Validation of growth and inactivation kinetics of *Listeria monocytogenes* in food products

MSc Food Safety Thesis

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ABSTRACT

Predictive modelling in food microbiology provides quantitative estimation of growth of micro-organisms. In the present study, the predicted growth kinetics of *Listeria monocytogenes* was validated in milk and ham incubated at 7°C. The prediction was in agreement with the growth study in BHI broth and ham, while it had a higher μ_{max} than the observed kinetics in milk of all three strains. Different size of inoculum of L6 and temperature of pre-culturing of FBR15 affected λ but not μ_{max}. Three *Listeria monocytogenes* strains were more heat resistant when they were inactivated in ham than in BHI broth and milk as heating media at 65°C and when they were grown in food product.

Keywords: Validation; *Listeria monocytogenes*; Food

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1. INTRODUCTION

Predictive microbiology using different mathematical models is widely used to quantitatively estimate microbial growth in foods under different physical or chemical conditions such as temperature, pH and water activity. It enables the prediction of microbial food safety and quality, surveillance of critical points within a food chain, optimization of safety controls from production to consumption and quantitative investigation of mechanisms and correlations between kinetic differences (Ross *et al.*, 2000; Zwietering *et al.*, 1990; Zwietering & den Besten, 2011). Also, it provides much faster results than microbiological challenge testing, storage testing and surveillance testing (Zwietering *et al.*, 1996).

Predictive growth models have been developed and applied to a wide range of pathogens, for example *Salmonella* (Gibson *et al.*, 1988; Silva *et al.*, 2009), *Bacillus cereus* (Bae *et al.*, 2012; Zwietering *et al.*, 1996), *Escherichia coli* O157:H7 (Ding *et al.*, 2012; Sutherland *et al.*, 1995), *Clostridium perfringens* (Juneja *et al.*, 2013; Smith & Schaffner, 2004), *Staphylococcus aureus* (Stewart *et al.*, 2002; Sutherland *et al.*, 1994) and *Yersinia enterocolitica* (Pin *et al.*, 2000; Sutherland & Bayliss, 1994). Among them, to estimate the specific growth rate of different micro-organisms, the Gamma concept is regarded as one of the best models since various variables as hurdle effects can be quantified. Besides, it enables the closest prediction of the growth data among other models including Pathogen Modeling Program (PMP, version5.0), Food MicroModel (FMM, version 2.5), GVdl Arrhenius equation, Patterson polynomial model, Duffy quadratic equation, Farber quadratic equation and Murphy polynomial model due to the smallest mean square error (MSE) (Te Giffel & Zwietering, 1999).

Listeria monocytogenes is a pathogen which has been found not only in environment, but also in many food products including milk and ham (Farber & Peterkin, 1991; Tompkin, 2002). It leads to a high mortality rate and is a serious threat to pregnant women, their unborn children, elderly people and immunocomprimised people (Allerberger & Wagner, 2010). According to the EFSA report in 2015, a total of 13 *Listeria* outbreaks with 1763 confirmed human listeriosis cases in Europe were reported in 2013. When combined with the data from previous years, it showed a statistically significant increasing trend from 2009 to 2013 (EFSA, 2015).

For *L. monocytogenes*, similar to other aforementioned pathogens, differences often occur between the prediction and the actual growth kinetics which are caused by several factors such as strain variability, biological variability, experimental variability and food product composition. Based on a previous study, strain variability is defined as the variability between strains from the same species; biological variability is defined as the variability between biologically independent reproductions of the same strain performed on different experimental days; and experimental variability is defined as the variability between parallel experimental replicates at the same time on the same experimental day (Aryani *et al.*, 2015a). By quantifying these variabilities, a more realistic prediction of growth kinetics could be achieved.

To check the accuracy of the predictive growth model proposed by Aryani *et al.*

(2015a), a validation study by microbial challenge testing in milk and ham was performed. Due to the wider acceptance of various bacterial growth experiments compared to other sigmoidal functions (Logistic, Richards, Schnute and Stannard) reported by Zwietering *et al.* (1990), Gompertz equation was regarded as a preferable model to be fitted to the growth data to obtain the real specific growth rate.

Furthermore, to investigate the other factors influencing thermal resistance (*D*-value), the thermal inactivation study was also conducted using milk and ham. The *D*-value,which was defined as the time required at a certain temperature to kill 90% of the micro-organisms, is frequently used to describe the thermal resistance of bacterial cells. In order to reduce the correlation between parameters β and δ to an acceptance level, a modified Weibull model was used to estimate *D*-values instead of Weibull model in previous studies (Aryani *et al.*, 2015b; Metselaar *et al.*, 2013).

The aim of this study was to validate the growth kinetics of *Listeria monocytogenes* in food products and study the effect of food product composition on the growth and inactivation of *Listeria monocytogenes*. During the course of this work, an effect of inoculum size and low temperature pre-culturing in growth kinetics was also quantified.

2. MATERIALS AND METHODS

2.1 *Listeria monocytogenes* strains

Three strains of *Listeria monocytogenes* were used in this study: L6 (origin: milk), which was the most heat resistant strain; FBR15 (origin: ice cream packaging machine), which had the slowest growth rate; and FBR17 (origin: frozen fried rice), which had lower pH_{min}, lower $a_{w\ min}$ and higher LA_{max} (LA, undissociated lactic acid concentration) (Aryani *et al.*, 2015b). The stock cultures containing 3:7 (v/v) of 0.3 ml glycerol (Sigma-Aldrich, Germany) and 0.7 ml overnight culture in Brain Heart Infusion (BHI) broth (Becton Dickinson, France) were kept frozen at -20°C in 1 ml cryovial tubes (Thermo Fisher Scientific Inc., Waltham, USA).

2.2 Food samples preparation

Skim milk (UHT, 0% fat) and ham purchased from Albert Heijn in Wageningen with the same batch number were used in this study.

Each slice of cooked shoulder ham (about 11.5 g) was put into the bottom of a stomacher bag or a vacuum bag separately. To remove the possible impacts from the competitive flora (Buchanan & Bagi, 1999), all the bags were gamma-irradiated with a dose of 10 kilo Gray (Synergy Health Ede B.V., Ede, The Netherlands) and then stored at 4°C until inoculation.

2.3 Growth experiments

2.3.1 Culture preparation

A loop of content from the stock culture was streaked onto a BHI agar plate (BHI broth with 1.5% (w/w) of bacteriological agar, Oxoid, UK) and incubated for 24h at 30°C (IKS, Technisch Bureau, Leerdam, The Netherlands). A single colony from BHI agar plate was transferred into a test tube pre-filled with 10 ml of BHI broth. The test tube was incubated for 17h at 30°C, 200 rpm (Forma Orbital Shakers, Thermo Electron Corporation, USA) until the stationary phase.

To consider the real situation in food cold chain and explore the impact of low temperature pre-culturing on growth kinetics, an additional experiment was required. Only FBR15, which had slowest growth rate among the three strains, was selected. A single colony of FBR15 from BHI agar plate was inoculated in a 100 ml Erlenmeyer flask pre-filled with 20 ml of BHI broth and incubated until reaching stationary phase for 10 days at 7°C.

2.3.2 Inoculation and storage

To validate the growth kinetics of three strains, three different growth media were selected: BHI broth, milk and ham.

For BHI broth, 50 microliters (µl) of the overnight culture from the test tube was inoculated in a 100 ml Erlenmeyer flask pre-filled with 50 ml of BHI broth and incubated until the stationary phase at 7°C.
For milk, a similar approach was followed. 50 µl of the overnight culture from the test tube was inoculated in a 100 ml Erlenmeyer flask pre-filled with 50 ml of UHT milk and incubated until the stationary phase at 7°C.
For ham, to obtain the same initial concentration as in BHI broth and in milk, a 100 times dilution in BHI broth was done. 0.5 ml of 100 times diluted overnight culture from the test tubes was inoculated on one side of ham in each stomacher bag. Then another 0.5 ml of diluted overnight culture was inoculated on the other side. After the inoculum was spread on most part of the surface of ham, the bags were enclosed with tapes and incubated until the stationary phase at 7°C.
According to a previous study (Gorski *et al.*, 2006), the concentration of overnight culture can be estimated as 9.6 logarithm colony forming units per milliliter (log CFU/ml). The initial cell concentration will therefore be approximately 6.6 log CFU/ml in BHI broth and milk, and 6.6 log CFU/g in ham. L6 was the only strain selected in this experiment which was conducted in duplicate on the same day considering the experimental variability.
To follow a longer growth history until the stationary phase and consider the worst case scenario, a smaller inoculum size was required in another experiment. To achieve it, 10,000 times further dilution of the overnight culture should be made before inoculation into growth media. For ham particularly, 1,000,000 times dilution in total was made before spreading it on the surface of the ham. Under this circumstance, the initial cell concentration of all three strains will therefore be around 2.6 log CFU/ml in BHI broth and milk, and 2.6 log CFU/g in ham. This inoculum size was also used in aforementioned FBR15 including the phase of low temperature pre-culturing in Section 2.3.1. These experiments were conducted in duplicate on the same day and reproduced one time on the other day considering the experimental and the biological variability.

2.3.3 Sampling and enumeration

The samples with an initial inoculum size of around 6.6 log CFU/ml were sampled daily. The samples with an initial inoculum size of around 2.6 log CFU/ml were sampled every 2 days, considering the probable increase of lag time from a previous study (Robinson *et al.*, 2001). For samples containing FBR15 in ham both with and without low temperature pre-culturing were taken every 4 days, considering slower growth rate and solid growth media as an inhibiting factor (Koutsoumanis *et al.*, 2004).
For BHI broth and milk samples, 1 ml of each sample was diluted in 9 ml of peptone physiological salt (PPS, Tritium Microbiologie). Then, further decimal dilutions were made and the appropriate dilutions were plated in duplicate on BHI agar plates using a spiral plater (Eddy Jet, IUL Instruments). For the initial time points where low concentrations of viable cells was expected, no dilution was made. In this case, 50 µl

of the sample was plated in duplicate on BHI agar plate using spiral plater, giving the detection limit of 1.3 log CFU/ml. All the plates were incubated for 24h at 30 °C, and the colonies were counted and reported in log CFU/ml.
Ham was diluted in 1:9 in PPS (a solution with two components, 0.85% (w/w) of sodium chloride (AnalaR NORMAPUR, VWR International, Belgium) and 0.1% (w/w) of neutralized bacteriological peptone (Oxoid, UK)). The mixture was then homogenized using a stomacher machine (400 Circulator, Seward, UK) for 1 min at 260 rpm. After homogenization, further dilutions were made and the appropriate dilutions were plated in duplicate using spiral plater. For the initial time points where low concentrations of viable cells was expected, one ml of the homogenized sample was evenly divided into two parts and spread plated onto two BHI agar plates. A detection limit of 1 log CFU/g was obtained using this method. All the plates were incubated for 24h at 30 °C, and the colonies were counted and reported in log CFU/g.

2.3.4 Maximum growth rate and lag time

The logarithms of colony counts were plotted against the time to obtain the growth curve of each growth experiment. The Gompertz model (Zwietering *et al.*, 1990) was used to fit each growth curve and to estimate the maximum growth rate (μ_{max}) and lag time (λ).

$$\ln\left(\frac{N_t}{N_0}\right) = A \cdot exp\left\{-exp\left[\frac{\mu_{max} \cdot e}{A}(\lambda - t) + 1\right]\right\} \quad (1)$$

where N_t is the bacterial concentration (CFU/ml) at time t (day); N_0 is the initial bacterial concentration (CFU/ml); A is the maximal value reached in the growth curve ($A = \ln(N_\infty / N_0)$); μ_{max} is the maximum specific growth rate (per day); λ is the lag time (day); t is the time (day).
Before the fitting procedure, the equation (Eq.(1)) was transformed from ln scale into log scale. Then it was done using Microsoft Excel Solver Add-in and confirmed using TableCurve 2D v5.1.

2.3.5 Effect of inoculum size and pre-culturing temperature on μ_{max} and λ

The μ_{max} and λ of strain L6 with 2 different initial inoculum size ($\pm$ 6.6 log CFU/ml(g) and $\pm$2.6 log CFU/ml(g)) in 3 different growth media (BHI broth, milk and ham) were compared using two independent samples *t*-tests.
The μ_{max} and λ of strain FBR15 in 3 different growth media (BHI broth, milk and ham) with 7°C and 30°C pre-culturing were compared using two independent samples *t*-tests.

2.3.6 Comparison of the prediction and the observed growth kinetics

The growth kinetics of *L. monocytogenes* in BHI, milk and ham were predicted using

the logistic equation (Augustin *et al.*, 2000).

$$N_t = \begin{cases} N_0, \ \mathrm{t} \le \lambda \\ \frac{N_0 \cdot exp[\mu_{max} \cdot (t-\lambda)]}{1+\frac{N_0}{N_{max}} \cdot \{exp[\mu_{max} \cdot (t-\lambda)]-1\}}, \ \mathrm{t} > \lambda \end{cases} \quad (2)$$

where N_t is the bacterial concentration (CFU/ml) at time t (day); N_0 is the initial bacterial concentration (CFU/ml); N_{max} is the maximum bacterial concentration (CFU/ml); μ_{max} is the maximum specific growth rate (per day); λ is the lag time (day); t is the time (day).

The specific values of N_0, N_{max} and λ of each strain in each medium were derived from the average values of observations in this study. The specific values of μ_{max} of each strain in BHI broth or milk were calculated using Gamma approach (Zwietering *et al.*, 1996) (Eqs.(3)–(6)), while the ones in ham were calculated using Eqs.(4)–(8) because of the presence of undissociated lactic acid.

$$\mu_{max} = \mu_{ref} \cdot \gamma(T) \cdot \gamma(pH) \cdot \gamma(a_w) \quad (3)$$

$$\gamma(T) = \frac{(T-T_{min})^2}{(T_{ref}-T_{min})^2} \quad (4)$$

$$\gamma(pH) = \frac{1-2^{\frac{(pH-pH_{min})}{pH_{min}-pH_{1/2}}}}{1-2^{\frac{(pH_{ref}-pH_{min})}{pH_{min}-pH_{1/2}}}} \quad (5)$$

$$\gamma(a_w) = \frac{1-\left(\frac{1-aw}{1-aw_{min}}\right)^a}{1-\left(\frac{1-aw_{ref}}{1-aw_{min}}\right)^a} \quad (6)$$

$$\gamma(LA) = 1 - \left(\frac{[LA]}{[LA_{max}]}\right)^a \quad (7)$$

$$\mu_{max} = \mu_{ref} \cdot \gamma(T) \cdot \gamma(pH) \cdot \gamma(a_w) \cdot \gamma(LA) \quad (8)$$

The average values of T_{min}, pH_{min}, $pH_{1/2}$, $a_{w\,min}$, a_{aw}, LA_{max} and $a_{[LA]}$ of each strain as well as T_{ref}(30 °C), pH_{ref}(7.3) and a_{wref}(0.997) were derived from the study of Aryani et al. (2015). The only incubation temperature (T) for growth in this study was 7 °C. The pH was measured using a pH-meter (Microprocessor pH meter, WTW, Germany), while the a_w was measured using an a_w-meter (LabMaster Aw, Novasina, Switzerland). Two pH buffer solutions (pH=4 and pH=7) (Merck KGaA, Darmstadt, Germany) were used to calibrate the pH-meter before every measurement. For BHI broth, the average pH was 7.43 and the average a_w was 0.995. For UHT milk, the average pH was 6.66 and the average a_w was 0.994. For ham, the average pH was 6.70 and the average a_w was 0.970. The lactic acid concentration in ham sample was not measured in this study. Therefore, 1.58 mM undissociated lactic acid was used in calculation for assumption.

In addition to the predicted curve of each strain in each growth medium, the 95% predicted confidence interval (CI) of each parameter were included to obtain the range within which the mean value would be most possibly located.

For further statistical analysis, the μ_{max} from the prediction and the obtained

experimental data of each strain in each growth media (BHI broth, milk and ham) were compared using one sample *t*-tests.

2.4 Inactivation experiments

2.4.1 Sample preparation and inactivation procedures

After cells entering the stationary phase, the thermal inactivation experiments were immediately started. Five different conditions, , namely BHI broth to BHI broth (grown in BHI broth and inactivated in BHI broth), BHI broth to milk, BHI broth to ham, milk to milk and ham to ham, were tested. All the experiments were done using a water bath (Julabo SW20, Julabo Labortechnik GmbH, Germany) set at 65 °C and 200 rpm.

For the inactivation experiments using liquid heating media (BHI broth and milk), three 250 ml Erlenmeyer flasks prefilled with 40 ml of heating media were pre-heated in the water bath at 65 °C. Two of them were used for inactivation experiment in duplicate, while the other one was used to measure the temperature of heating media using a thermocouple (PeakTech 3150, Thermocouple K-type). When its temperature was stable at 65 ± 0.3 °C, the inactivation was immediately started by inoculating 400 µl of the stationary phase culture into the pre-heated media. For the starting time point of t = 0, similar dilution (1:100) was made and plated in duplicate on BHI agar plates. At the other time points, one ml of the sample was diluted in 9 ml of PPS. Then, further decimal dilutions were made and plated in duplicate on BHI agar plates using a spiral plater. For the sampling points where low concentration of the cells was expected, , one ml of the sample was transferred into a sterile cup placed in ice bucket for few seconds to stop the inactivation. From this sample, two different plating methods were used. For a relatively higher bacterial concentration, 100 µl of the sample was spread plated onto BHI agar plate in duplicate. A detection limit of 1 log CFU/ml was obtained. For a relatively lower bacterial concentration, one ml of the sample was evenly divided into three parts, spread plated onto three BHI agar plates. A detection limit of 0 log CFU/ml was obtained.

For BHI broth to ham inactivation experiment, each side of sliced ham was inoculated with 0.5 ml of the stationary phase culture. After the inoculum was spread on most part of the surface of ham, the bags were vacuumed sealed using a vacuum sealer (Princess, the Netherlands). To measure the heating up time, a blank sample without inoculation was also prepared. After the water bath reached the desired temperature at 65 ± 0.3 °C, the vacuum bag containing the blank sample was immersed in water with a thermocouple attached to the surface of the sample. When its temperature reached 65°C, the heating up time was recorded and the inactivation experiment was immediately started. Since every experiment was conducted using 5 sampling points including the starting time point of t = 0, four bags of samples were immersed in the water together at 65°C. At each time point considering the adjusted heating up time in Section 2.4.2, one bag was taken out of water bath and put into ice water for few

seconds to stop the inactivation. The bag was cut open and the ham was diluted using PPS. The mixture was then homogenized using a stomacher machine for 1 min at 260 rpm. Further decimal dilutions were then prepared and the appropriate was plated in duplicate on BHI agar plates using a spiral plater. A detection limit of 1.3 log CFU/ml was obtained using this method.

For ham to ham inactivation experiment, the samples were directly taken from growth experiment in Section 2.3.2. All heating procedures followed similar procedure as previously described.

All the plates were incubated for 4-5 days at 30 °C. The colonies were counted and reported in log CFU/g. Each experiment was conducted in duplicate on the same day and reproduced one time on the other day considering the experimental and the biological variability.

2.4.2 Adjustment of heating up time

During the heating up period, the effect of the increasing temperature from room temperature to the desired heating temperature of 65°C had the killing power to *Listeria* that cannot be totally neglected. To quantify this effect, Eq.(9) was used to evaluate the equivalent killing power at other temperatures that was lower than 65°C.

$$\log\frac{t}{F} = \frac{65-T}{z} \tag{9}$$

where T is the temperature (°C); t is the thermal death time at temperature T (s); F is the thermal death time at temperature 65°C (s); z is the temperature for one $\log_{10}$ reduction in the D-value (°C).

By adding these F-values of each temperature point together, the total F was given by the equation below (Earle & Earle, 2004).

$$\mathrm{F} = t_1 \times 10^{\frac{T_1-65}{z}} + t_2 \times 10^{\frac{T_2-65}{z}} + \cdots \tag{10}$$

By excluding the total F-value from the initially recorded heating up time, a more accurate adjusted heating up time was applied.

