

# The application of the WLF equation to predict lag time as a function of temperature for three psychrotrophic bacteria

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## Abstract

Many mathematical models for microbial growth rates or lag times have been proposed. Most of these models either predict well, but are empirical and offer no insight into mechanism or they reflect mechanism but are too complex or do not fit the data adequately. The Williams-Landel-Ferry (WLF) equation (used by polymer chemists) is empirical, but shows some relation to first principles, and hence may offer some insight into mechanism. This model also has only three parameters and therefore a fairly simple form. The WLF equation was fit to lag times derived from three datasets previously developed in our laboratory. The fits obtained with the WLF equation were as good as the best fits obtained with other models (e.g Arrhenius, Davey, response surface and square root). The WLF model was able to account for 98 to 99% of the variance in the three datasets, indicating a very good fit overall. The parameter estimates of the WLF model were not as highly correlated as those of some of the other models. Many of the models, including the WLF equation did not predict well at very long lag times. Weighted least squares non-linear regression improved the fit for these long lag times.

**Keywords:** Lag time; Williams-Landel-Ferry (WLF) model; *Listeria monocytogenes*; *Yersinia enterocolitica*

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## 1. Introduction

The objective of predictive food microbiology is to improve food safety and quality through the application of mathematics and statistics. Many mathematical models which predict growth rates or lag times as a function of temperature have

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been proposed in the literature (Broughall et al., 1983; Ratkowsky et al., 1991; Alber and Schaffner, 1992). Most of these models suffer from one of several defects:

The models may predict well, but are empirical and offer no insight into mechanism. Examples of these sorts of models include the response surface (or polynomial) type models, the Davey model, and the square root model (Davey, 1989; Ratkowsky, 1992). It should also be noted that lag time is a complex phenomenon which can be influenced by many factors including treatment of the inoculum and inoculum size. Until the effect of all these factors are well understood, a model incorporating at least some empirical components may be needed.

Other models may reflect mechanism, but are too complex or do not fit the data adequately. The Arrhenius model is an example of a simple model with does not fit growth or lag time data over the entire growth range (Ratkowsky et al., 1982). An example of a complex model is the non-linear Arrhenius model, commonly called the Schoolfield model. This model describes most datasets well, but does this using six parameters (Adair et al., 1989).

An alternative to simple, empirical models and complex models derived from first principles may be the Williams-Landel-Ferry (WLF) equation. This equation was developed to predict polymer viscosity as a function of temperature (Williams et al., 1955). It has been applied in a number of situations, including the study of chemical reactions in food (Peleg, 1992) and the thermal inactivation of bacterial spores (Sapru and Labuza, 1993). It has been noted that the WLF equation is particularly applicable where a reaction rate is diffusion limited (Karel, 1993). This model also has a fairly simple form.

The WLF equation applies in situations where a reaction is dependent upon viscosity (Ferry, 1980). If it is assumed that at low temperatures, the reduced fluidity of the bacterial membrane limits the diffusion of nutrients into the cells, and that slower diffusion results in longer lag times, then the WLF equation should apply. To confirm this mechanism, experiments at different nutrient concentrations could be carried out.

The objective of this work was to evaluate the fit of the Arrhenius (Eqn. 1), Davey (Eqn. 2), response surface (Eqn. 3) square root (Eqn. 4) and WLF (Eqn. 5) models to lag times derived from three datasets previously developed in our laboratory (Alber and Schaffner, 1992; Duh and Schaffner, 1993).

$$\ln(l) = a + \frac{b}{T} \quad (1)$$

$$\ln(l) = a + \frac{b}{T} + \frac{c}{T^2} \quad (2)$$

$$\ln(l) = a + bT + cT^2 \quad (3)$$

$$\ln(l) = (b(T - T_0))^2 \quad (4)$$

$$\ln(l) = \frac{a(T - T_0)}{b + T - T_0} \quad (5)$$

where  $l$  = lag time;  $T$  = temperature;  $a, b, c, T_0$  = regression constants.

These datasets consist of of plate count measurements and incubation times at more than 15 temperatures for each of three psychrotrophic bacteria: *Listeria monocytogenes* Scott A PFEI, *L. innocua* PFEI and *Yersinia enterocolitica* serotype 08.

## 2. Materials and methods

### 2.1. Microbiological methods

The microbiological methods have been described in the literature (Alber and Schaffner, 1992; Duh and Schaffner, 1993) but can be summarized as follows:

Stock cultures were maintained on brain hearth infusion (BHI) broth (Difco) plus 10% glycerol and stored at  $-20^{\circ}\text{C}$ . One loopful of stock culture was reactivated by streaking on a BHI agar slant. One loopful of the slant culture was transferred to a tube containing 5 ml sterile BHI broth, and incubated at  $30^{\circ}\text{C}$  for 16 h.

