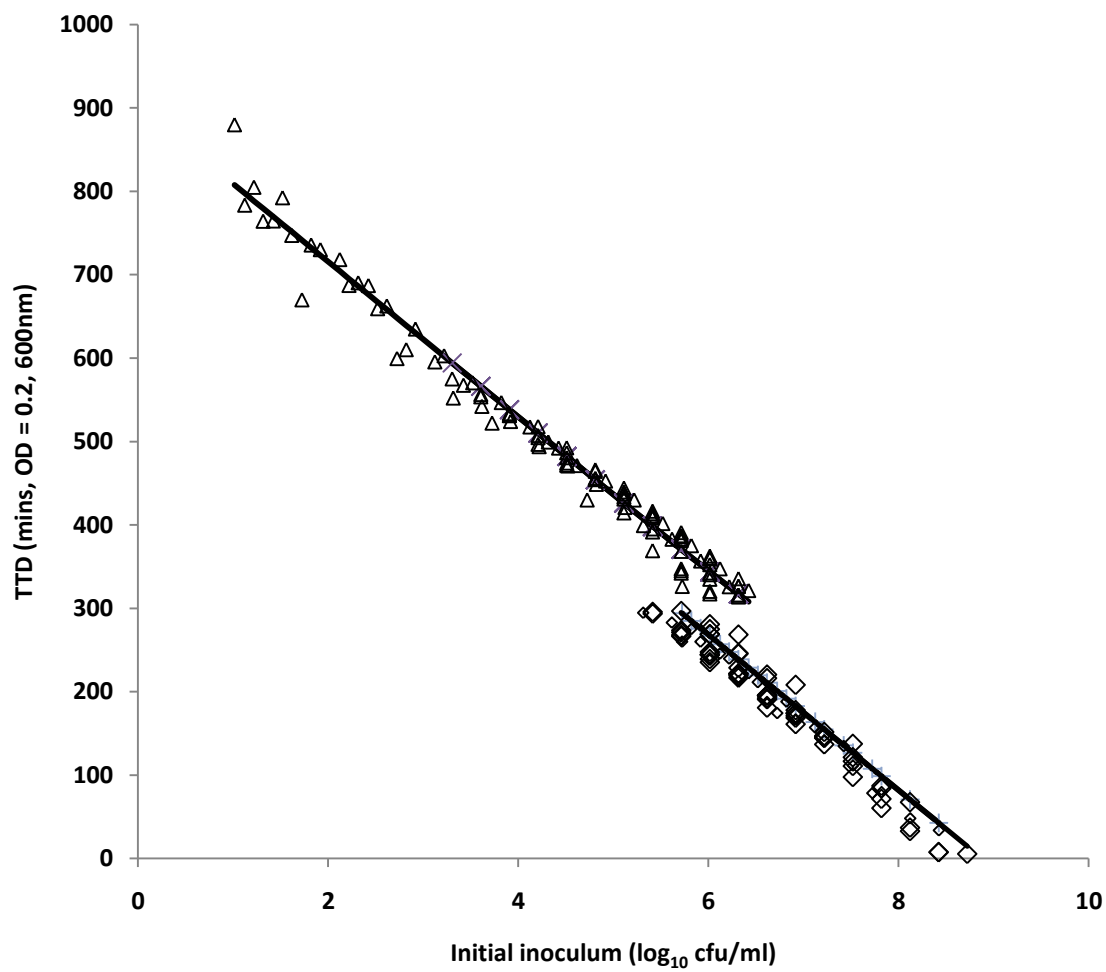


Figure 7

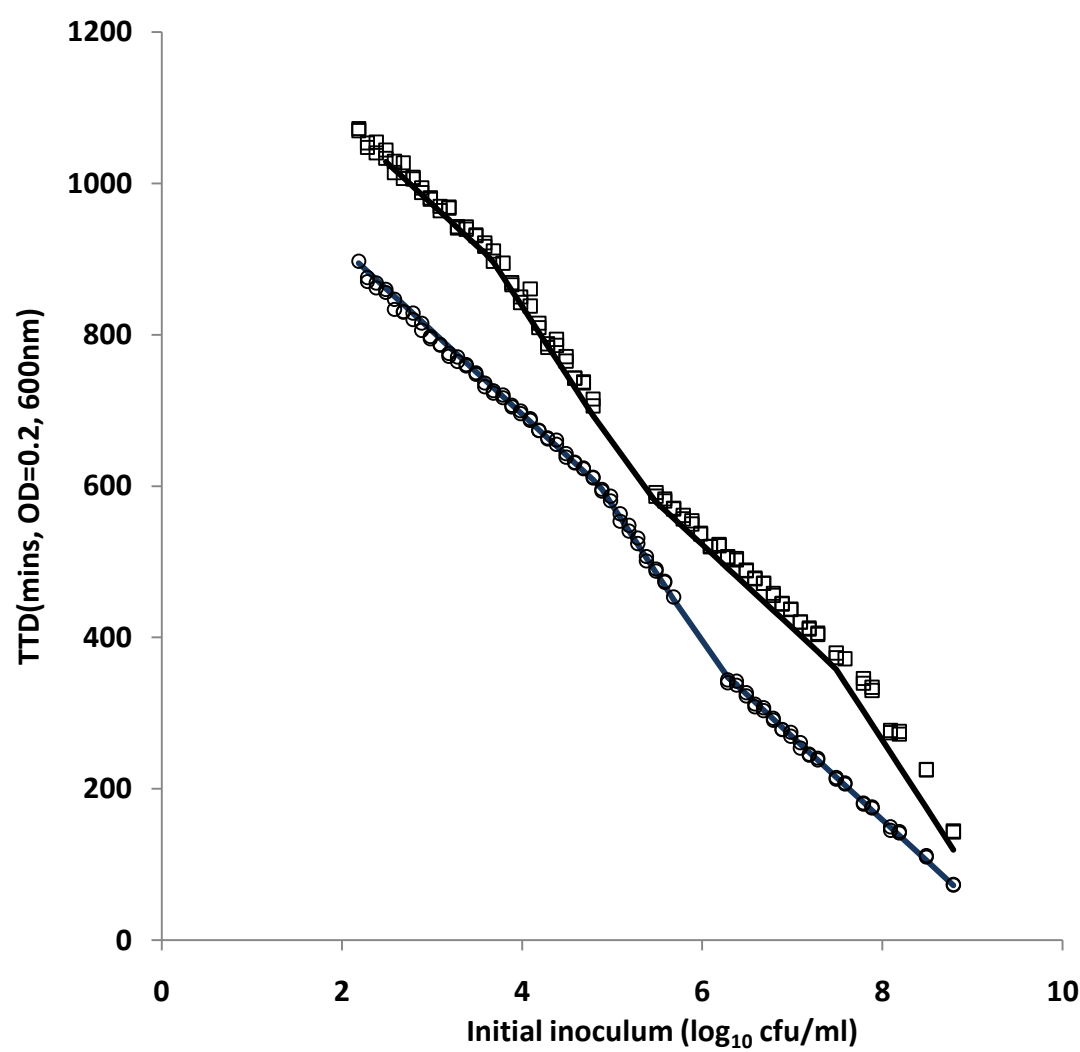
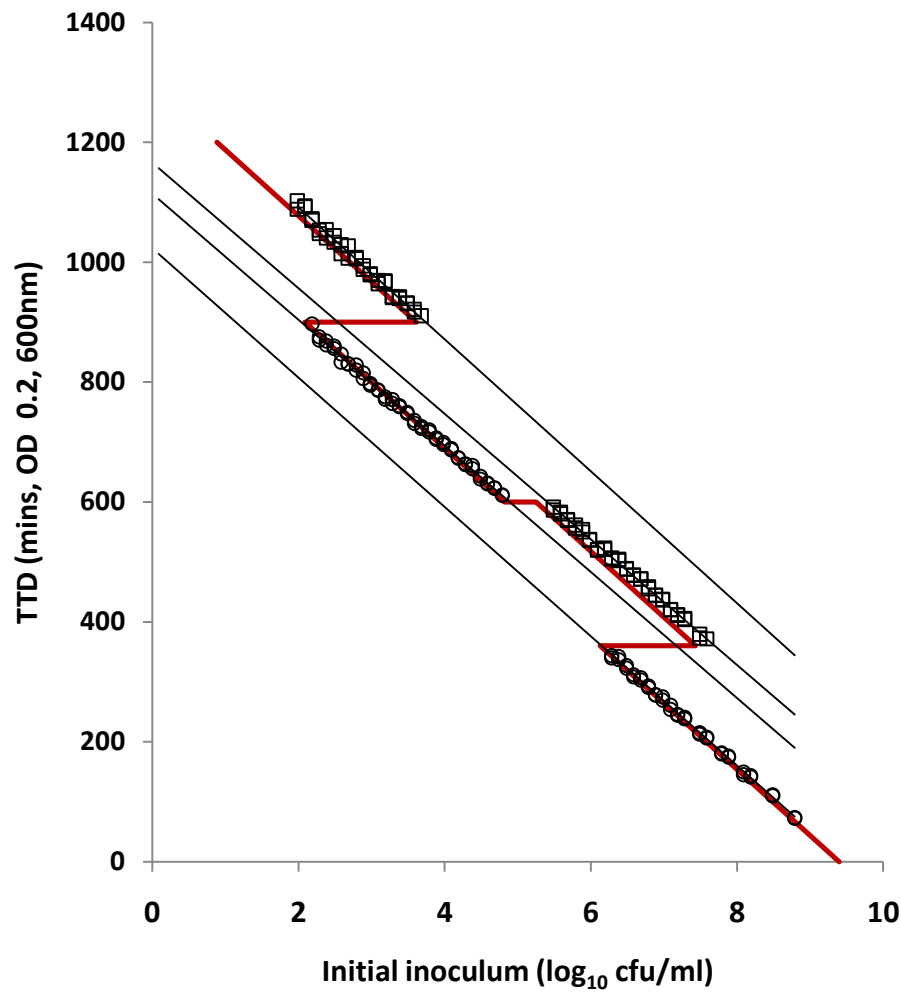


Figure 8





DSPACE

<https://dspace.org/>

Modelling of bacterial growth with shifts in temperature using automated methods with *Listeria monocytogenes* and *Pseudomonas aeruginosa* as examples

Salih, Magdi; Mytilinaios, Ioannis; Schofield, Hannah K.; Lambert, Ronald J. W.

2012-04-02T00:00:00Z

Elsevier Science B.V., Amsterdam.

<http://dx.doi.org/10.1016/j.ijfoodmicro.2012.01.011>

M. Salih, I. Mytilinaios, H.K. Schofield and R.J.W. Lambert. Modelling of bacterial growth with shifts in temperature using automated methods with *Listeria monocytogenes* and *Pseudomonas aeruginosa* as examples. *International Journal of Food Microbiology*, Volume 155, Issues 1–2, 2 April 2012, Pages 29-35.