In this study, the heating up time ranged from 30 s to 43 s. To give an example of the adjustment of L6, 1°C increase in 1 second from room temperature of 25°C to heating temperature of 65°C with the total heating up process of 40 s was assumed. Thus t_1=t_2=⋯=t_{40}=1s; T_1=25°C, T_2=26°C, ⋯, T_{40}=64°C; z=5.7°C. The z-value used here was derived from the study of Aryani et al. (2015). Then the total F was calculated as 2.0 s (Eq.(10)). Therefore by deducting it from the initial 40 s, the adjusted heating up time was 38.0 s (Mullan, 2007).

However this ideal situation rarely happened in real situation. In most cases of this study, the higher the surface temperature of ham was, the slower increase of surface temperature was observed. Therefore if the precise heating up time was required, observation and record of the variations of temperature during the whole process was necessary.

2.4.3 *D*-value estimation

The logarithms of colony counts were plotted against the inactivation time to obtain the inactivation curve of each inactivation experiment. The modified Weibull model (Metselaar *et al.*, 2013) was fitted to the data of each individual experiment and to estimate the delta (*Δ*) decimal reduction time (*Δ* = 2, 3, 4, 5, 6).

$$\log N_t = \log N_0 - \Delta \cdot \left(\frac{t}{\Delta D}\right)^{\beta} \quad (11)$$

where N_t is the bacterial concentration (CFU/ml) at time t (s); N_0 is the initial bacterial concentration (CFU/ml); Δ is the number of decimal reductions; ΔD is the time needed to reduce the initial number of micro-organism with Δ decimals (s); β is a fitting parameter that defines the shape of a curve; and t is the time (s).

Δ was set at one value of 2, 3, 4, 5 and 6, based on the reduction range of the experiment. The fitting procedure was done using Microsoft Excel Solver Add-in. The *D*-value was then calculated as *ΔD* / *Δ*.

2.4.4 Effect of heating media and growth media on inactivation kinetics

The *D*-values of 3 different strains (L6, FBR15, FBR17) grown in BHI, and then heated in 3 different media (BHI broth, milk and ham) at 65 °C were compared using ANOVA. The *D*-values of 3 different strains (L6, FBR15, FBR17) grown in BHI and food matrix (milk or ham), and then heated in the same food matrix at 65 °C were compared using ANOVA and followed with a post hoc Tukey HSD test.

2.5 VBA based tool to predict growth kinetics of *Listeria monocytogenes*

In order to give a quick overview of the predicted growth curves of *Listeria monocytogenes* for the customers, like food factories who need food safety control, a preliminary generic tool using VBA based Microsoft Excel program was designed. It could help the customers to evaluate the safety of production process and find critical points in the production line without large time investment.

3. RESULTS

3.1 Growth experiments

3.1.1 Determination of μ_{max} and λ

The average of estimated μ_{max}s and λs are shown in Table 1-5. The zeros were used as the lower limits of 95% confidence intervals when they showed negative numbers based on calculation. As expected, L6 and FBR17 had a similar μ_{max}, while FBR15 grew much slower than those two strains. Also, L6 and FBR17 had a similar λ, while FBR15 had a longer lag time in food media (milk and ham) than those two strains (Table 2, 3 and 4). In general, strains grew fastest in BHI broth, intermediate in milk and slowest in ham at the same incubation temperature of 7 °C.

Table 1 The average μ_{max} and λ of L6 with large inoculum size (approximately 6.6 log CFU/ml) in three growth media at 7 °C

Growth medium	μ_{max} (h^{-1})		λ (day)	
	Excel Solver fitting*	TableCurve fitting**	Excel Solver fitting*	TableCurve fitting**
BHI broth	0.082 (0.00076)	0.082 (0.051, 0.11)	0.313 (0.12)	0.313 (0, 0.86)
Milk	0.061 (0.0056)	0.061 (0.050, 0.072)	0.322 (0.14)	0.322 (0.0306, 0.614)
Ham	0.037 (0.0018)	0.037 (0.022, 0.052)	1.52 (0.11)	1.52 (0.342, 2.71)

* Value within bracket is the standard deviation

** Value within bracket is the 95% confidence interval

Table 2 The average μ_{max} and λ of L6 with small inoculum size (approximately 2.6 log CFU/ml) in three growth media at 7 °C

Growth medium	μ_{max} (h^{-1})		λ (day)	
	Excel Solver fitting*	TableCurve fitting**	Excel Solver fitting*	TableCurve fitting**
BHI broth	0.092 (0.0022)	0.092 (0.066, 0.12)	1.35 (0.45)	1.35 (0.371, 2.33)
Milk	0.058 (0.0017)	0.058 (0.045, 0.072)	0.848 (0.35)	0.848 (0, 1.87)
Ham	0.054 (0.0044)	0.054 (0.044, 0.064)	2.64 (0.64)	2.64 (1.81, 3.47)

* Value within bracket is the standard deviation

** Value within bracket is the 95% confidence interval

Table 3 The average μ_{max} and λ of FBR17 with small inoculum size (approximately 2.6 log CFU/ml) in three growth media at 7 °C

Growth medium	μ_{max} (h^{-1})		λ (day)	
	Excel Solver fitting*	TableCurve fitting**	Excel Solver fitting*	TableCurve fitting**
BHI broth	0.092 (0.0093)	0.092 (0.071, 0.11)	1.41 (0.46)	1.41 (0.672, 2.15)
Milk	0.062 (0.0028)	0.062 (0.046, 0.078)	0.626 (0.24)	0.626 (0, 1.58)

Ham	0.045 (0.0078)	0.045 (0.039, 0.051)	1.81 (0.25)	1.81 (1.06, 2.56)

* Value within bracket is the standard deviation

** Value within bracket is the 95% confidence interval

Table 4 The average μ_{max} and λ of FBR15 with small inoculum size (approximately 2.6 log CFU/ml) in three growth media at 7 °C

Growth medium	μ_{max} (h^{-1})		λ (day)	
	Excel Solver fitting*	TableCurve fitting**	Excel Solver fitting*	TableCurve fitting**
BHI broth	0.050 (0.0013)	0.050 (0.043, 0.057)	1.51 (0.13)	1.51 (0.670, 2.36)
Milk	0.044 (0.0022)	0.044 (0.038, 0.050)	1.82 (0.26)	1.82 (1.11, 2.52)
Ham	0.028 (0.0033)	0.028 (0.020, 0.037)	6.30 (2.0)	6.30 (4.26, 8.33)

* Value within bracket is the standard deviation

** Value within bracket is the 95% confidence interval

Table 5 The average μ_{max} and λ of FBR15 with small inoculum size (approximately 2.6 log CFU/ml) and 7°C pre-culturing for 10 days in three growth media at 7 °C

Growth medium	μ_{max} (h^{-1})		λ (day)	
	Excel Solver fitting*	TableCurve fitting**	Excel Solver fitting*	TableCurve fitting**
BHI broth	0.052 (0.0019)	0.052 (0.044, 0.060)	1.12 (0.22)	1.12 (0.180, 2.06)
Milk	0.046 (0.0017)	0.046 (0.040, 0.052)	1.09 (0.28)	1.09 (0.316, 1.86)
Ham	0.022 (0.0012)	0.022 (0.019, 0.025)	2.20 (0.67)	2.20 (0.57, 3.83)

* Value within bracket is the standard deviation

** Value within bracket is the 95% confidence interval

3.1.2 Effect of inoculum size and pre-culturing temperature on μ_{max} and λ

The results showed that inoculum size had no obvious effect on μ_{max} of L6 in all three growth media. However, small inoculum size resulted in longer lag time. When L6 was grown in BHI broth, the lag time of smaller inoculum size (1.35 day) was 4.3 times longer than that of the larger inoculum size (0.313 day). When L6 was grown in milk, the lag time of smaller inoculum size (0.848 day) was 2.6 times longer than that of the larger inoculum size (0.322 day). When L6 was grown in ham, the lag time of smaller inoculum size (2.64 day) was 1.7 times longer than that of the larger inoculum size (1.52 day) (Table 1 and 2).

Similarly, the results showed that 7°C pre-culturing for 10 days of FBR15 had no effect on μ_{max} in all three growth media. However, 7°C pre-culturing led to shorter lag time. When BHI broth was used as the growth media for FBR15, the lag time with 30°C pre-culturing (1.51 day) was approximately 1.3 times longer than that with 7°C pre-culturing (1.12 day). When milk was used as the growth media for FBR15, the lag

time with 30°C pre-culturing (1.82 day) was approximately 1.7 times longer than that with 7°C pre-culturing (1.09 day). When ham was used as the growth media for FBR15, the lag time with 30°C pre-culturing (6.30 day) was approximately 2.9 times longer than that with 7°C pre-culturing (2.20 day) (Table 4 and 5).
The result for the two independent samples *t*-test was provided in Appendix 7.3.

3.1.3 Validation of growth kinetics and effect of food matrix

Table 6 - 8 show the predicted γ factors of three strains grown in different food products. As expected, the predicted γ factors in BHI broth and milk were almost similar. However, the γ (a_w) factors in ham were 25% to 30% lower than the ones in BHI broth and milk. Therefore the low a_w could be regarded as one of the inhibiting factor in ham as compared to BHI and milk.
For BHI broth and ham, the growth kinetics of all three strains was in agreement to the prediction kinetics (Fig.1 and Fig.3). However For milk, the growth kinetics of L6, FBR17 and FBR15 did not fall into the 95% predicted confidence interval of the prediction curves, although the effect was strain dependent (Fig.2). The observed μ_{max} of L6 in milk was 32% lower than the prediction with the predicted γ factor of 0.085, while the μ_{max} of FBR17 and FBR15 were 14% and 27% lower than the prediction with the predicted γ factor of 0.069 and 0.066 (Appendix 7.4.2).
For most cases, the differences obtained from the *t*-tests (Appendix 7.4) corresponded with the results in Fig.1 - 3.

Table 6 Predicted γ factors of L6, FBR17, and FBR15 in BHI broth

Strain	*γ (T)**	*γ (pH)**	*γ (a_w)**
L6	0.088 (0.082, 0.094)	1.00 (1.00, 1.00)	0.99 (0.98, 0.99)
FBR17	0.073 (0.068, 0.078)	1.00 (1.00, 1.00)	0.98 (0.98, 0.99)
FBR15	0.068 (0.058, 0.079)	1.00 (1.00, 1.00)	0.99 (0.99, 0.99)

* Value within bracket is the 95% confidence interval

Table 7 Predicted γ factors of L6, FBR17, and FBR15 in milk

Strain	*γ (T)**	*γ (pH)**	*γ (a_w)**
L6	0.088 (0.082, 0.094)	0.99 (0.99, 0.99)	0.98 (0.97, 0.98)
FBR17	0.073 (0.068, 0.078)	0.97 (0.97, 0.98)	0.97 (0.96, 0.98)
FBR15	0.068 (0.058, 0.079)	0.98 (0.98, 0.98)	0.99 (0.98, 0.99)

* Value within bracket is the 95% confidence interval

Table 8 Predicted γ factors of L6, FBR17, and FBR15 in ham

Strain	*γ (T)**	*γ (pH)**	*γ (a_w)**	*γ (LA)**
L6	0.088 (0.082, 0.094)	0.99 (0.99, 0.99)	0.70 (0.67, 0.72)	0.78 (0.69, 0.84)
FBR17	0.073 (0.068, 0.078)	0.98 (0.97, 0.98)	0.70 (0.67, 0.72)	0.60 (0.53, 0.66)
FBR15	0.068 (0.058, 0.079)	0.98 (0.98, 0.98)	0.75 (0.72, 0.77)	0.52 (0.52, 0.52)

* Value within bracket is the 95% confidence interval

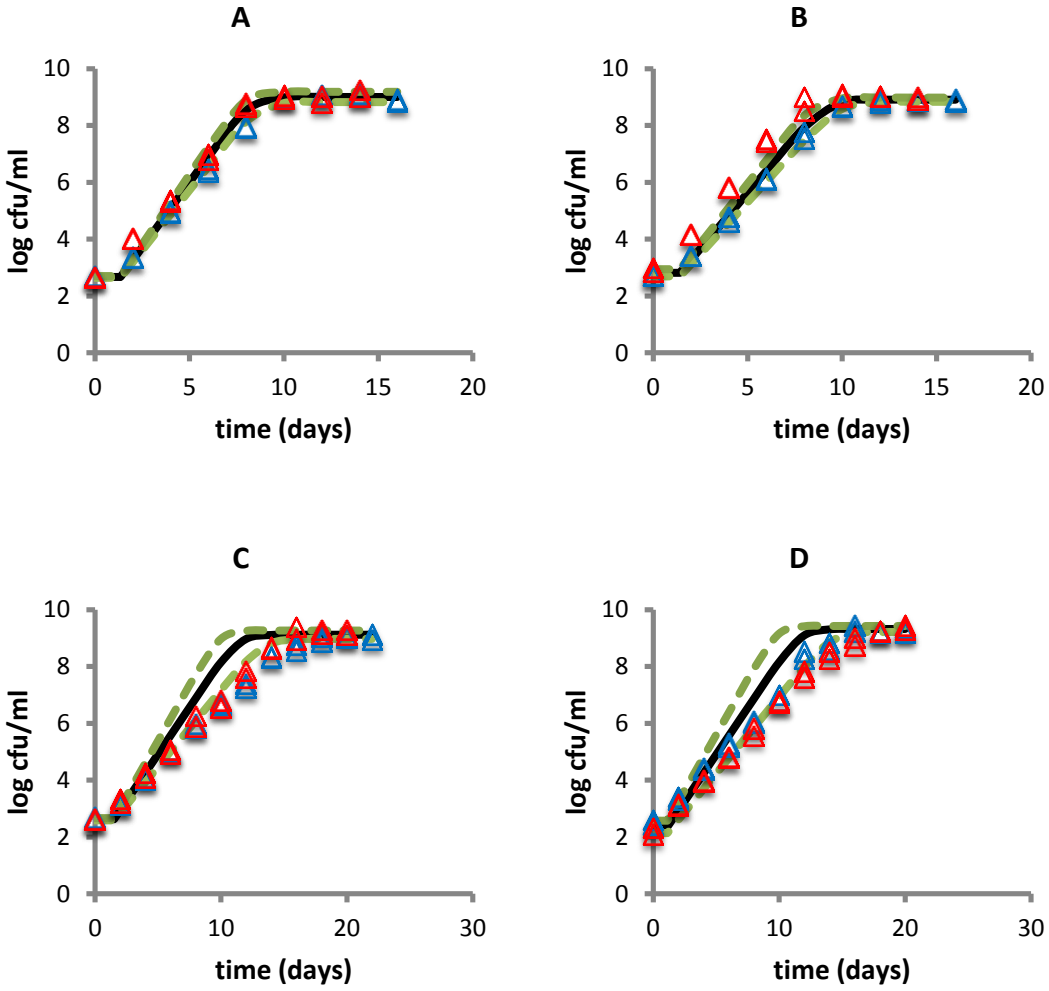
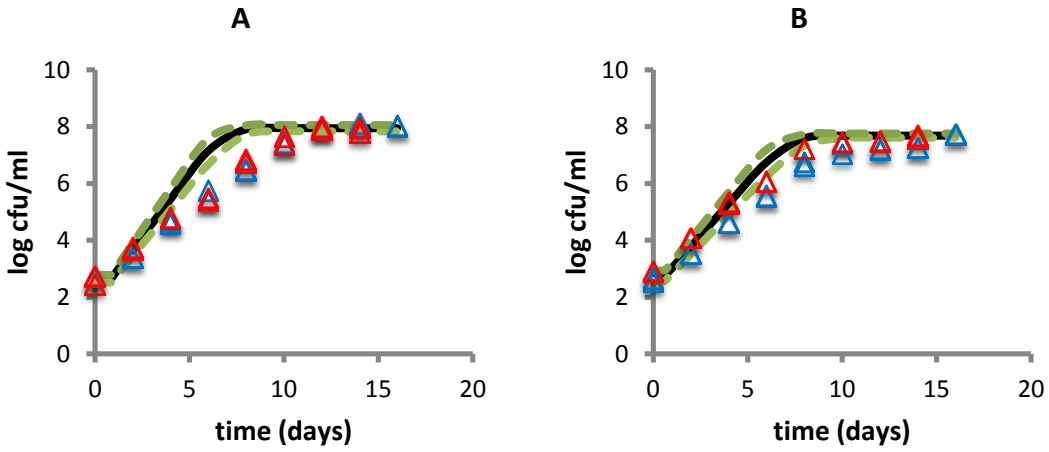


Fig.1. Growth kinetics in BHI broth compared with predicted growth curves and their 95% predicted confidence intervals of L6 (A), FBR17 (B), FBR15 (C), and FBR15 with ten-day 7°C pre-culturing (D) at 7°C. — Prediction curve, -- 95% predicted confidence interval, Δ first reproduction, and Δ second reproduction.



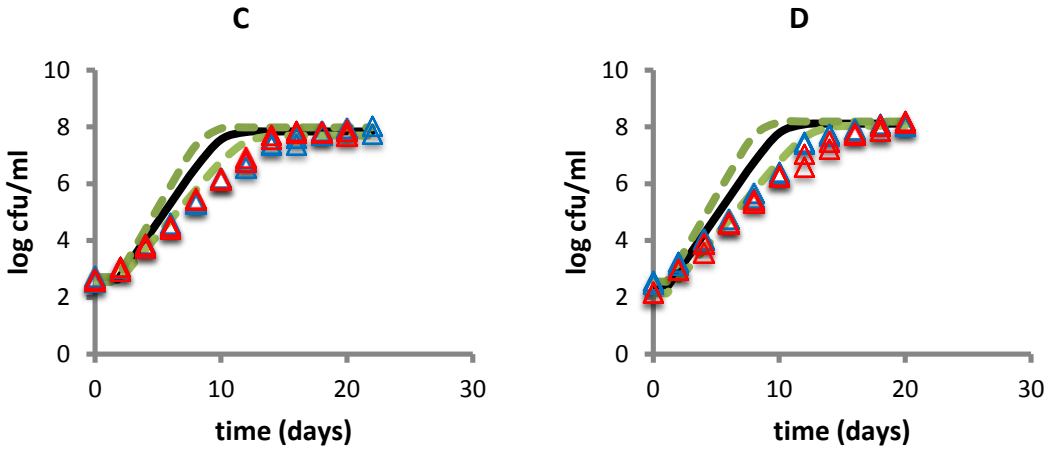


Fig.2. Growth kinetics in milk compared with predicted growth curves and their 95% predicted confidence intervals of L6 (A), FBR17 (B), FBR15 (C), and FBR15 with ten-day 7°C pre-culturing (D) at 7°C. — Prediction curve, -- 95% predicted confidence interval, Δ first reproduction, and Δ second reproduction.