One ml of this preinoculum, containing about  $10^9$  colony forming units (CFU) per ml, was diluted with 9 ml sterile BHI broth and thoroughly mixed. A micropipette was used to deliver 0.05 ml of this inoculum to 5 ml sterile BHI broth. Fifteen to 30 tubes were inoculated in this fashion for incubation at each temperature. This procedure yielded a starting concentration of about  $10^6$  CFU/ml.

The samples were incubated at one of 17 temperatures from 2 to  $46^{\circ}\text{C}$ . The sampling times varied according to the temperature under study.

Samples were diluted in 0.1% peptone water until a concentration of about  $10^3$  cells/ml was obtained. One hundredth ml of diluted cell suspension was transferred to separate, duplicate BHI agar plates and spread using a glass rod. Plate count was determined by standard procedures after incubation at  $37^{\circ}\text{C}$  for 24 to 48 h.

### 2.2. Statistical methods

The method for determining lag time estimates is summarized below. The Genstat 5 software (Numerical Algorithms Group, Downers Grove, IL) was used for all statistical analyses. The Gompertz equation was fitted to each growth curve, for each organism, at each temperature. The minimum and maximum of the second derivative of the fitted Gompertz function are the points where the slope of the growth curve shows the point of greatest increase, and the point of greatest decrease. These two points may be used to mathematically define the beginning and end of the exponential phase of growth (Buchanan and Cygnarowicz, 1990). Each possible pair of points within the exponential phase of growth was used to determine a line. The intersection of the slope of each line with the initial number of organisms was taken to be one estimate of the lag time. The average of all positive estimates was used to determine the lag time used for further modeling. Negative lag times were discarded since they represent a biological impossibility.

A subset of all the lag times estimated was used for subsequent analysis. This subset consisted of only those measurements where lag time decreased with increasing temperature (i.e. lag times below the optimum lag time temperature). There are two reasons for confining our analysis to this subset: Firstly, lag times at relatively high temperatures will be very short, and accurate estimates of these lag times are of little practical significance in food microbiology; Secondly, the rate limiting step hypothesized above (that reduced membrane fluidity reduces diffusion of essential nutrients into the cell) may no longer be rate limiting, and hence the WLF model may not apply.

Non-linear regression was used to fit the equations to lag time vs. temperature data. In each case the natural logarithm of the lag time was used as the response variable to be modeled. This transformation was chosen because it is the one typically used with the WLF equation. The same transformation was used with the other models to make model to model comparisons easier.

### 3. Results and discussion

A summary of the non-linear regression results (Table 1) reveals a number of interesting findings:

The results of the model fitting are expressed as "percentage variance accounted for" by the model. The percent variance accounted for is another term for the adjusted  $r^2$  statistic which can be expressed as:

$$\text{Percentage variance accounted for} = 100 \times \left( \frac{1 - \text{residual mean square error}}{\text{total mean square error}} \right) \quad (6)$$

This statistic is usually a better guide to the fit of the model than the unadjusted  $r^2$  statistic. The Arrhenius model does explain most of the lag time percent

Table 1  
Summary of non-linear regression results for the three data sets and five models tested

	<i>Listeria innocua</i>			<i>Listeria monocytogenes</i>			<i>Yersinia enterocolitica</i>		
	% variance	r.m.s. <sup>a</sup>	parameters correlated?	% variance	r.m.s. <sup>a</sup>	parameters correlated?	% variance	r.m.s. <sup>a</sup>	parameters correlated?
Arrhenius	95.7	0.2028	yes	94.9	0.3033	yes	98.6	0.006763	yes
Davey	99.5	0.0251	yes	98.2	0.1067	yes	98.5	0.007386	yes
Response surface	99.6	0.0208	yes	98.2	0.1078	yes	98.4	0.007526	yes
Square root	99.4	0.0269	no	96.2	0.2254	no	97.7	0.011410	no
WLF	99.0	0.0473	no	98.1	0.1120	no	98.5	0.007268	no

<sup>a</sup> Root mean square error.

variance of the data (> 94%) for each of the three organisms tested. Its performance is especially good for the *Y. enterocolitica* data set, where it appears to explain as much of the variability as any of the other four models. Its ability to model the lag time behavior seen in the *Listeria* data sets is considerably worse than the other models.