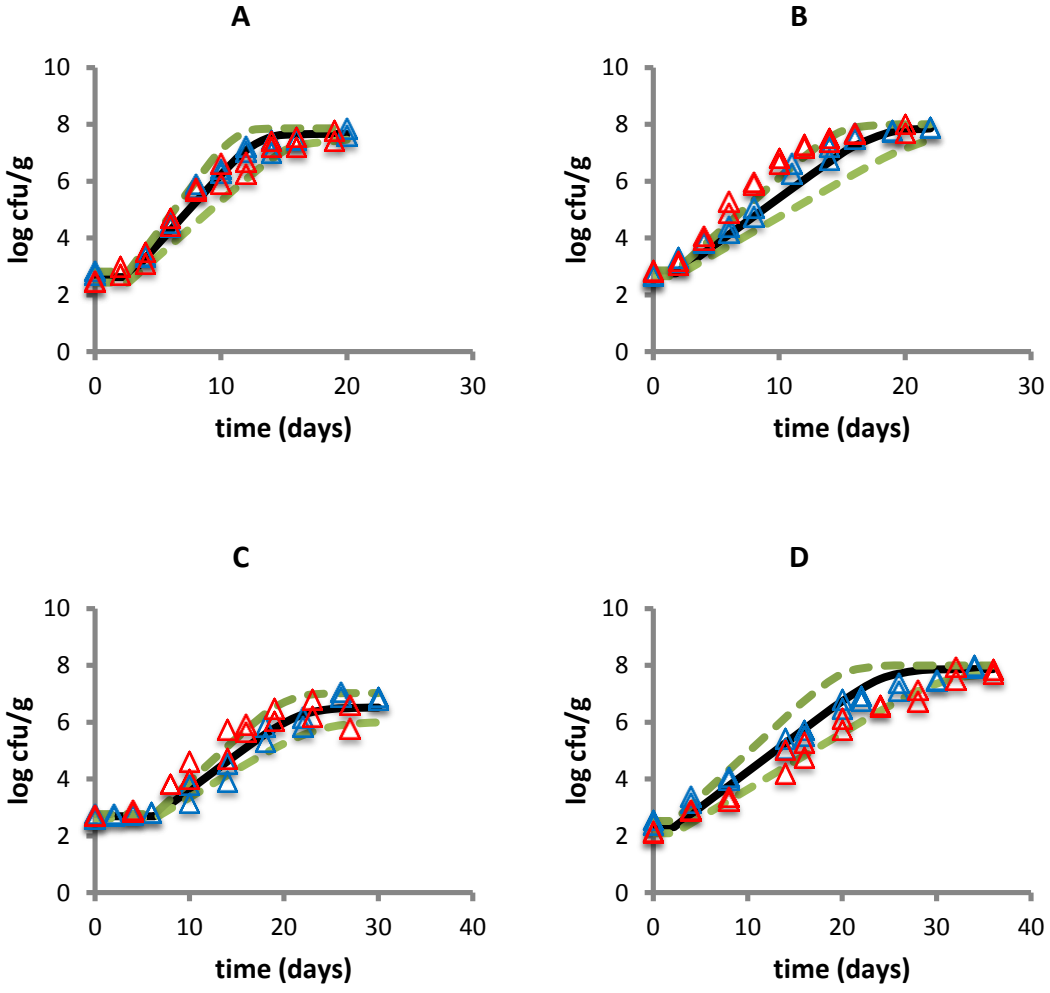


Fig.3. Growth kinetics in ham compared with predicted growth curves and their 95%

predicted confidence intervals of L6 (A), FBR17 (B), FBR15 (C), and FBR15 with ten-day 7°C pre-culturing (D) at 7°C. — Prediction curve, -- 95% predicted confidence interval, Δ first reproduction, and Δ second reproduction.

3.2 Inactivation experiments

3.2.1 Effect of heating media on inactivation kinetics

Fig.4 shows similar thermal inactivation kinetics in both BHI broth and milk as heating media, while it was higher in ham as heating media. The same outcome was observed when the *D*-values of BHI, milk and ham were compared. For both strains L6 and FBR17, the *D*-values in ham were about 5 times higher than the ones in BHI broth and milk. For strain FBR15, the *D*-value in ham was about 9.5 times higher than the ones in BHI broth and milk (Fig.5 and Table 12).

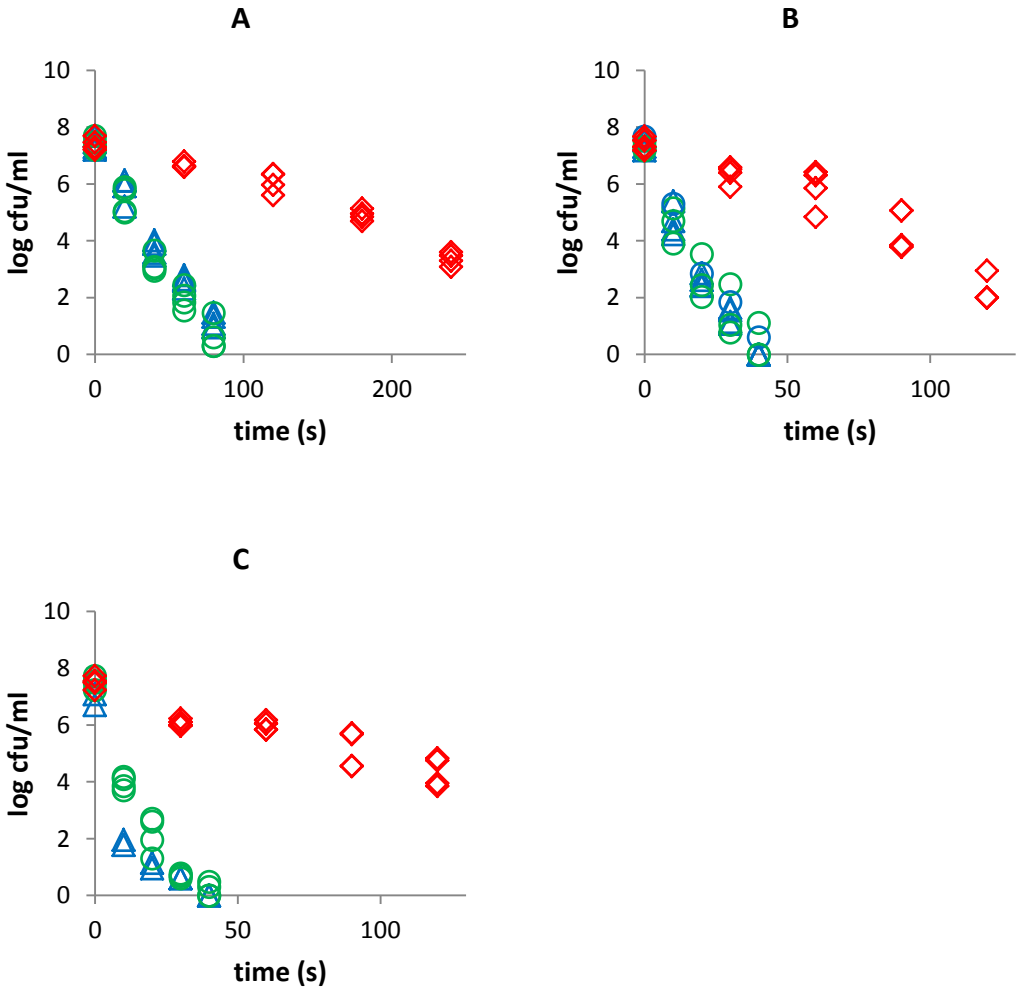


Fig.4. Inactivation kinetics from BHI broth as growth medium to three heating media (BHI broth, milk and ham) of L6 (A), FBR17 (B), and FBR15 (C) at 65°C. Δ BHI broth to BHI broth, ○ BHI broth to milk and ◊ BHI broth to ham.

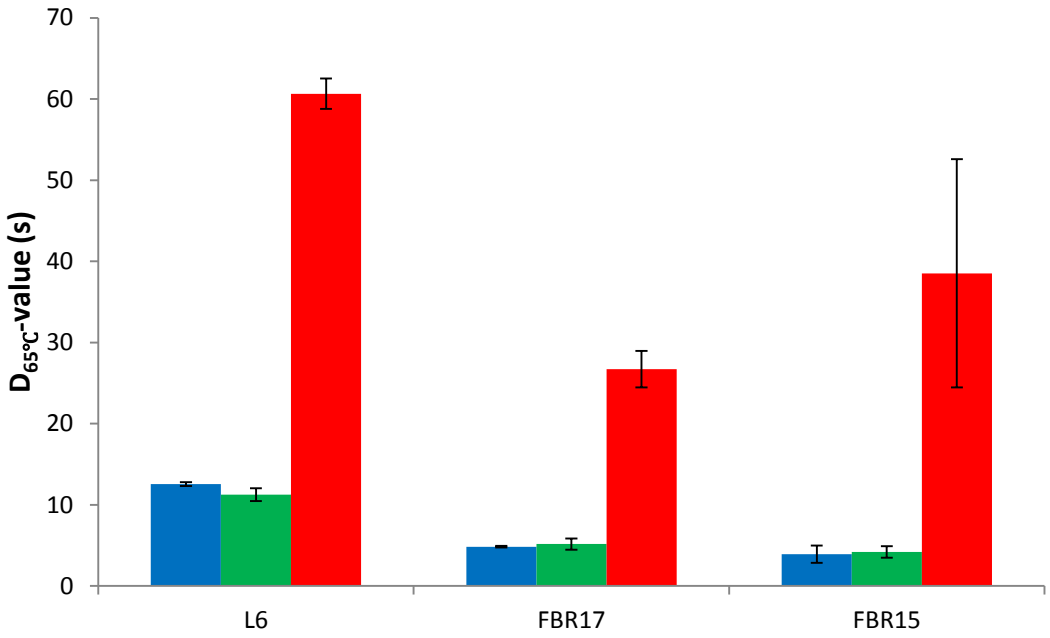
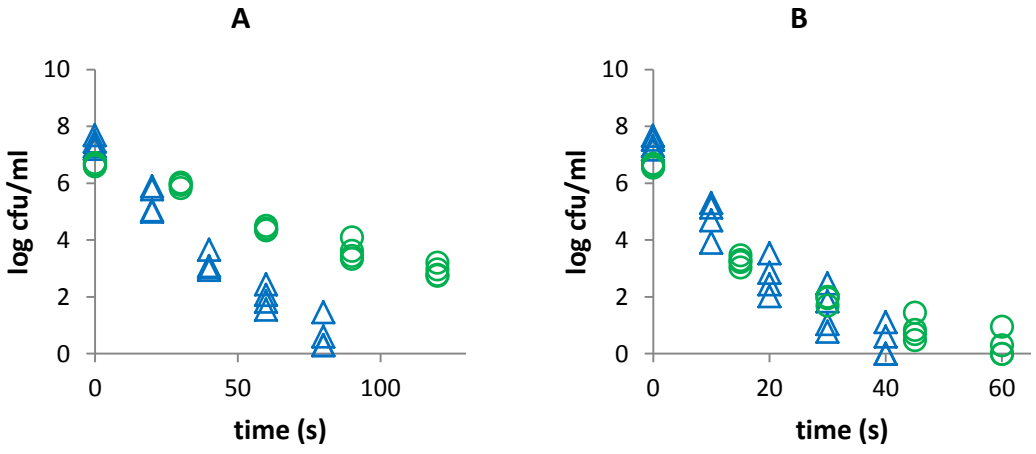


Fig.5. Average *D*-values with standard deviations from BHI broth as growth medium to three heating media (BHI broth, milk and ham) of L6, FBR17, and FBR15 at 65°C. ■ BHI broth to BHI broth, ■ BHI broth to milk and ■ BHI broth to ham.

3.2.2 Effect of growth media on inactivation kinetics

The thermal inactivation kinetics of FBR17 heated in milk were similar for both cells grown in BHI or in milk (Fig.6B). However, the figures were different for L6 and FBR15 since both strains grown in milk were more resistant than the one grown in BHI (Fig.6A and C). Similar conclusion was obtained when the *D*-value data were compared (Fig.7 and Table 13).



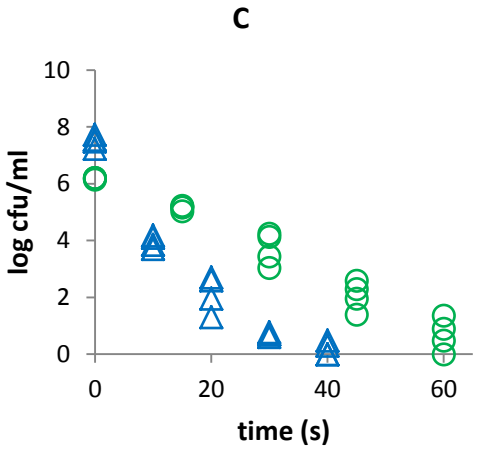


Fig.6. Inactivation kinetics from two growth media (BHI broth and milk) to milk as heating medium of L6 (A), FBR17 (B), and FBR15 (C) at 65°C. Δ BHI broth to milk and ○ milk to milk.

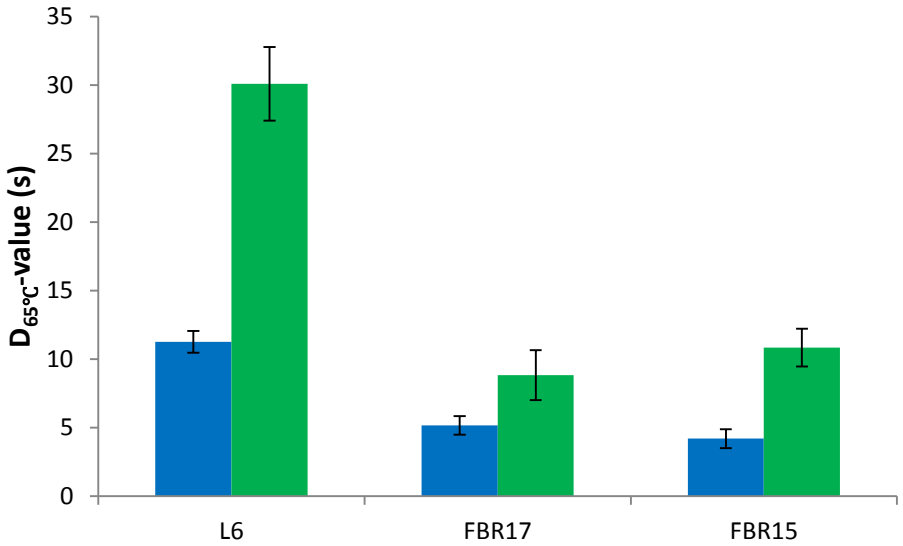


Fig.7. Average *D*-values with standard deviations from two growth media (BHI broth and milk) to milk as heating medium of L6, FBR17, and FBR15 at 65°C. ■ BHI broth to milk and ■ milk to milk.

For ham, the inactivation kinetics of FBR17 and FBR15 grown in BHI and ham was also similar (Fig.8B and C). However strain L6 grown in ham was more resistant than that of grown in BHI (Fig.8A). Similar outcome was obtained when the *D*-value data were compared (Fig.9 and Table 14).

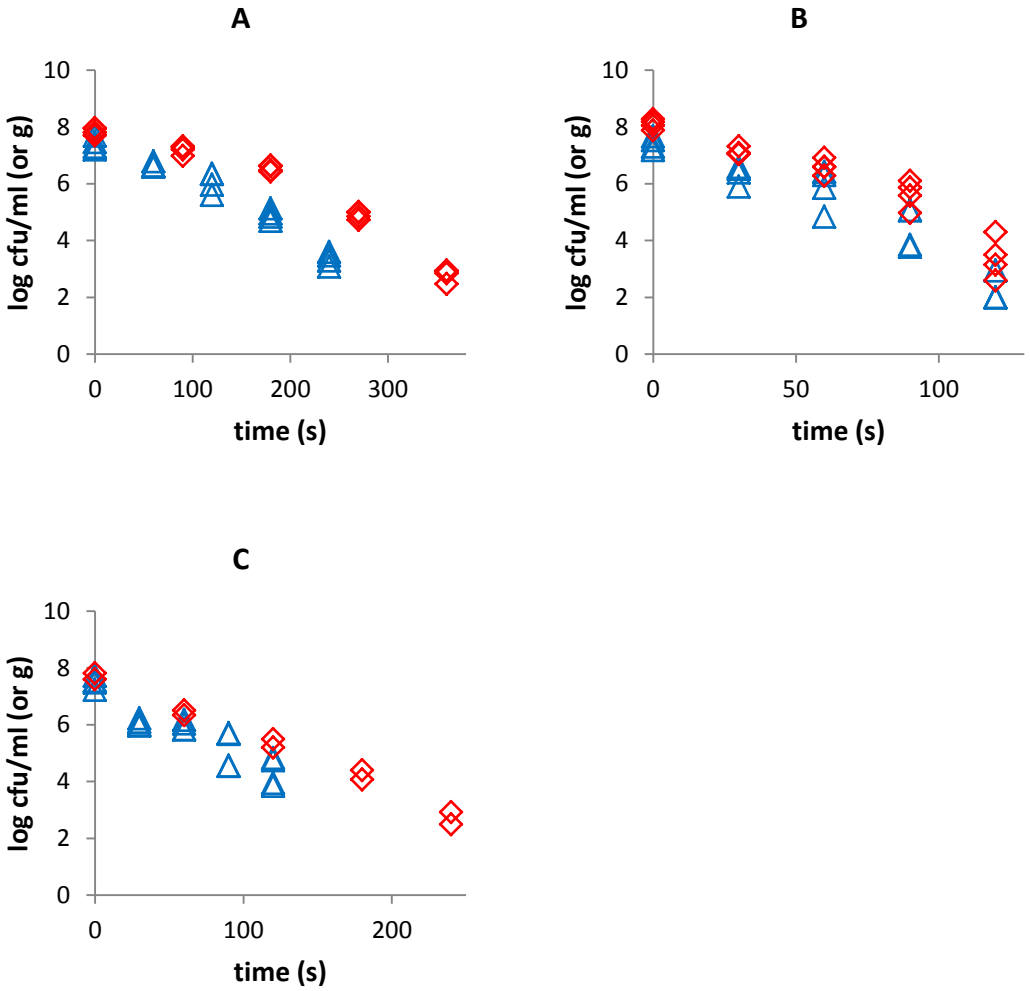


Fig.8. Inactivation kinetics from two growth media (BHI broth and ham) to ham as heating medium of L6 (A), FBR17 (B), and FBR15 (C) at 65°C. Δ BHI broth to ham and ◊ ham to ham.

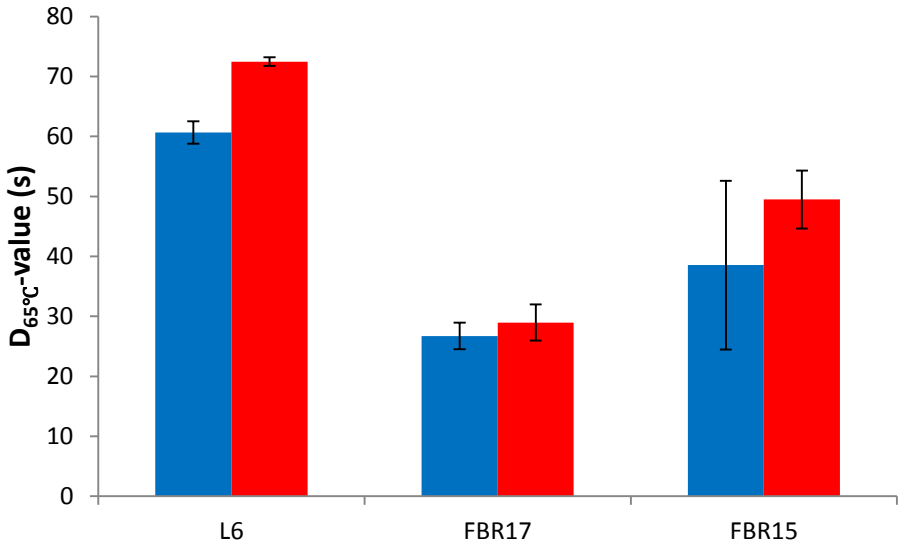


Fig.9. Average *D*-values with standard deviations from two growth media (BHI broth and ham) to ham as heating medium of L6, FBR17, and FBR15 at 65°C. ■ BHI broth to ham and ■ ham to ham.

3.3 VBA based tool to predict growth kinetics of *Listeria monocytogenes*

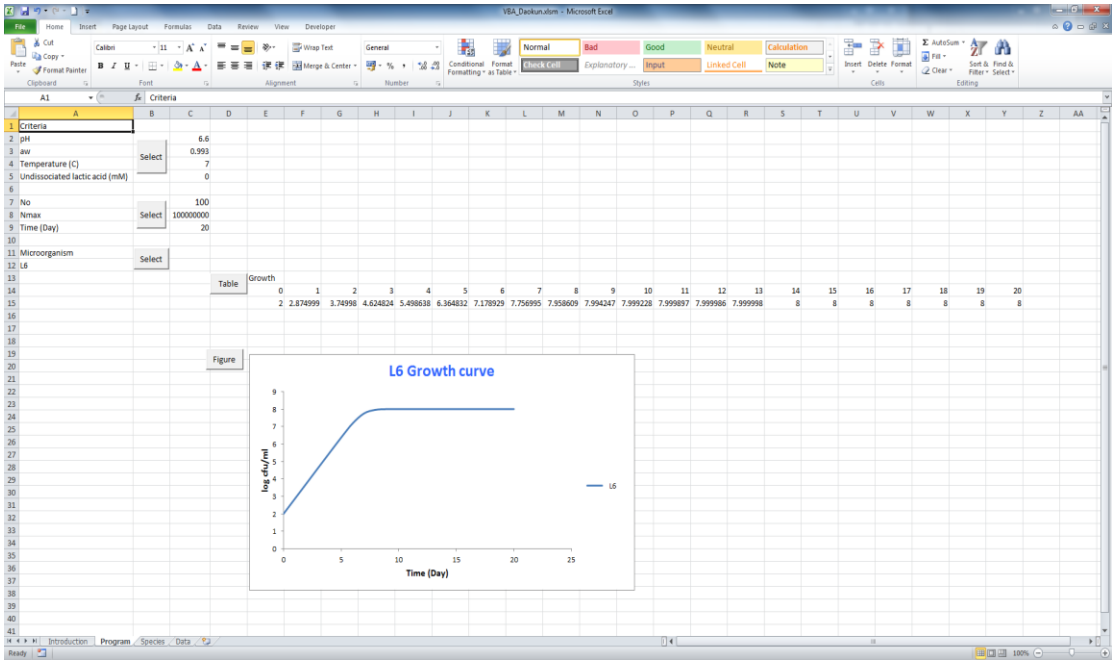


Fig.10. The screenshot of an example of L6 predicted growth curve.

Twenty *Listeria monocytogenes* strains under certain growth condition with four factors including temperature, pH, water activity and undissociated lactic acid concentration were selected. The prediction used was based on Gamma concept aforementioned in 2.3.6. Therefore the specific values of N_0, N_{max} and t (growing time) of each strain should be established. This program provided not only the predicted growth curve, but also the specific values of each time point in a table (Fig.10).

4. DISSCUSSION

4.1 Effect of inoculum size and pre-culturing temperature on μ_{max} and λ

In the present study, small inoculum size of L6 extended the lag time under suboptimal growth condition at 7°C. This result was in agreement with the result of a study using Scott A grown at 7°C (Augustin *et al.*, 2000) and Scott A and V7 pre-cultured at 4°C and grown at 14°C (Gay *et al.*, 1996). Similarly, a study using tryptone soya broth (TSB) with 1.2 M NaCl as an inhibiting factor also reported an increased lag time of NCTC 11994 as the inoculum size became smaller. However, it was also reported that the lag times were little affected by the inoculum size under optimum growth conditions (Robinson *et al.*, 2001). This was supported by a previous study using NCTC 11994 grown at 30°C (Duffy *et al.*, 1994). Larger inoculum size resulting in short lag time might be attributed to the contribution of subpopulation group with shortest lag time since they began to propagate faster than the other subpopulations (Baranyi, 1998).

Apart from the inoculum size, low temperature pre-culturing could shorten the lag time under suboptimal growth condition. Previous study reported that additional pre-culturing at 4°C could induce a great reduction in the lag time for NCTC 11994 incubated at 30°C (Walker *et al.*, 1990). Similarly, another study using Scott A grown in UHT milk and canned dog food at 5°C showed significant increase of lag time when higher pre-culturing temperature was used (Buchanan & Klawitter, 1991). This report was in agreement with current finding since the lag time of FBR15 grown in BHI, milk and ham at 7°C reduced when the cells were pre-cultured at the same temperature prior to inoculation.

In the present study, inoculum size of L6 had no influence on the maximum specific growth rate (μ_{max}). This result was in agreement with the result of a previous study using NCTC 11994 grown at 30°C (Duffy *et al.*, 1994). Moreover, low temperature pre-culturing (7°C) also did not affect maximum growth rate of FBR15 in the current study as well. Likewise, no obvious difference in μ_{max} was observed for Scott A grown at 5°C when it was pre-cultured at two different temperatures (30°C and 4°C) (Buchanan & Klawitter, 1991). However, these results contradicted with some other studies, which reported that low temperature pre-culturing could result in higher maximum growth rate (Membré *et al.*, 1999; Walker *et al.*, 1990).

4.2 Validation of growth kinetics and effect of food matrix

From this validation study, it was suggested that the growth rates were similar between the prediction and actual behavior in BHI broth. The only inhibiting factor was low incubation temperature (7°C) since BHI broth was the ideal media for *Listeria monocytogenes*. Likewise, all the strains had a similar growth kinetics to the prediction in ham, although it had more inhibiting factors such as nitrate (Junttila *et*

al., 1989). For meat products, a previous literature study reported that the μ_{max} of *Listeria* predicted using Gamma concept were smaller than the observed μ_{max} under unfavorable conditions, like low temperatures (Te Giffel & Zwietering, 1999), which was not in agreement with the result of our study.

However the observed growth rate was smaller than prediction in milk, because the essential nutrients for *Listeria* might not be sufficiently available. For example, the lactose in milk could not be utilized by *Listeria monocytogenes* (Pine *et al.*, 1989). Moreover, the milk used in this study was the skim milk containing no fat according to the description in the label. Based on a previous study using F 5069 strain, the growth rate in whole milk was significantly higher than in skim milk and 11% nonfat milk solids at 10°C and 4°C (Donnelly & Briggs, 1986). Therefore milkfat as a food product factor that could influence the growth rate was excluded by using skim milk in this study. Another study using NCTC 5348 reported lower observed μ_{max} than the prediction in pasteurized milk and UHT milk at low temperatures (Murphy *et al.*, 1996), which correspond to the finding of current study.

4.3 Effect of heating media and growth media on inactivation kinetics

The *D*-values of three strains inactivated in ham were much higher than the ones in BHI broth and milk from the same growth media (BHI broth). This result was in agreement with the result of a previous study using *L. innocua* M1 inactivated at 65°C. The average *D*-value was 1.71 min in ground chicken breast meat, which was much higher than the average *D*-value of 0.252 min in 0.1% peptone-agar solution (Murphy *et al.*, 2000). The increased thermal tolerance in ham might be induced by sub-lethal heat shock due to relatively much longer heating procedure compared to heating in BHI broth and milk (Carlier *et al.*, 1996; Fedio & Jackson, 1989; Pagán *et al.*, 1997).

5. CONCLUSION

- The growth study showed that the inoculum size of L6 grown at 7°C had no obvious effect on maximum growth rate. However, small inoculum size extended the lag time under suboptimal growth condition at 7°C.
- Ten-day pre-culturing at 7°C of FBR15 grown at 7°C had no significant effect on maximum growth rate, while it shortened the lag time.
- The validation study of all three strains grown at 7°C indicated that the growth rates were similar between the prediction and actual behavior in both BHI broth and ham. However the observed growth rates were 14% to 32% smaller than the prediction in milk based on different strains.
- The inactivation study revealed that $D_{65°C}$-values of L6 and FBR17 inactivated in ham were 5 times higher than the ones inactivated in BHI broth and milk. $D_{65°C}$-value of FBR15 inactivated in ham was 9.5 times higher than the ones inactivated in BHI broth and milk. $D_{65°C}$-values of all three strains grown in milk and ham were higher than the one grown in BHI broth.

6. RECOMMENDATION

In the present study, the maximum growth rate and lag time were estimated by fitting growth curves with Gompertz model. However a study reported that the maximum specific growth rates were systematically overestimated by Gompertz function in certain previous studies (Membré *et al.*, 1999). Therefore the Baranyi model (Eq.⑿) was suggested to be used (den Besten *et al.*, 2006).

$$\log N_t = \log N_0 + \frac{\mu}{\ln 10} \cdot A_t - \frac{1}{\ln 10} \cdot \ln\left[1 + \frac{exp(\mu \cdot A_t) - 1}{10^{(\log N_{final} - \log N_0)}}\right] \quad ⑿$$

where N_t is the bacterial concentration (CFU/ml) at time t (min); N_0 is the initial bacterial concentration (CFU/ml); N_{final} is the final bacterial concentration (CFU/ml); μ is the maximum specific growth rate (per min); t is the time (min); A_t is defined by Eq.⒀.

$$A_t = \mathrm{t} + \frac{1}{\mu} \cdot \ln[exp(-\mu \cdot t) + exp(-\mu \cdot \lambda) - exp(-\mu \cdot t - \mu \cdot \lambda)] \quad ⒀$$

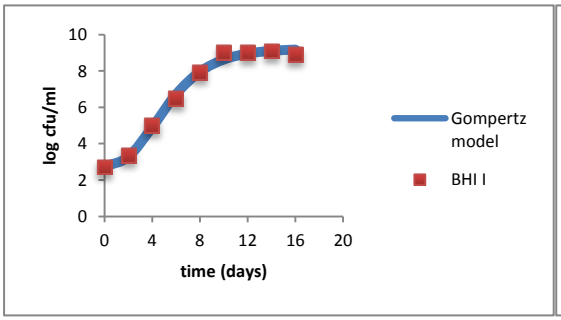
where λ is the duration of the lag period of the growth curve (min).

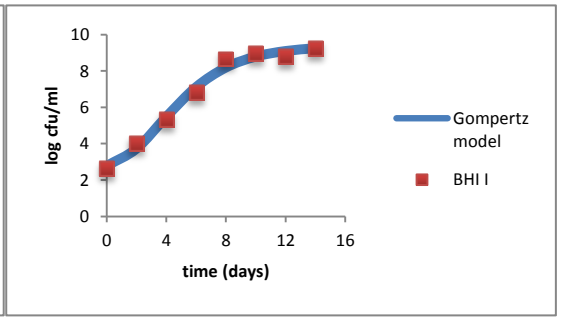
In addition, it might also be useful to investigate the effect of shaking condition on growth in BHI broth. In current study, all the strains were incubated statically at 7°C. Based on a previous study using Scott A and F6861 at pH 4.5 and 20°C, larger growth rate was observed under the growth condition filled with air than with nitrogen (George & Lund, 1992). However, another study showed that there was no obvious difference of the maximum specific growth rate or lag phase of NCTC 11994 between duration for non-aerated and aerated condition at 30°C (Duffy *et al.*, 1994).

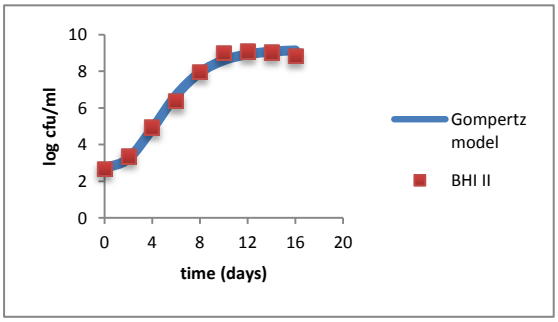
7. APPENDIX

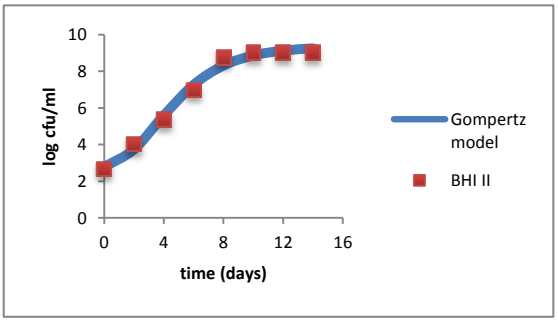
7.1 Figures of fitting Gompertz model to growth curves using Excel Solver Add-in