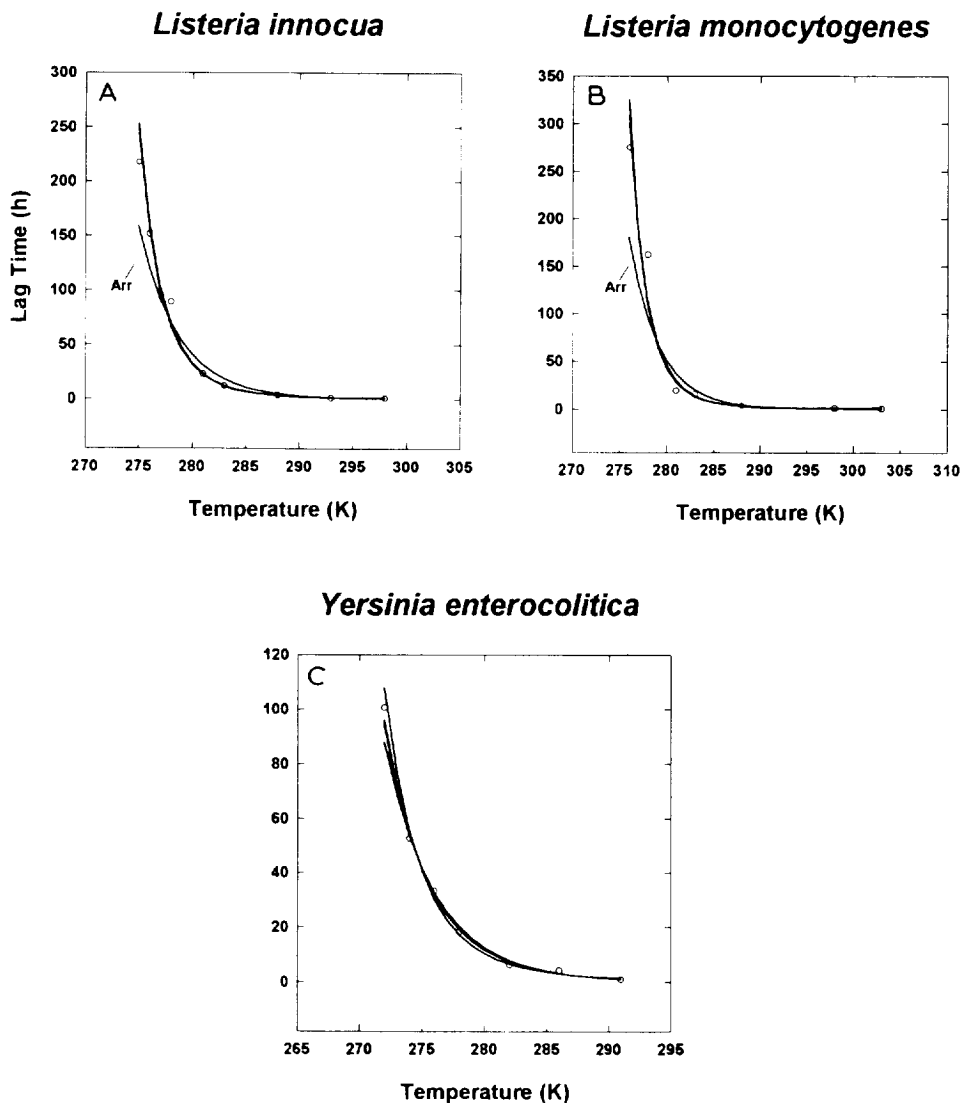


Fig. 1. The effect of incubation temperature on lag time for growth of three psychrotrophic bacteria: (A) *Listeria innocua*, (B) *Listeria monocytogenes* and (C) *Yersinia enterocolitica*. The data points, which were derived from viable counts, are represented by ○, regression lines are shown as solid lines, where "Arr" indicates Arrhenius model.

Each of the other four models (Davey, response surface, square root and WLF) can explain 99% or more of the variability of the *L. innocua* lag time data. Three of the models (Davey, response surface and WLF) can explain 98% or more of the