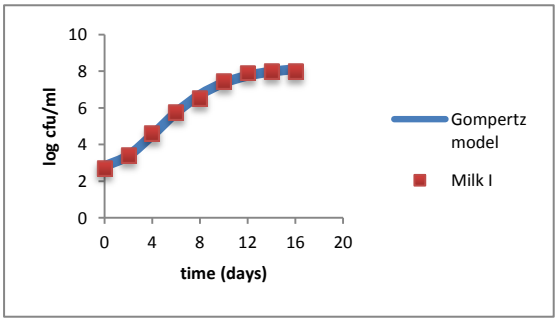
7.1.1 L6 growth curves at 7°C

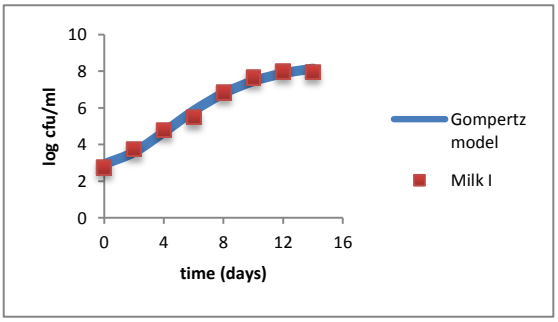


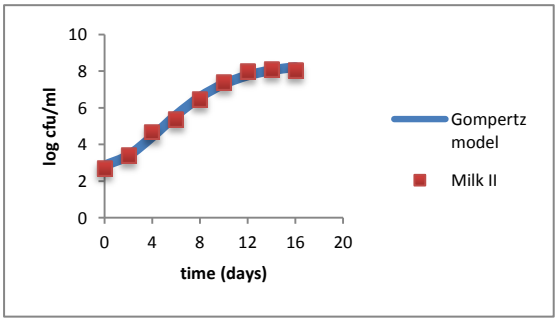


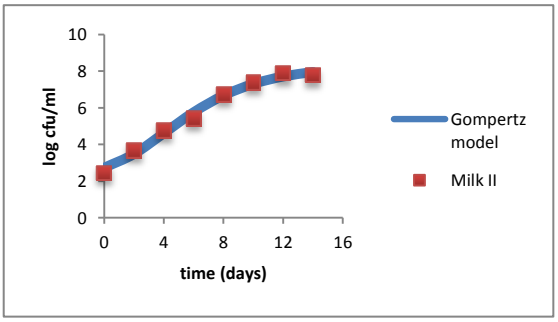


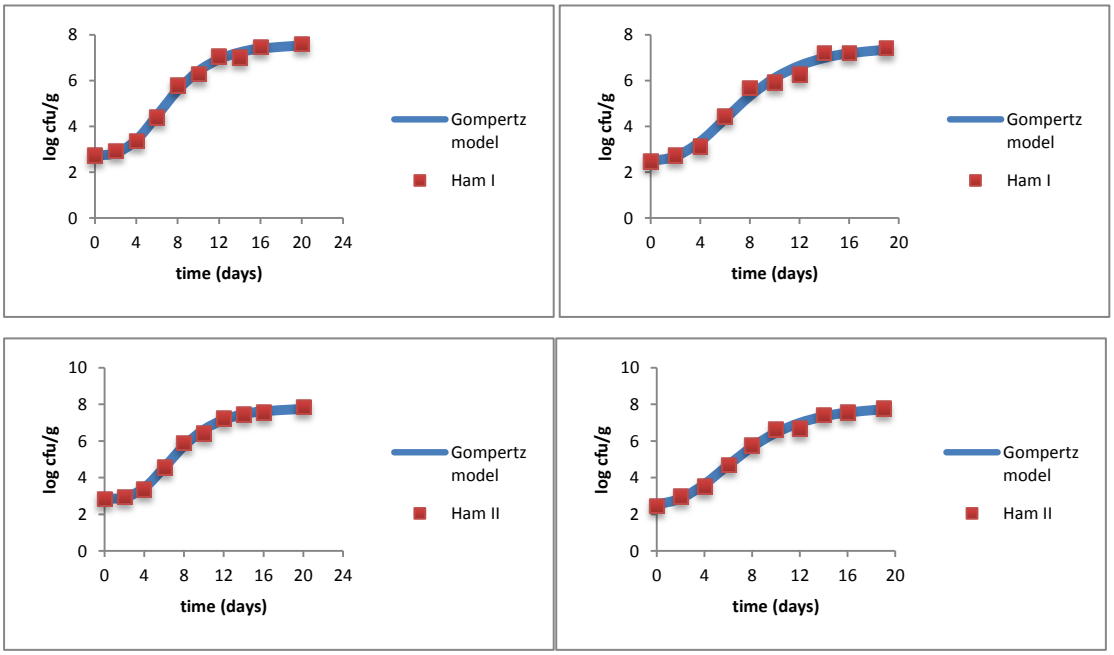




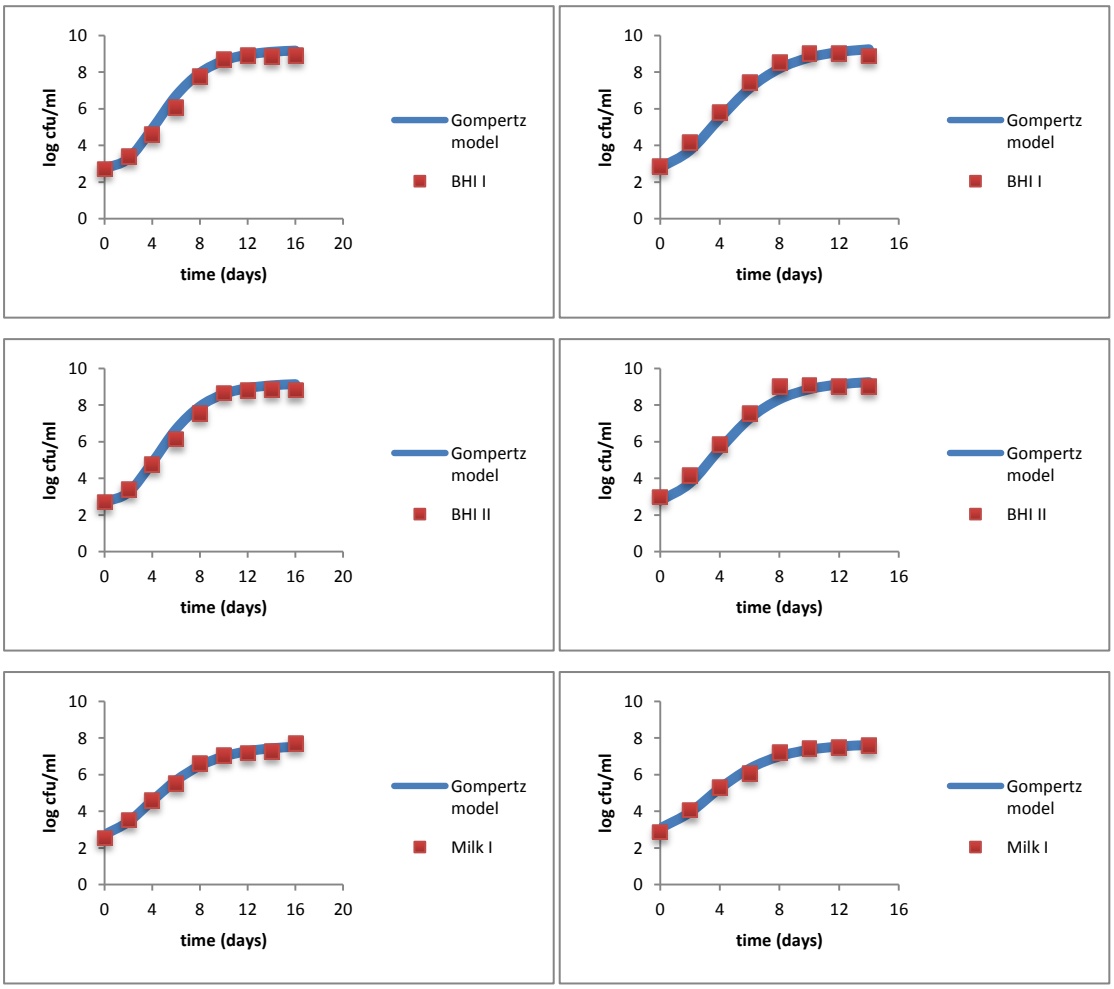


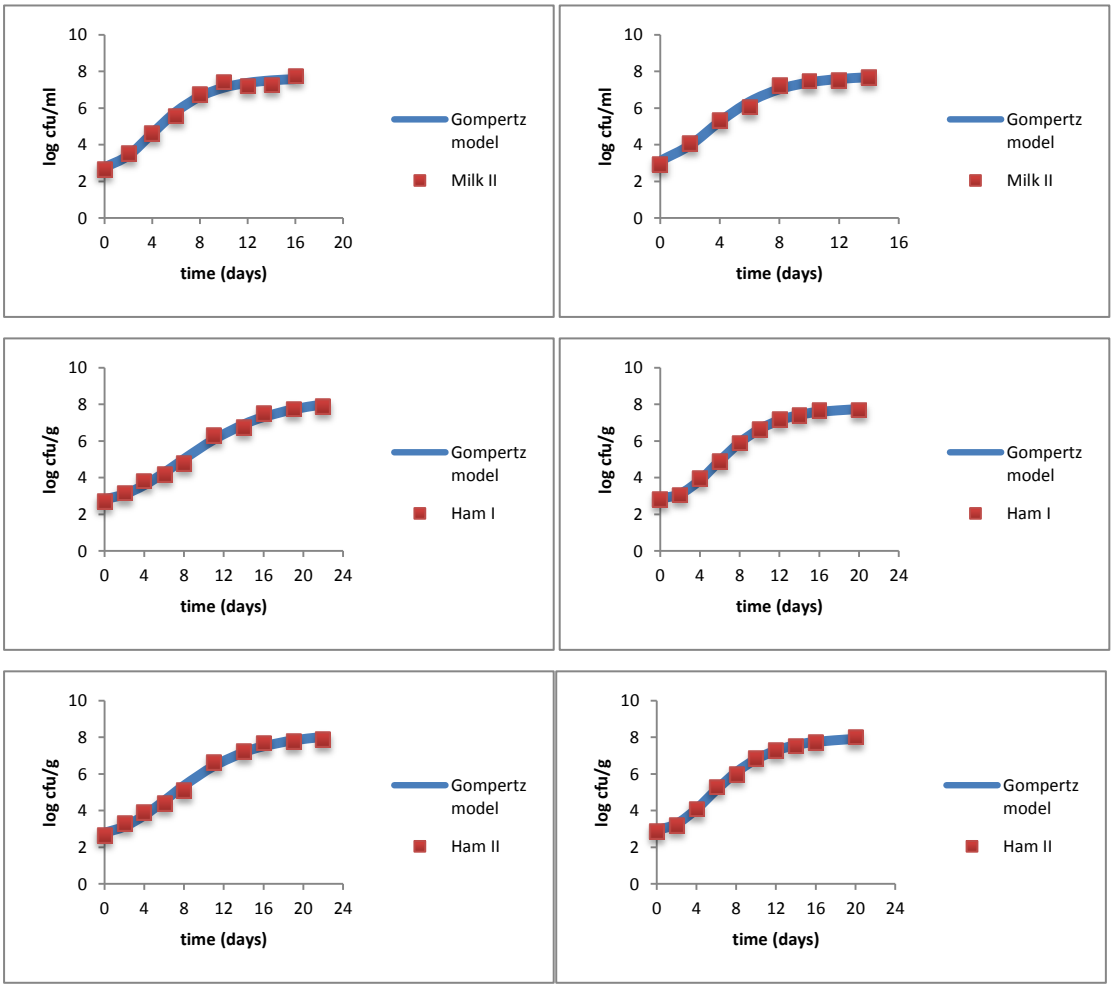




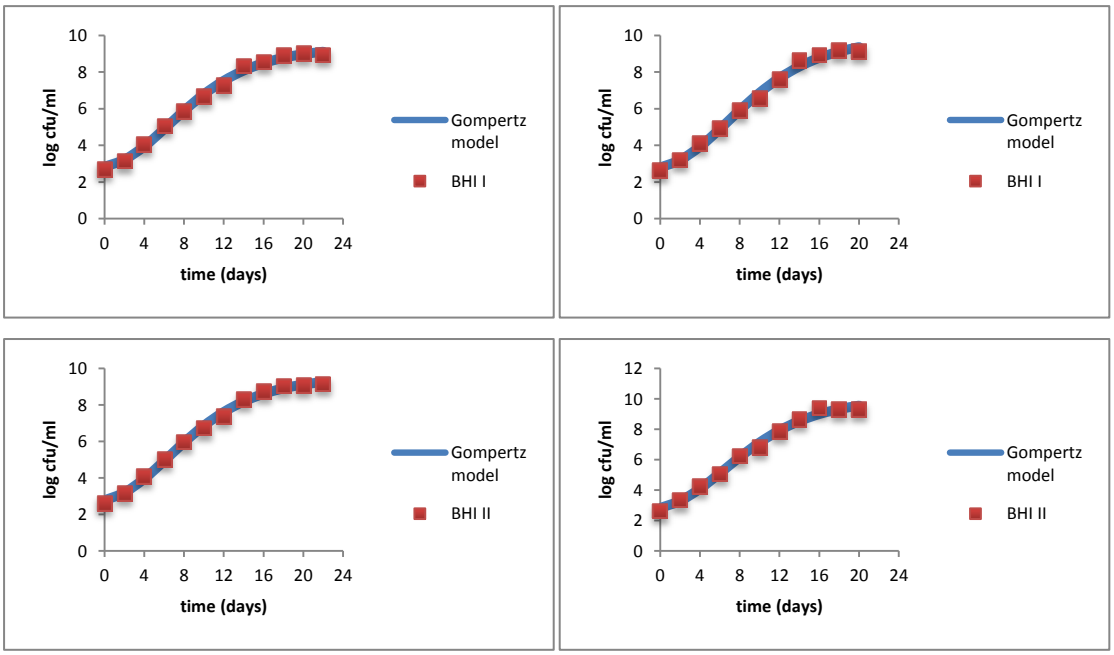


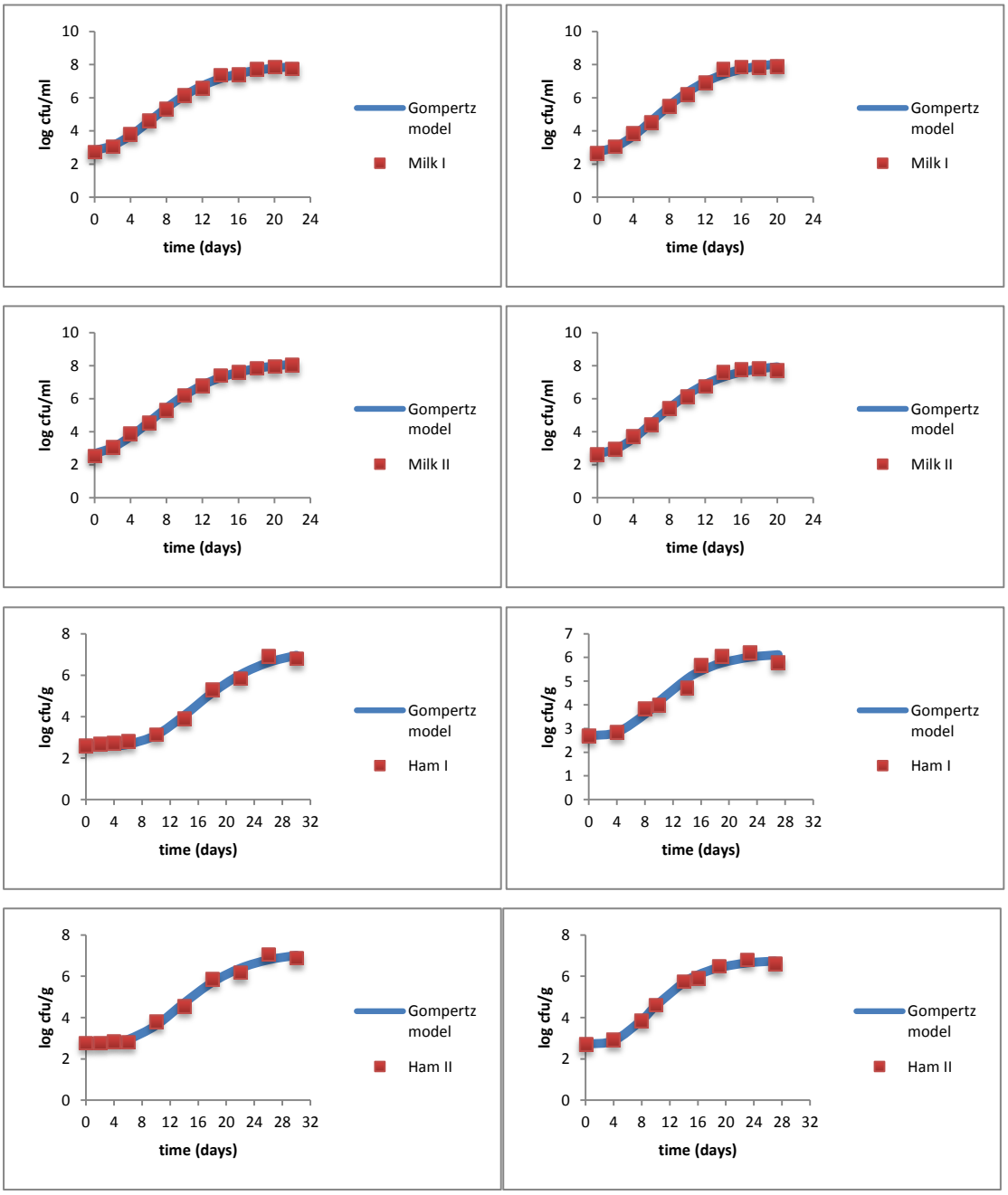
7.1.2 FBR17 growth curves at 7°C



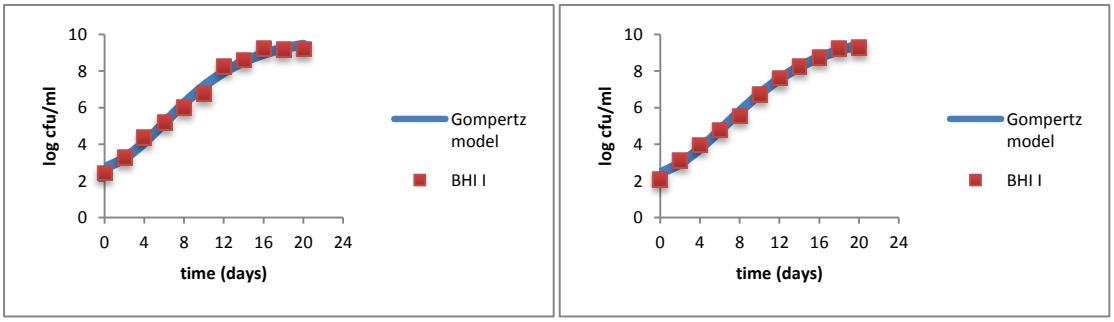


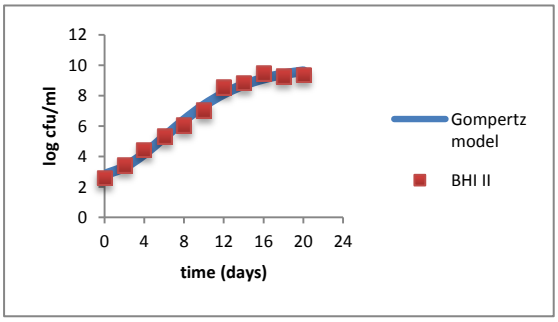
7.1.3 FBR15 growth curves at 7°C

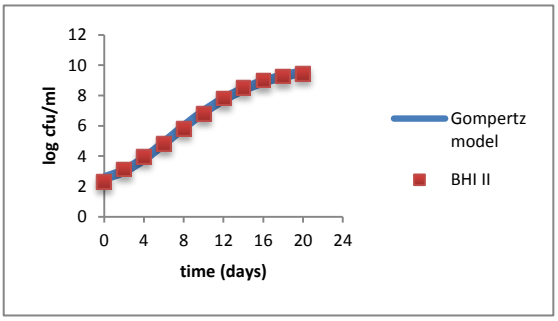




7.1.4 FBR15 growth curves at 7°C with 7°C pre-culturing for 10 days




log cfu/ml
time (days)
Gompertz model
BHI II


log cfu/ml
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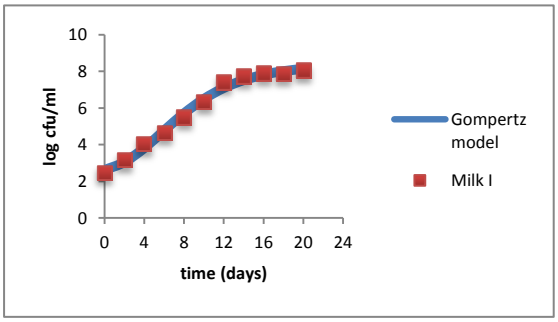
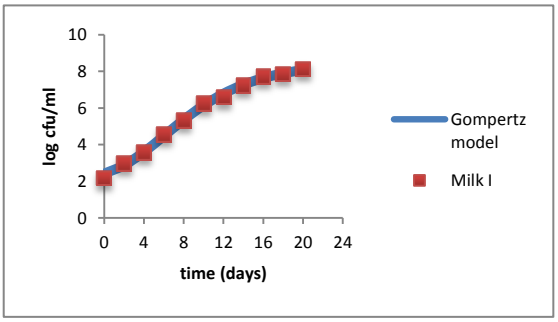
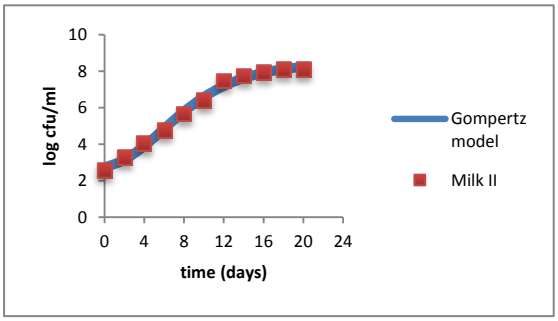
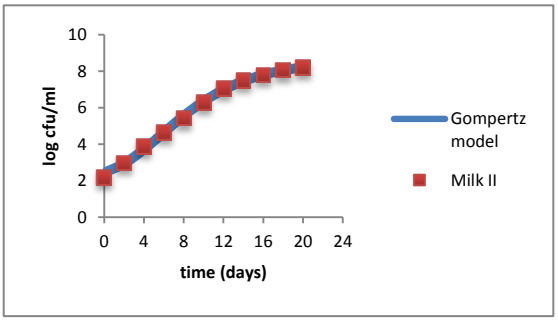
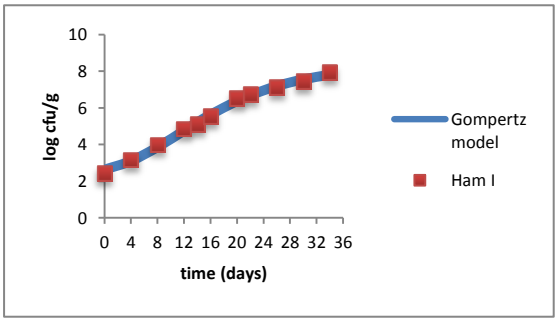
Gompertz model
BHI II

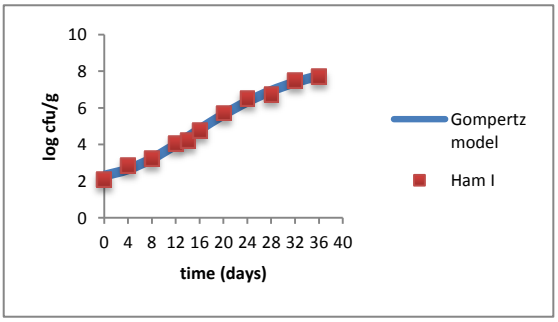

log cfu/ml
time (days)
Gompertz model
Milk I

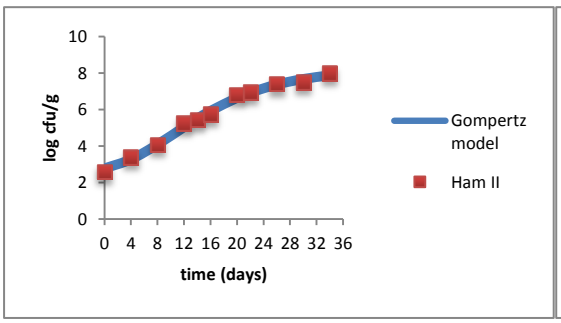

log cfu/ml
time (days)
Gompertz model
Milk I

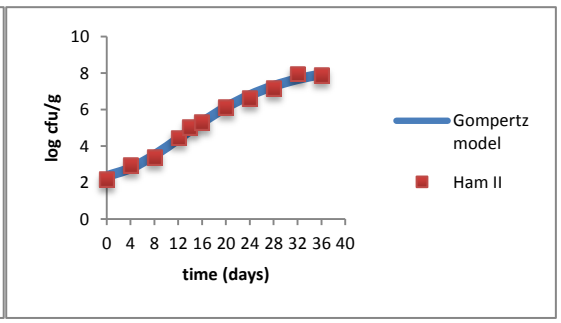

log cfu/ml
time (days)
Gompertz model
Milk II


log cfu/ml
time (days)
Gompertz model
Milk II


log cfu/g
time (days)
Gompertz model
Ham I


log cfu/g
time (days)
Gompertz model
Ham I


log cfu/g
time (days)
Gompertz model
Ham II


log cfu/g
time (days)
Gompertz model
Ham II

7.2 Tables of μ_{max} and λ derived from growth curves fitted by Gompertz model using TableCurve 2D

In these tables, 'a' is *A*, 'b' is μ_{max} (per day), and 'c' is λ (day) in Section 2.3.4

7.2.1 L6 growth parameters at 7°C with larger inoculum size

BHI I

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	5.965594882	0.166365322	35.85840369	5.537939206	6.393250559	0.00000
b	1.951062916	0.212535758	9.179927829	1.404722357	2.497403475	0.00026
c	0.225779118	0.168699312	1.338352334	-0.20787627	0.659434505	0.23841

BHI II

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	5.605005260	0.242133028	23.14845402	4.982582495	6.227428024	0.00000
b	1.976976342	0.354555077	5.575935789	1.065563499	2.888389184	0.00256
c	0.401007802	0.258908025	1.548842691	-0.26453647	1.066552069	0.18210

Milk I

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	4.677583550	0.081048746	57.71321297	4.469241115	4.885925986	0.00000
b	1.551124432	0.103063425	15.05019293	1.286191463	1.816057401	0.00002
c	0.418130419	0.101067218	4.137151760	0.158328863	0.677931974	0.00902

Milk II

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	4.629147563	0.101776049	45.48366364	4.367523900	4.890771226	0.00000
b	1.361832111	0.101603038	13.40345862	1.100653188	1.623011035	0.00004
c	0.226460455	0.125899790	1.798735767	-0.09717526	0.550096168	0.13197

Ham I

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	6.984315373	1.710543110	4.083098131	2.235086334	11.73354441	0.01506
b	0.849559494	0.108799509	7.808486486	0.547483631	1.151635358	0.00145
c	1.446661936	0.422893012	3.420869803	0.272522703	2.620801169	0.02676

Ham II

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	5.423788972	0.734739890	7.381917121	3.383824003	7.463753940	0.00180
b	0.909494305	0.150829621	6.029944897	0.490724142	1.328264468	0.00381
c	1.602621896	0.429309557	3.733021707	0.410667479	2.794576314	0.02024

7.2.2 L6 growth parameters at 7°C

BHI I

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	15.02675623	0.364439397	41.23252410	14.13500515	15.91850731	0.00000
b	2.222511366	0.196003098	11.33916450	1.742909063	2.702113668	0.00003

c	1.724176225	0.299343800	5.759852797	0.991708333	2.456644116	0.00119

BHI II

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	15.02445194	0.419091933	35.85001466	13.99897093	16.04993296	0.00000
b	2.237960907	0.228584023	9.790539521	1.678635954	2.797285861	0.00007
c	1.750033681	0.344622485	5.078118105	0.906772838	2.593294523	0.00227

BHI III

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	15.55578996	0.702911018	22.13052516	13.74889966	17.36268026	0.00000
b	2.129753815	0.280163926	7.601813148	1.409569515	2.849938116	0.00063
c	0.923086901	0.465550189	1.982787083	-0.27364796	2.119821763	0.10421

BHI IV

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	15.41324717	0.639572172	24.09930864	13.76917455	17.05731978	0.00000
b	2.245566436	0.295127591	7.608798707	1.486916808	3.004216063	0.00062
c	0.997699800	0.442876322	2.252772954	-0.14075003	2.136149631	0.07402

Milk I

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	12.79346477	0.304023808	42.08047015	12.04954531	13.53738423	0.00000
b	1.454154944	0.090606780	16.04907436	1.232448141	1.675861747	0.00000
c	1.131143114	0.261224969	4.330149299	0.491948643	1.770337585	0.00493

Milk II

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	13.23703892	0.511064848	25.90089884	11.98650829	14.48756955	0.00000
b	1.364768648	0.117247056	11.64011019	1.077875438	1.651661858	0.00002
c	1.108682535	0.386285266	2.870113446	0.163476541	2.053888529	0.02842

Milk III

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	13.29611604	0.798554122	16.65023780	11.24336731	15.34886476	0.00001
b	1.379351738	0.151701714	9.092525743	0.989390067	1.769313408	0.00027
c	0.763666554	0.475343878	1.606555988	-0.45824379	1.985576897	0.16906

Milk IV

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	13.48220949	0.802160537	16.80737068	11.42019018	15.54422880	0.00001
b	1.380486507	0.154725950	8.922139467	0.982750789	1.778222225	0.00029
c	0.390122317	0.492898254	0.791486505	-0.87691299	1.657157620	0.46453

Ham I

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	11.19112454	0.273613423	40.90122630	10.54413161	11.83811748	0.00000
b	1.343459171	0.098024929	13.70528069	1.111667046	1.575251296	0.00000
c	3.002982061	0.298893643	10.04699209	2.296210907	3.709753216	0.00002

Ham II

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	11.39527316	0.206566169	55.16524421	10.90682178	11.88372453	0.00000

b	1.434203369	0.080971025	17.71255058	1.242737321	1.625669417	0.00000
c	3.268067036	0.221948389	14.72444582	2.743242493	3.792891579	0.00000

Ham III

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	11.53176928	0.549933292	20.96939656	10.23138369	12.83215488	0.00000
b	1.206309644	0.144618761	8.341308140	0.864340617	1.548278672	0.00007
c	2.436594652	0.545975595	4.462827050	1.145567523	3.727621782	0.00293

Ham IV

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	12.46532510	0.364339618	34.21347690	11.60379880	13.32685139	0.00000
b	1.228184615	0.086612914	14.18015579	1.023377618	1.432991612	0.00000
c	1.838238918	0.338619029	5.428634428	1.037532152	2.638945685	0.00098

7.2.3 FBR17 growth parameters at 7°C

BHI I

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	14.82754268	0.376342270	39.39908924	13.90666632	15.74841904	0.00000
b	2.082576144	0.176512499	11.79846276	1.650665619	2.514486669	0.00002
c	1.913839130	0.299057264	6.399574129	1.182072367	2.645605894	0.00069

BHI II

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	14.73173970	0.366196256	40.22908337	13.83568974	15.62778966	0.00000
b	1.972429890	0.156762218	12.58230408	1.588846562	2.356013218	0.00002
c	1.654755692	0.292121897	5.664606828	0.939959161	2.369552223	0.00130

BHI III

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	14.54172329	0.306759748	47.40427443	13.75317225	15.33027433	0.00000
b	2.299888543	0.173851487	13.22904154	1.852989067	2.746788018	0.00004
c	0.902559411	0.238908850	3.777840004	0.288424659	1.516694163	0.01292

BHI IV

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	14.42867883	0.441384948	32.68955796	13.29406270	15.56329496	0.00000
b	2.472489378	0.291417995	8.484340079	1.723375570	3.221603185	0.00037
c	1.171112527	0.347441571	3.370674737	0.277985532	2.064239522	0.01988

Milk I

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	11.68179910	0.324202798	36.03238213	10.88850343	12.47509477	0.00000
b	1.388850608	0.113929191	12.19047201	1.110075922	1.667625295	0.00002
c	0.637875482	0.335263365	1.902610151	-0.18248442	1.458235382	0.10579

Milk II

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	11.50199045	0.407652183	28.21520633	10.50450149	12.49947941	0.00000
b	1.487616835	0.171082184	8.695334615	1.068993812	1.906239857	0.00013

c	0.961824719	0.438446631	2.193709907	-0.11101554	2.034664976	0.07071

Milk III

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	11.08940608	0.374910809	29.57878465	10.12566716	12.05314500	0.00000
b	1.543882849	0.163810430	9.424814095	1.122794733	1.964970966	0.00023
c	0.451194869	0.373831214	1.206948087	-0.50976886	1.412158600	0.28143

Milk IV

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	11.14858390	0.382245122	29.16605932	10.16599153	12.13117627	0.00000
b	1.520872852	0.158800838	9.577234411	1.112662301	1.929083403	0.00021
c	0.452360381	0.373660500	1.210618681	-0.50816451	1.412885277	0.28014

Ham I

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	13.10806581	0.624815894	20.97908509	11.63061100	14.58552063	0.00000
b	0.872949334	0.066240093	13.17856451	0.716316405	1.029582264	0.00000
c	1.857215139	0.475938290	3.902218369	0.731799917	2.982630361	0.00588

Ham II

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	12.87559688	0.503902248	25.55177501	11.68405741	14.06713636	0.00000
b	0.967112672	0.078683805	12.29112745	0.781055040	1.153170305	0.00001
c	1.525330001	0.469336145	3.249973432	0.415526373	2.635133630	0.01406

Ham III

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	11.49937937	0.114886445	100.0934390	11.22771609	11.77104264	0.00000
b	1.249099224	0.034724022	35.97219352	1.166989960	1.331208487	0.00000
c	2.129483218	0.124499965	17.10428766	1.835087582	2.423878855	0.00000

Ham IV

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	11.81430042	0.185819450	63.57946078	11.37490725	12.25369360	0.00000
b	1.225205189	0.051752185	23.67446318	1.102830719	1.347579660	0.00000
c	1.733829775	0.197029045	8.799868926	1.267930116	2.199729433	0.00005

7.2.4 FBR15 growth parameters at 7°C

BHI I

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	15.43735500	0.395562835	39.02630287	14.54252970	16.33218030	0.00000
b	1.161657970	0.064213173	18.09064894	1.016397681	1.306918260	0.00000
c	1.625186996	0.338834438	4.796404423	0.858690246	2.391683746	0.00098

BHI II

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	16.03285236	0.349213740	45.91128735	15.24287599	16.82282872	0.00000
b	1.183925522	0.054549870	21.70354418	1.060525142	1.307325903	0.00000
c	1.461266322	0.286820689	5.094703342	0.812432847	2.110099797	0.00065

BHI III

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	16.80946175	0.707435502	23.76112268	15.17811256	18.44081094	0.00000
b	1.191386531	0.079652692	14.95726631	1.007707093	1.375065968	0.00000
c	1.604736769	0.413314472	3.882604839	0.651631889	2.557841650	0.00466

BHI IV

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	17.14093283	0.719134860	23.83549148	15.48260487	18.79926079	0.00000
b	1.235110764	0.087503065	14.11505714	1.033328335	1.436893194	0.00000
c	1.358586298	0.435066495	3.122709552	0.355321164	2.361851433	0.01417

Milk I

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	12.20469539	0.240946190	50.65319923	11.65963724	12.74975354	0.00000
b	0.998389873	0.048344312	20.65165140	0.889027442	1.107752304	0.00000
c	1.832320000	0.279034226	6.566649639	1.201100727	2.463539273	0.00010

Milk II

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	13.25363916	0.215655678	61.45740884	12.76579212	13.74148619	0.00000
b	1.027473681	0.038599891	26.61856449	0.940154662	1.114792701	0.00000
c	1.452024552	0.226086344	6.422433699	0.940581710	1.963467393	0.00012

Milk III

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	12.97113693	0.405133031	32.01698194	12.03689849	13.90537538	0.00000
b	1.096104492	0.074205857	14.77113169	0.924985479	1.267223506	0.00000
c	1.896818931	0.369890235	5.128058955	1.043850521	2.749787342	0.00090

Milk IV

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	12.76821291	0.378497812	33.73391475	11.89539539	13.64103043	0.00000
b	1.107618379	0.073305287	15.10966578	0.938576086	1.276660673	0.00000
c	2.079604957	0.354019838	5.874261086	1.263233748	2.895976166	0.00037

Ham I

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	11.07912712	0.802297707	13.80924690	9.181994511	12.97625973	0.00000
b	0.641824296	0.069952363	9.175162444	0.476413242	0.807235350	0.00004
c	8.737180772	0.817373718	10.68933412	6.804399059	10.66996249	0.00001

Ham II

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	10.31922891	0.562034225	18.36049914	8.990229154	11.64822867	0.00000
b	0.647763586	0.070756799	9.154789237	0.480450342	0.815076830	0.00004
c	7.106961611	0.793942884	8.951477184	5.229585017	8.984338206	0.00004

Ham III

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	8.085801410	0.665111263	12.15706582	6.458332778	9.713270042	0.00002
b	0.631546288	0.136697184	4.620038766	0.297060329	0.966032247	0.00362

c	4.706421452	1.332581477	3.531807648	1.445712045	7.967130859	0.01234

Ham IV

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	9.427262085	0.236928429	39.78949315	8.847519105	10.00700506	0.00000
b	0.799642812	0.060790919	13.15398469	0.650892793	0.948392830	0.00001
c	4.640978167	0.439682263	10.55529996	3.565114428	5.716841905	0.00004

7.2.5 FBR15 growth parameters at 7°C with 7°C pre-culturing for 10 days

BHI I

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	17.11677558	0.776897077	22.03223064	15.32524771	18.90830345	0.00000
b	1.258546098	0.103892730	12.11389963	1.018969034	1.498123162	0.00000
c	1.043292099	0.502853593	2.074743254	-0.11629036	2.202874562	0.07170

BHI II

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	17.04723378	0.798185658	21.35747944	15.20661435	18.88785320	0.00000
b	1.308053475	0.117948174	11.09006977	1.036064499	1.580042451	0.00000
c	1.282698999	0.531614620	2.412836200	0.056793489	2.508604508	0.04232

BHI III

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	18.54268958	0.655513086	28.28729127	17.03107370	20.05430547	0.00000
b	1.195419633	0.059998958	19.92400662	1.057061789	1.333777478	0.00000
c	0.845887231	0.334497279	2.528831430	0.074535125	1.617239338	0.03532

BHI IV

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	18.09232154	0.474746806	38.10941186	16.99755345	19.18708964	0.00000
b	1.247418056	0.050817763	24.54689049	1.130232085	1.364604027	0.00000
c	1.300348463	0.257810146	5.043821912	0.705837201	1.894859725	0.00100

Milk I

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	13.60367959	0.467529211	29.09696181	12.52555530	14.68180389	0.00000
b	1.126221943	0.085743803	13.13473276	0.928496378	1.323947508	0.00000
c	1.283430270	0.426035779	3.012494099	0.300990003	2.265870536	0.01675

Milk II

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	13.55920866	0.389523989	34.80968834	12.66096473	14.45745258	0.00000
b	1.127319338	0.071746486	15.71253723	0.961871644	1.292767032	0.00000
c	1.362349304	0.354652678	3.841361953	0.544518763	2.180179845	0.00494

Milk III

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	14.50976221	0.384768196	37.71039909	13.62248516	15.39703926	0.00000

b	1.039566377	0.048335701	21.50721614	0.928104050	1.151028704	0.00000
c	0.910247138	0.288820386	3.151602802	0.244226134	1.576268142	0.01357

Milk IV

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	14.71526035	0.340406412	43.22850524	13.93028176	15.50023894	0.00000
b	1.088104443	0.047561403	22.87788789	0.978427651	1.197781235	0.00000
c	0.787746694	0.266402975	2.956974091	0.173420334	1.402073055	0.01823

Ham I

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	13.66200205	0.500661721	27.28789015	12.50747405	14.81653005	0.00000
b	0.525760588	0.027906547	18.84004475	0.461407976	0.590113200	0.00000
c	1.675228436	0.618341382	2.709229051	0.249330654	3.101126218	0.02669

Ham II

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	13.04301650	0.476178813	27.39100551	11.94494619	14.14108681	0.00000
b	0.552716894	0.035219243	15.69360502	0.471501174	0.633932615	0.00000
c	1.698953243	0.702818050	2.417344352	0.078251916	3.319654569	0.04202

Ham III

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	15.17096923	0.908426002	16.70028070	13.07613511	17.26580334	0.00000
b	0.483905879	0.028825771	16.78726580	0.417433532	0.550378225	0.00000
c	3.107674745	0.740349255	4.197579351	1.400426304	4.814923186	0.00301

Ham IV

Parm	Value	Std Error	t-value	95% Confidence Limits		P>|t|
a	14.65942063	0.661052619	22.17587560	13.13503056	16.18381070	0.00000
b	0.534793379	0.034693046	15.41500216	0.454791072	0.614795687	0.00000
c	2.326332410	0.762120991	3.052445001	0.568878255	4.083786565	0.01576

7.3 Tables of two independent samples t-test using SPSS

In these tables, 'Difference' is the difference of μ_{max} or λ between two different initial inoculum sizes of L6 in Section 2.3.5.

7.3.1 Effect of inoculum size

Grown in BHI broth

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Max growth rate	Equal variances assumed	1,600	,275	-5,971	4	,004	-,010206	,001709	-,014951	-,005460
	Equal variances not assumed			-8,221	3,922	,001	-,010206	,001241	-,013679	-,006732
Lag time	Equal variances assumed	154,666	,000	-3,032	4	,039	-1,03536	,34143	-1,98332	-,08739
	Equal variances not assumed			-4,291	3,723	,015	-1,03536	,24126	-1,72534	-,34537

Grown in milk

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Max growth rate	Equal variances assumed	16,865	,015	,945	4	,398	,002574	,002723	-,004987	,010136
	Equal variances not assumed			,639	1,092	,631	,002574	,004032	-,039510	,044659
Lag time	Equal variances assumed	2,351	,200	-1,963	4	,121	-,52610	,26807	-1,27038	,21818
	Equal variances not assumed			-2,644	3,993	,057	-,52610	,19898	-1,07892	,02673

Grown in ham

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Max growth rate	Equal variances assumed	3,815	,123	-5,184	4	,007	-,017646	,003404	-,027097	-,008196
	Equal variances not assumed			-6,949	3,999	,002	-,017646	,002539	-,024698	-,010595
Lag time	Equal variances assumed	4,415	,104	-2,322	4	,081	-1,11183	,47877	-2,44112	,21746
	Equal variances not assumed			-3,400	3,336	,036	-1,11183	,32703	-2,09567	-,12799

7.3.2 Effect of low temperature pre-culturing

Grown in BHI broth

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Max growth rate	Equal variances assumed	,322	,591	-2,138	6	,076	-,002472	,001157	-,005303	,000358
	Equal variances not assumed			-2,138	5,223	,083	-,002472	,001157	-,005408	,000463
Lag time	Equal variances assumed	2,404	,172	3,155	6	,020	,39439	,12501	,08850	,70029
	Equal variances not assumed			3,155	4,826	,026	,39439	,12501	,06953	,71926

Grown in milk

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Max growth rate	Equal variances assumed	1,215	,313	-1,129	6	,302	-,001579	,001399	-,005004	,001845
	Equal variances not assumed			-1,129	5,671	,305	-,001579	,001399	-,005052	,001894
Lag time	Equal variances assumed	,420	,541	3,791	6	,009	,72926	,19235	,25860	1,19992
	Equal variances not assumed			3,791	5,979	,009	,72926	,19235	,25820	1,20032

Grown in ham

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Max growth rate	Equal variances assumed	3,227	,123	3,665	6	,011	,006496	,001772	,002159	,010832
	Equal variances not assumed			3,665	3,785	,024	,006496	,001772	,001463	,011528
Lag time	Equal variances assumed	9,023	,024	3,898	6	,008	4,09587	1,05076	1,52474	6,66700
	Equal variances not assumed			3,898	3,681	,021	4,09587	1,05076	1,07592	7,11582

7.4 Tables of one sample t-test using SPSS

In these tables, 'Difference' is the difference of μ_{max} between experiments and prediction of L6 (A), FBR17 (B), FBR15 (C), and FBR15 with ten-day 7°C pre-culturing (D) at 7°C in Section 2.3.6.

7.4.1 Grown in BHI broth

A

One-Sample Test

	Test Value = 0.0883335779339713					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
Max growth rate	3,315	3	,045	,003706	,00015	,00726

B

One-Sample Test

	Test Value = 0.0764160869650177					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
Max growth rate	3,339	3	,044	,015536	,00073	,03034

C

One-Sample Test

	Test Value = 0.0634359132988522					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
Max growth rate	-21,413	3	,000	-,013727	-,01577	-,01169

D

One-Sample Test

	Test Value = 0.0634359132988522					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
Max growth rate	-11,690	3	,001	-,011254	-,01432	-,00819

7.4.2 Grown in milk

A

One-Sample Test

	Test Value = 0.0843815825727740					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
Max growth rate	-31,300	3	,000	-,026270	-,02894	-,02360

B

One-Sample Test

	Test Value = 0.0713136541405381					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
Max growth rate	-6,622	3	,007	-,009426	-,01396	-,00490

C

One-Sample Test

	Test Value = 0.0608650651659737					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
Max growth rate	-15,248	3	,001	-,016807	-,02031	-,01330

D

One-Sample Test

	Test Value = 0.0608650651659737					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
Max growth rate	-17,659	3	,000	-,015228	-,01797	-,01248

7.4.3 Grown in ham

A

One-Sample Test

	Test Value = 0.0376543503137072					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
Max growth rate	7,525	3	,005	,016639	,00960	,02368

B

One-Sample Test

	Test Value = 0.0238316363502864					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
Max growth rate	5,409	3	,012	,021110	,00869	,03353

C

One-Sample Test

	Test Value = 0.0189478251204742					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Max growth rate	5,642	3	,011	,009393	,00410	,01469

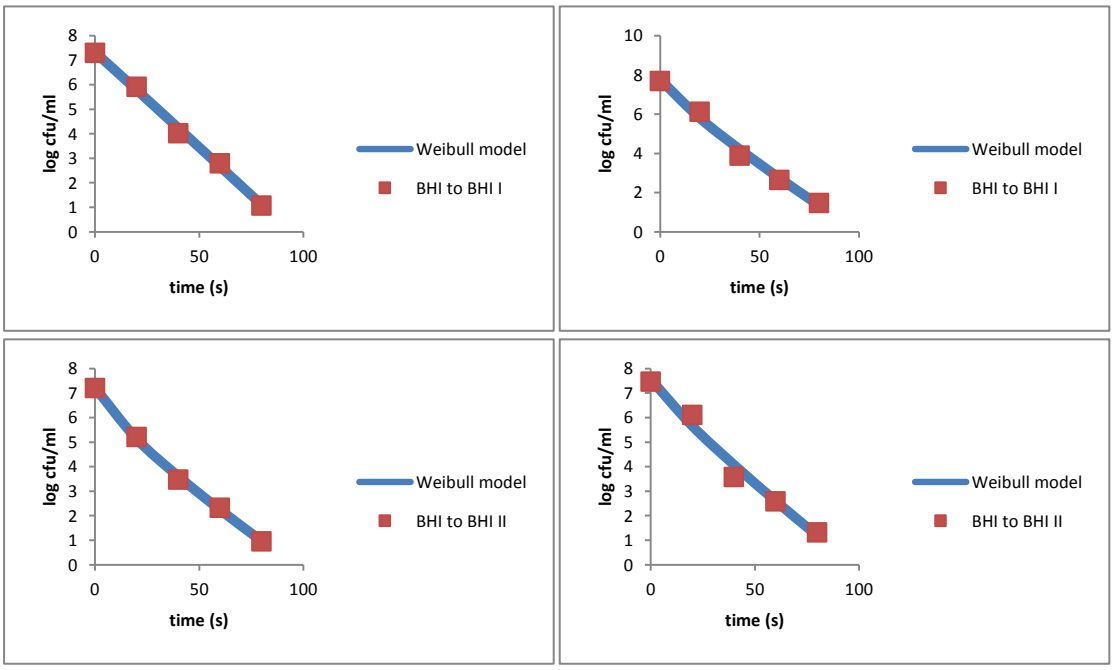
D

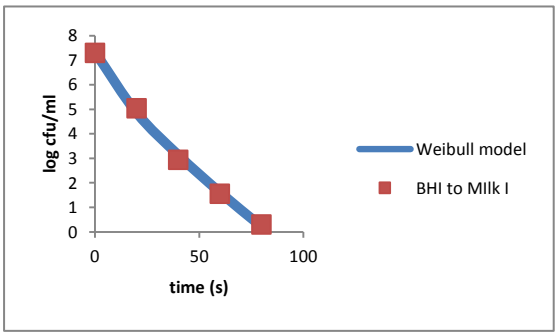
One-Sample Test

	Test Value = 0.0189478251204742					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Max growth rate	4,769	3	,018	,002898	,00096	,00483

7.5 Figures of fitting modified Weibull model to inactivation curves using Excel Solver Add-in

7.5.1 L6 inactivation curves at 65°C

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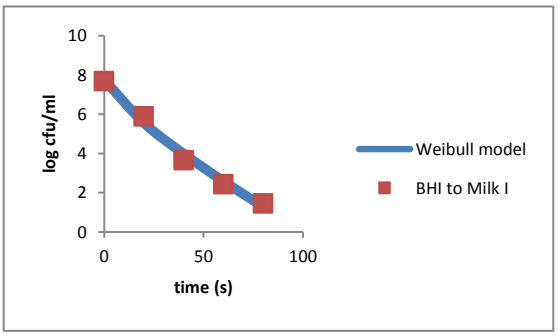
Weibull model
BHI to MIlk I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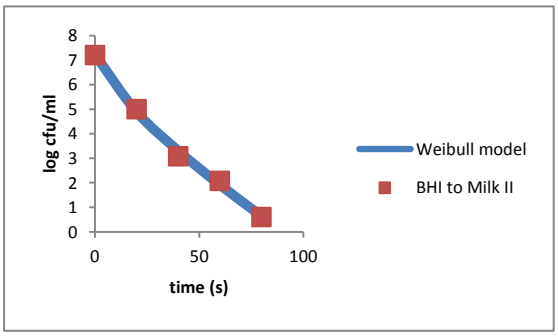
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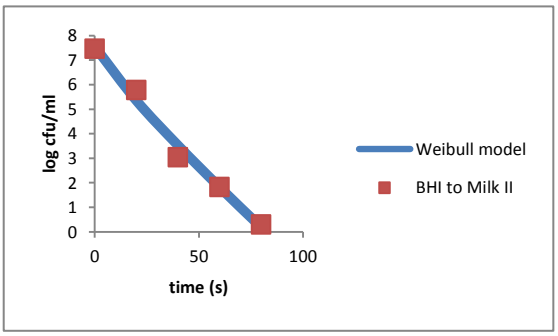
Weibull model
BHI to Milk II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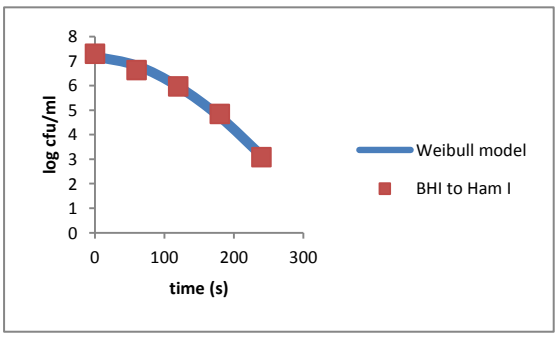
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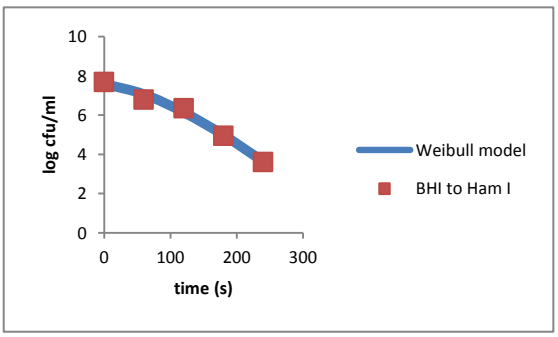
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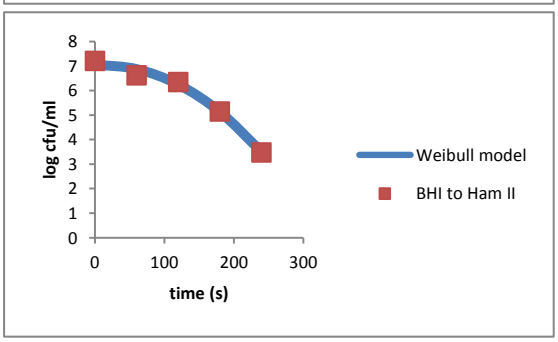
Weibull model
BHI to Ham I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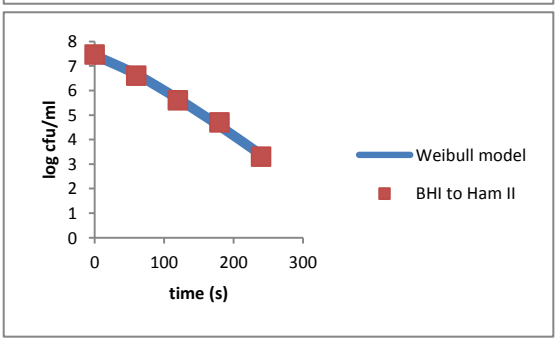
Weibull model
BHI to Ham II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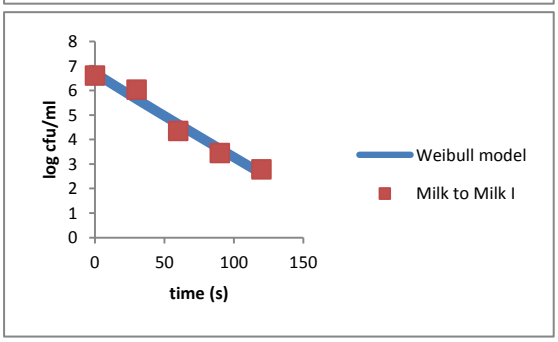
BHI to Ham II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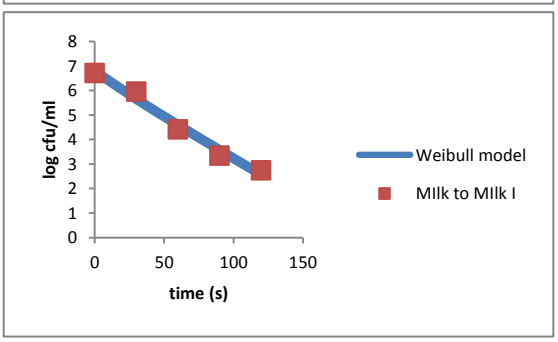
Weibull model
Milk to Milk I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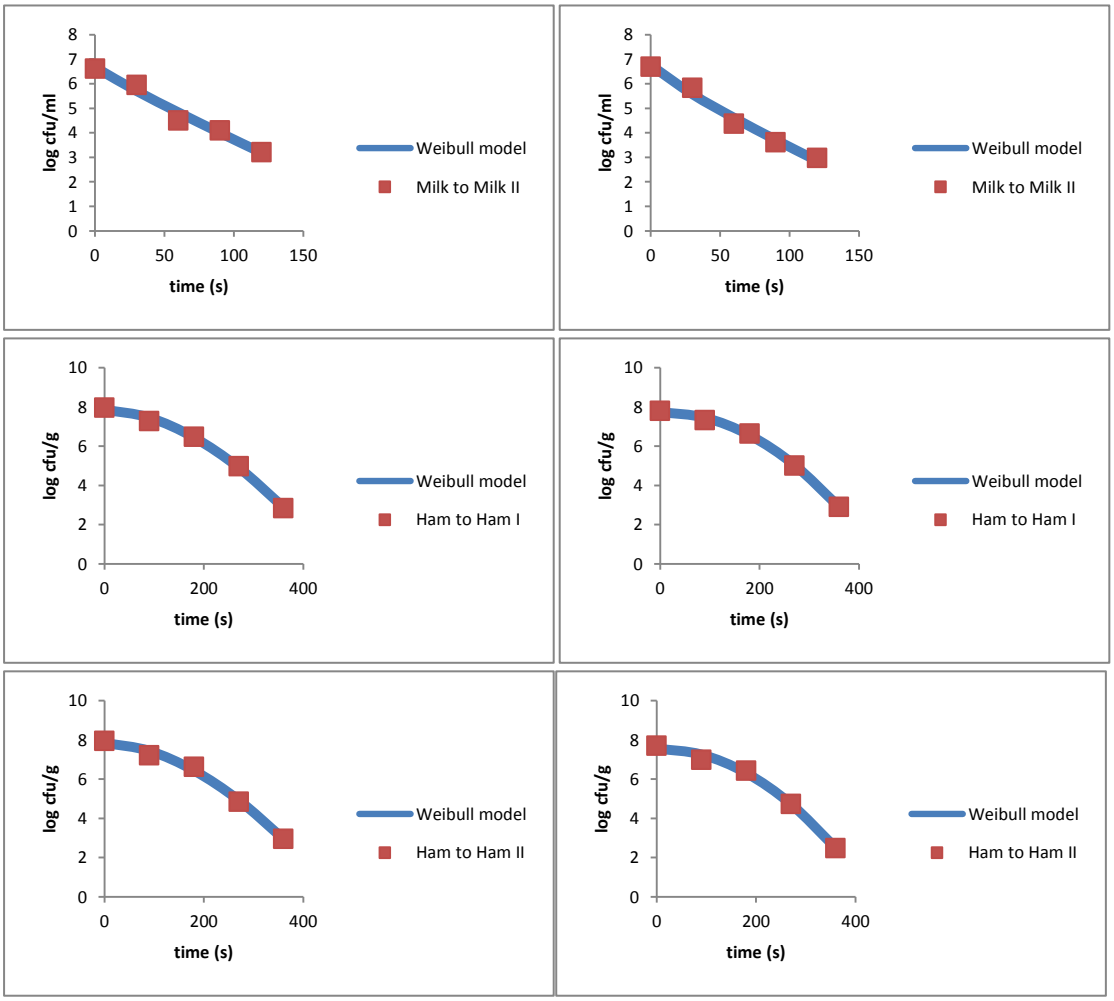
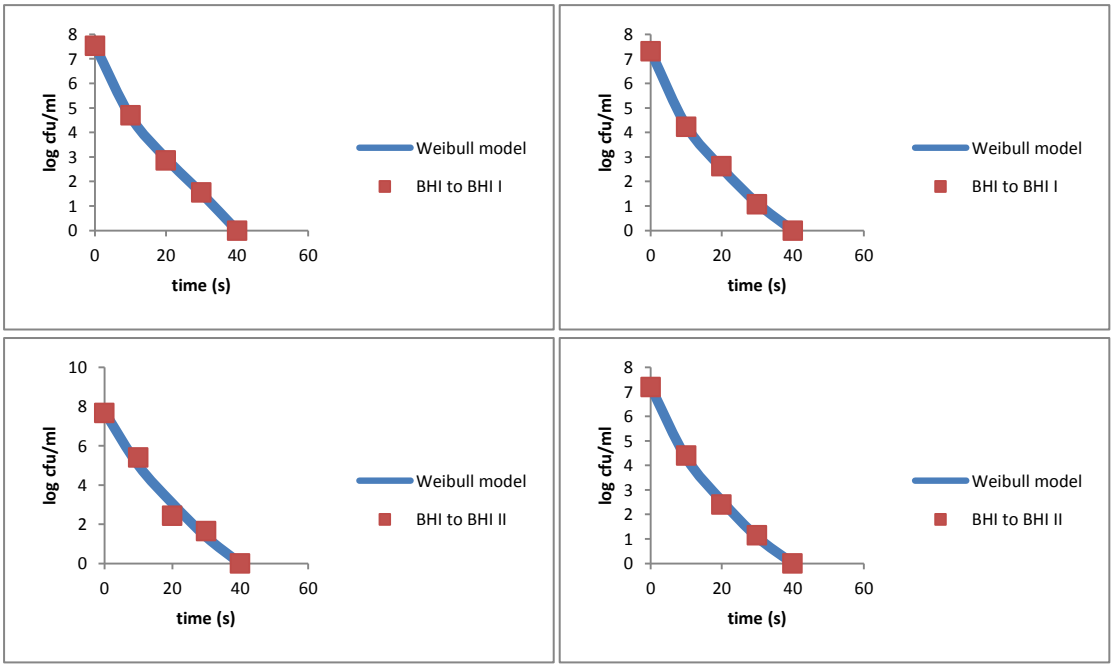
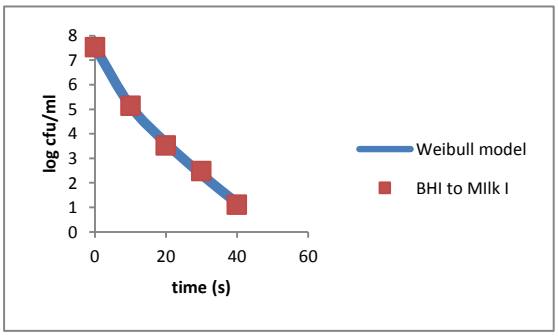
time (s)
Weibull model
MIlk to MIlk I



7.5.2 FBR17 inactivation curves at 65°C

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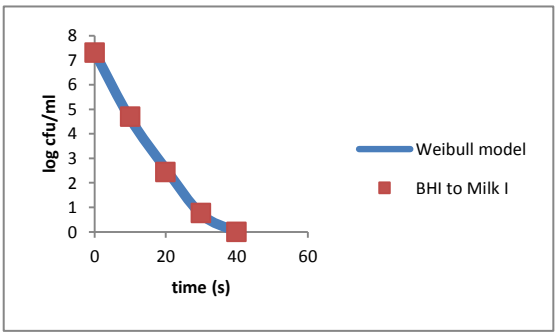
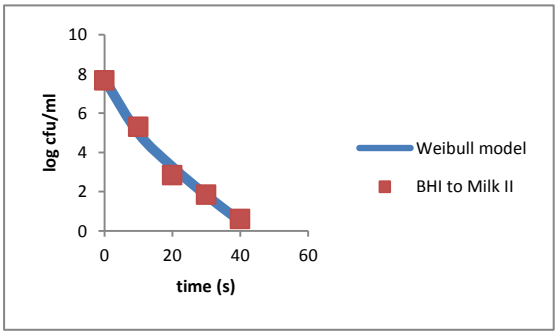
Weibull model
BHI to MIlk I


log cfu/ml
time (s)
Weibull model
BHI to Milk I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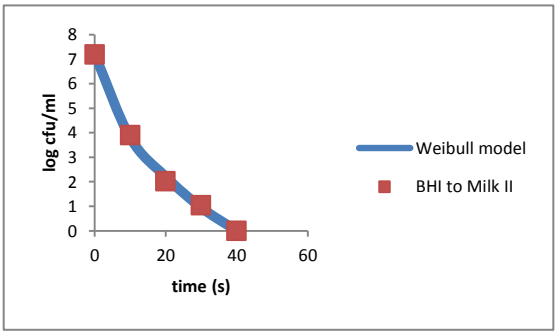
Weibull model
BHI to Milk II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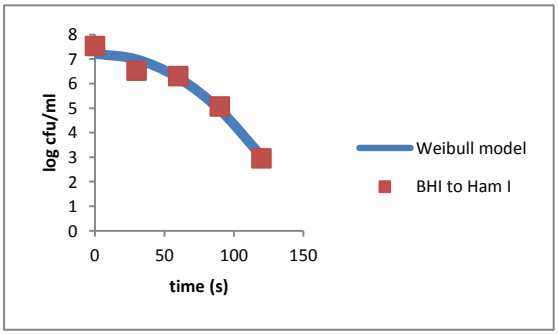
Weibull model
BHI to Milk II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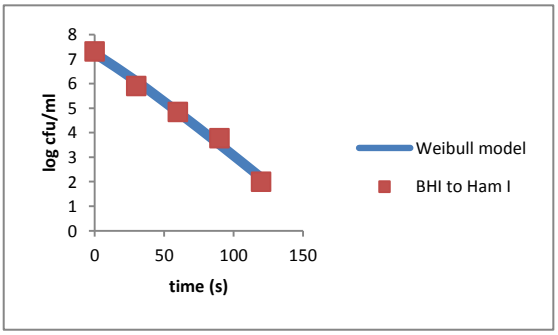
Weibull model
BHI to Ham I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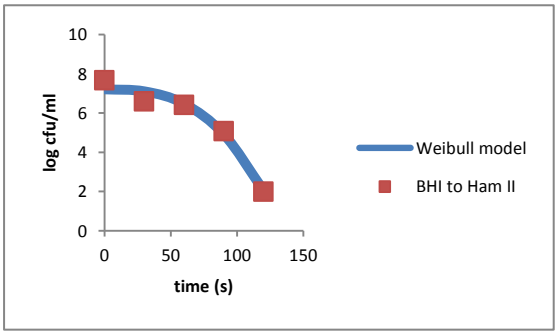
Weibull model
BHI to Ham I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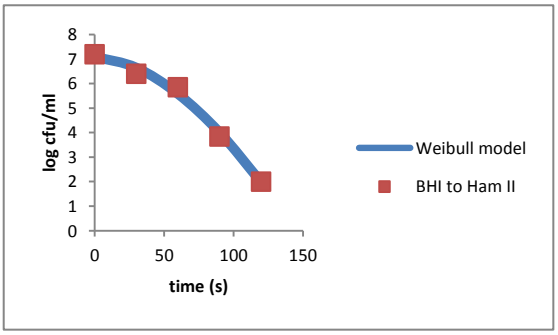
Weibull model
BHI to Ham II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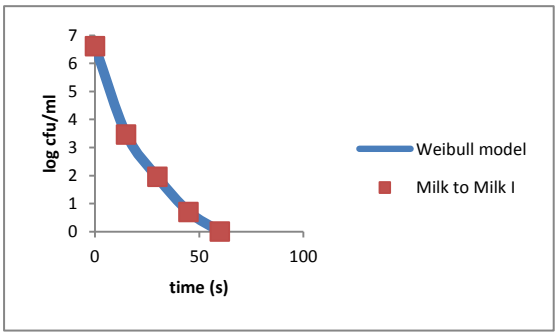
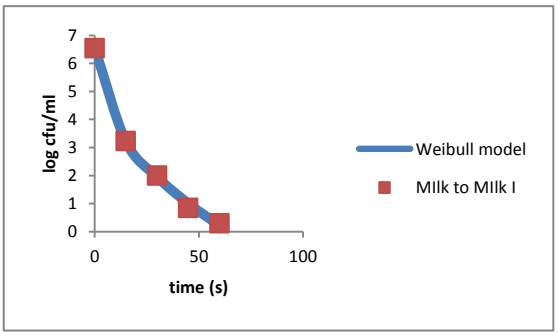
time (s)
Weibull model
BHI to Ham II


log cfu/ml
time (s)
Weibull model
Milk to Milk I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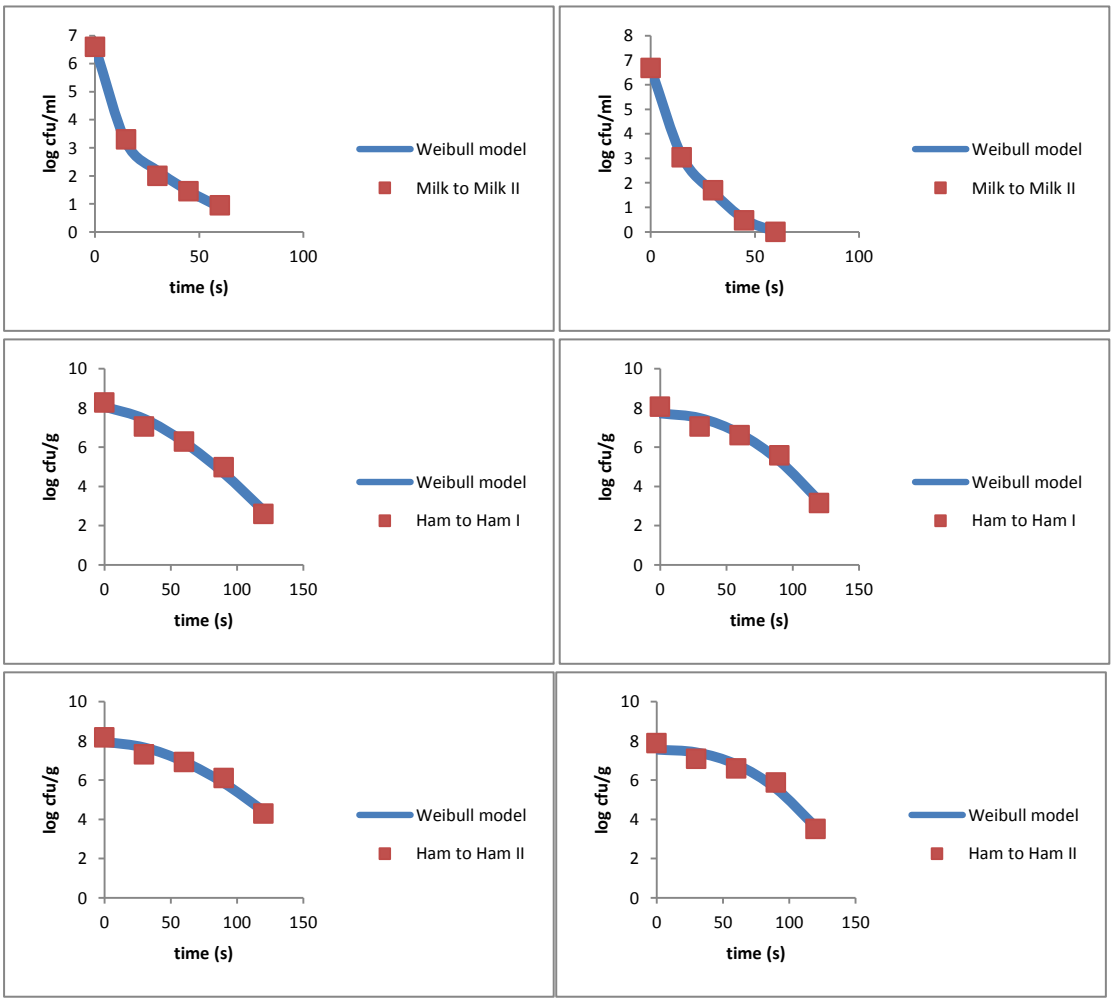
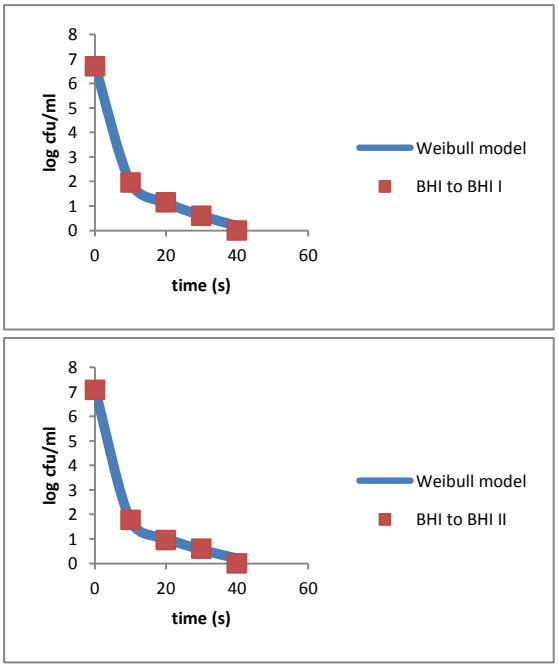
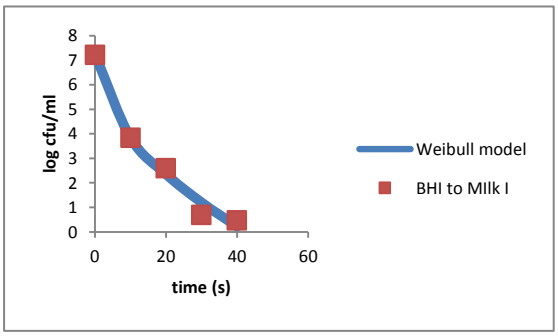
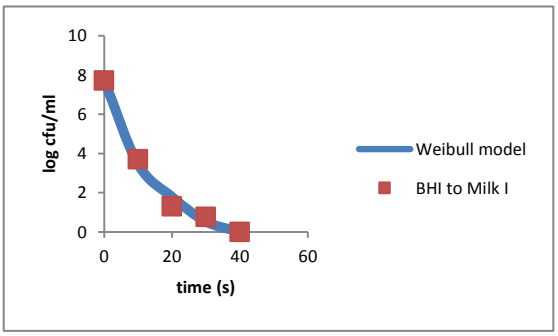
time (s)
Weibull model
MIlk to MIlk I



7.5.3 FBR15 inactivation curves at 65°C




log cfu/ml
time (s)
Weibull model
BHI to MIlk I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time (s)
Weibull model
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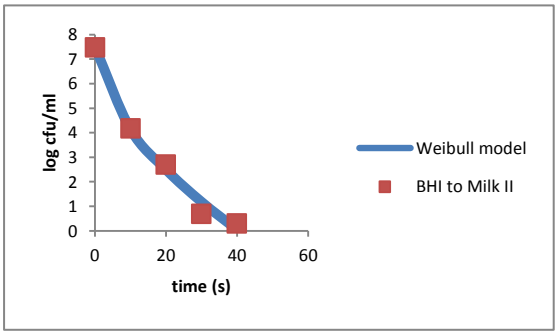
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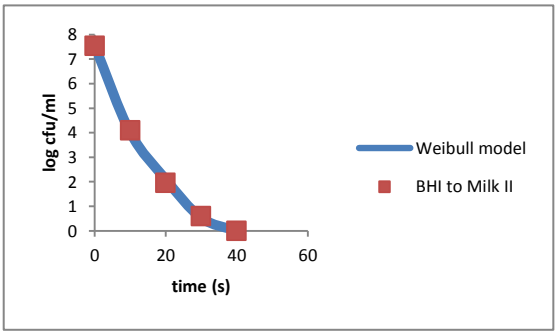
time (s)
Weibull model
BHI to Milk II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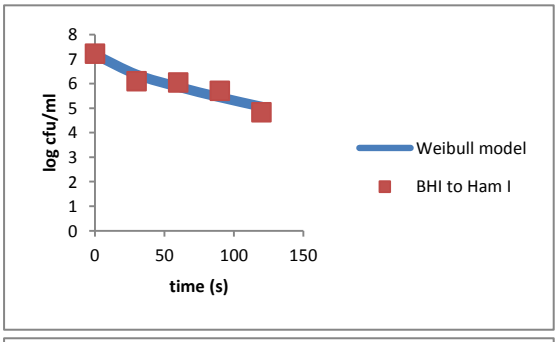
time (s)
Weibull model
BHI to Milk II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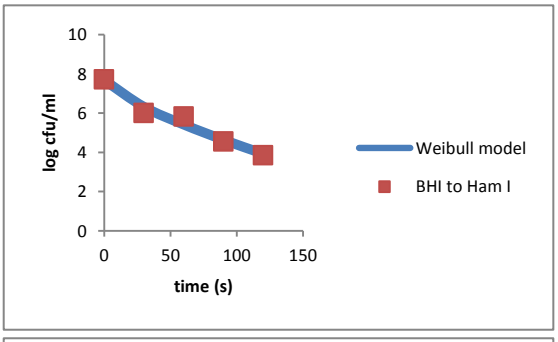
time (s)
Weibull model
BHI to Ham I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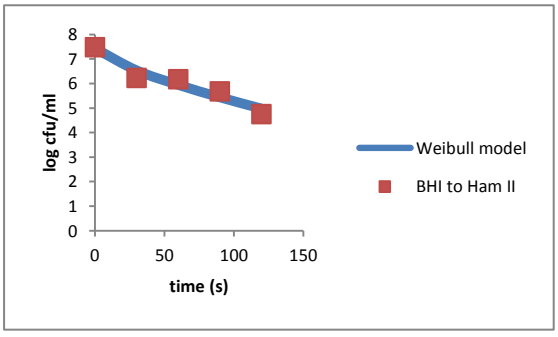
time (s)
Weibull model
BHI to Ham I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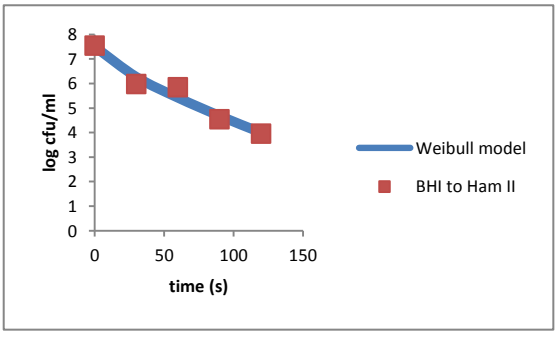
time (s)
Weibull model
BHI to Ham II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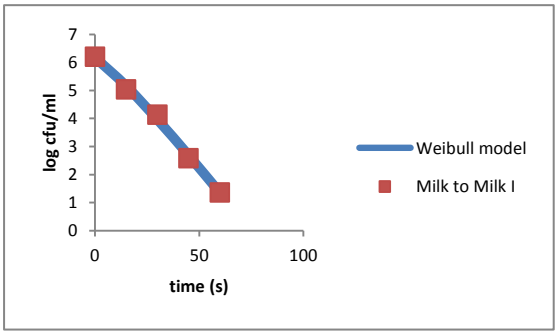
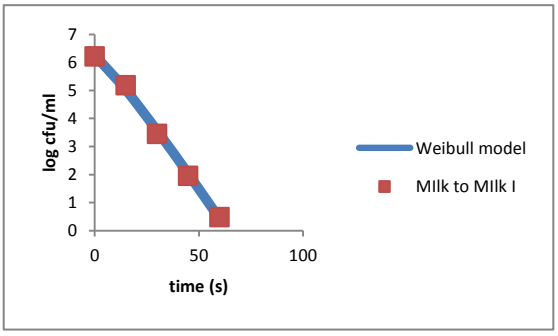
time (s)
Weibull model
BHI to Ham II


log cfu/ml
time (s)
Weibull model
Milk to Milk I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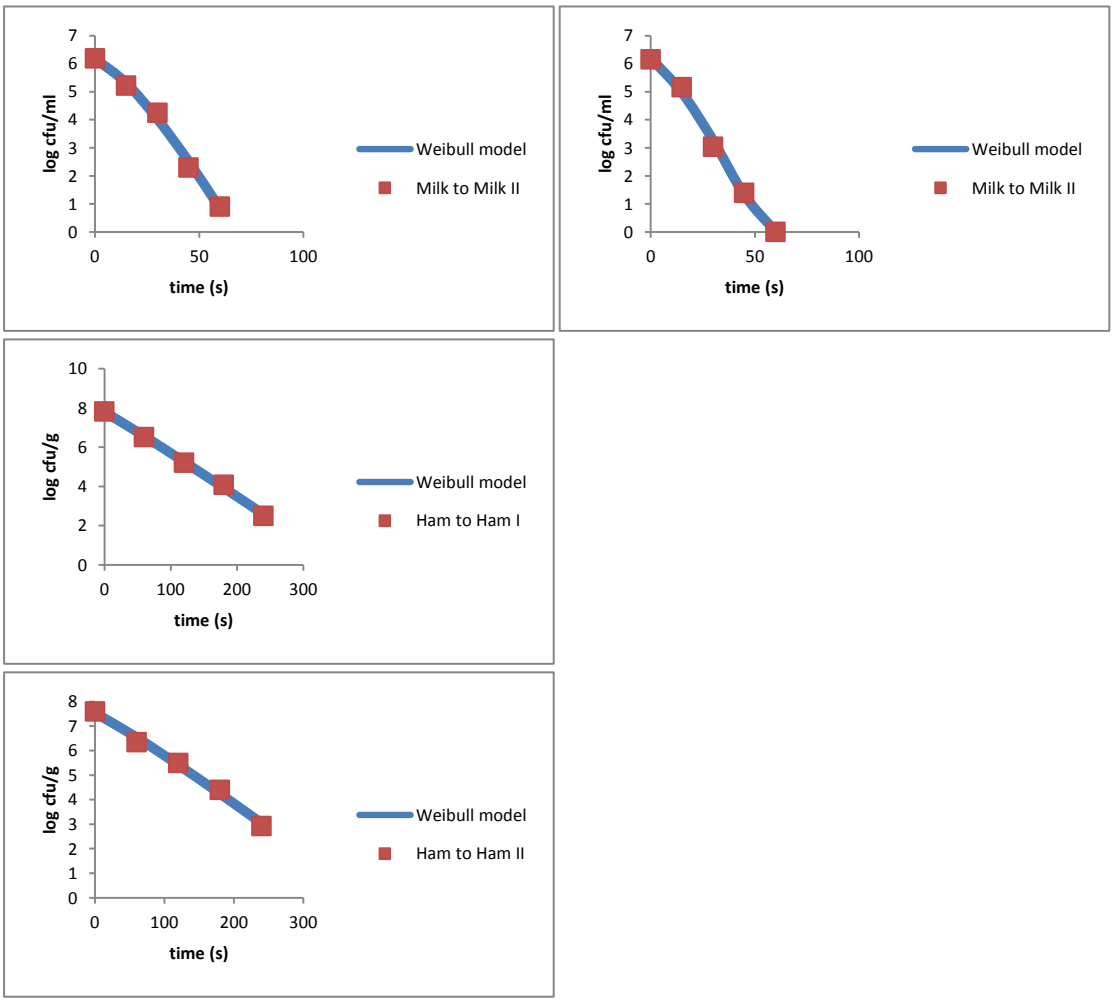
time (s)
Weibull model
MIlk to MIlk I



7.6 Determination of D-value

The inactivation curves of L6, FBR17, FBR15 from three different growth media (BHI broth, milk and ham) into three different heating media (BHI broth, milk and ham) at 65 °C were presented in Appendix 7.5.

Table 9 until Table 11 showed the average of estimated *D*-values. The results that were below the detection limits mentioned in Section 2.4.1 should not be taken into account when *D*-values were calculated. As expected, L6 was the most heat resistant strain. FBR17 was slightly more heat resistant than FBR15 from BHI broth to BHI broth and from BHI broth to milk, while more heat sensitive from BHI broth to ham, from milk to milk, and from ham to ham (Table 9, 10 and 11).

Table 9 The average *D*-values of L6 from three kinds of growth media to three types of heating media at 65 °C

Growth medium to heating medium	*D*-value (s)*
BHI broth to BHI broth	12.6 (0.25)
BHI broth to milk	11.3 (0.79)
BHI broth to ham	60.6 (1.9)
Milk to milk	30.1 (2.7)

Ham to ham	72.5 (0.71)

* Value within bracket is the standard deviation

Table 10 The average *D*-values of FBR17 from three kinds of growth media to three kinds of heating media at 65 °C

Growth medium to heating medium	*D*-value (s)*
BHI broth to BHI broth	4.8 (0.090)
BHI broth to milk	5.2 (0.68)
BHI broth to ham	26.7 (2.2)
Milk to milk	8.8 (1.8)
Ham to ham	29.0 (3.0)

* Value within bracket is the standard deviation

Table 11 The average *D*-values of FBR15 from three kinds of growth media to three kinds of heating media at 65 °C

Growth medium to heating medium	*D*-value (s)*
BHI broth to BHI broth	3.9 (1.1)
BHI broth to milk	4.2 (0.70)
BHI broth to ham	38.5 (14)
Milk to milk	10.8 (1.4)
Ham to ham	49.5 (4.8)

* Value within bracket is the standard deviation

7.7 Tables of ANOVA using SPSS

7.7.1 Effect of heating media on inactivation kinetics

Table 12 Randomized complete block design ANOVA table of average *D*-values from BHI broth as growth medium to three heating media (BHI broth, milk and ham) of L6, FBR17, and FBR15 at 65°C.

Tests of Between-Subjects Effects

Dependent Variable: D - value

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	11994,647[a]	8	1499,331	59,994	,000
Intercept	11270,667	1	11270,667	450,983	,000
Medium	9347,892	2	4673,946	187,022	,000
Strain	1660,837	2	830,419	33,228	,000
Medium * Strain	978,103	4	244,526	9,784	,000
Error	624,784	25	24,991		
Total	25571,030	34			
Corrected Total	12619,431	33			

a. R Squared = ,950 (Adjusted R Squared = ,935)

Multiple Comparisons

Dependent Variable: D - value
Tukey HSD

(I) Heating medium	(J) Heating medium	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
BHI	Milk	,875	2,1405	,912	-4,457	6,206
	Ham	-34,214*	2,1405	,000	-39,545	-28,882
Milk	BHI	-,875	2,1405	,912	-6,206	4,457
	Ham	-35,088*	2,0409	,000	-40,172	-30,005
Ham	BHI	34,214*	2,1405	,000	28,882	39,545
	Milk	35,088*	2,0409	,000	30,005	40,172

Based on observed means.
The error term is Mean Square(Error) = 24,991.

*. The mean difference is significant at the .05 level.

7.7.2 Effect of growth media on inactivation kinetics

Table 13 Randomized complete block design ANOVA table of average *D*-values from two growth media (BHI broth and milk) to milk as heating medium of L6, FBR17, and FBR15 at 65°C.

Tests of Between-Subjects Effects

Dependent Variable: D - value

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1782,900[a]	5	356,580	153,029	,000
Intercept	3302,290	1	3302,290	1417,199	,000
Medium	565,495	1	565,495	242,686	,000
Strain	959,779	2	479,889	205,948	,000
Medium * Strain	257,626	2	128,813	55,281	,000
Error	41,943	18	2,330		
Total	5127,133	24			
Corrected Total	1824,843	23			

a. R Squared = ,977 (Adjusted R Squared = ,971)

Table 14 Randomized complete block design ANOVA table of average *D*-values from two growth media (BHI broth and ham) to ham as heating medium of L6, FBR17, and FBR15 at 65°C.

Tests of Between-Subjects Effects

Dependent Variable: D - value

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	6553,214[a]	5	1310,643	31,265	,000
Intercept	43781,139	1	43781,139	1044,375	,000
Medium	357,464	1	357,464	8,527	,010
Strain	6034,969	2	3017,485	71,980	,000
Medium * Strain	106,823	2	53,411	1,274	,307
Error	670,734	16	41,921		
Total	53430,895	22			
Corrected Total	7223,948	21			

a. R Squared = ,907 (Adjusted R Squared = ,878)

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