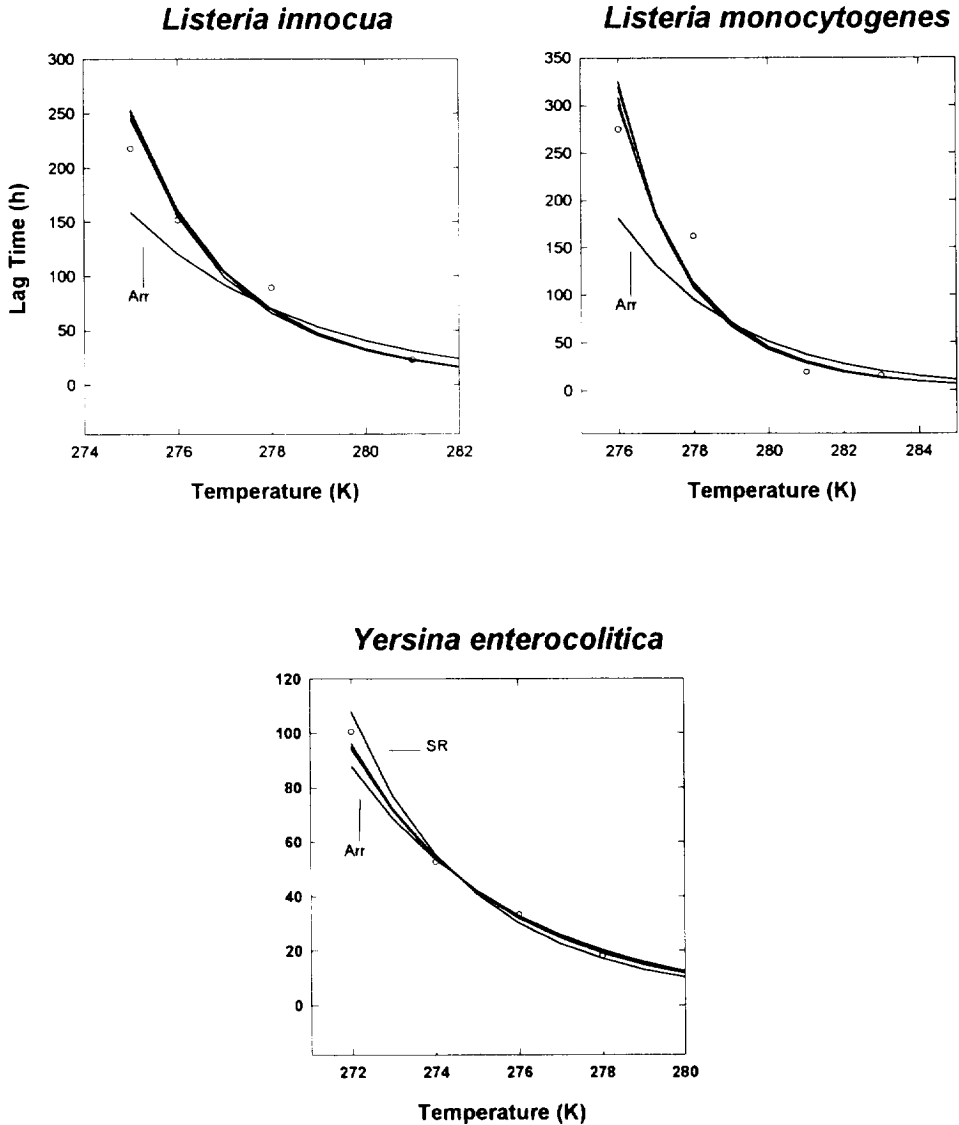


Fig. 2. The effect of incubation temperature in the range of -1 to 10°C on lag time for growth of three psychrotrophic bacteria: (A) *Listeria innocua*, (B) *Listeria monocytogenes* and (C) *Yersinia enterocolitica*. The data points, which were derived from viable counts, are represented by  $\circ$ , regression lines are shown as solid lines, where "Arr" indicates Arrhenius model and "SR" indicates square root model.

variability of the *L. monocytogenes* and the *Y. enterocolitica* data sets, while the square root model doesn't perform as well.

It is also interesting to note that the way the Arrhenius, Davey, and response surface models are formulated tends to result in parameter estimates that are highly (0.999) correlated (results not shown). The model parameter estimates produced by non-linear regression of the square root and WLF models results in parameter estimates that are not as highly correlated. Ratkowsky (1989) makes several pertinent comments regarding parameter correlation that should be noted here: First, it is best to use non-linear regression models that estimate well, and which also have parameter estimates which are not highly correlated. Second, correlated parameters can be reparameterized to reduce correlation. And lastly, high correlation may be indicative of over-parameterization of the model.

The graphical results of the non-linear regression are shown in Figs. 1 and 2. Fig. 1 shows when the lag time is plotted as a function of temperature, all the data sets show the same trend. Below a certain temperature, which was dependent upon the bacterium, the lag times increased rapidly with decreasing temperature. Above that same temperature, the lag times were largely unchanged, and were quite close to zero (i.e. a very short lag time). When the results of the predictions are plotted on the same axes as the data, the predictions appear to be quite close to the actual data, for most of the equations, and any of the organisms. As discussed above, the Arrhenius model gives the poorest fit to the *Listeria* data, and the plot of this model is different from the other models shown in the first two panels of Fig. 1.

Even though all the models (except Arrhenius) seem to show a good fit from Fig. 1, and high correlations from Table 1, an examination of Fig. 2 shows these conclusions may be misleading. When only the data and predictions for the lowest four temperatures are shown (Fig. 2) a clear difference between the predictions and the data emerge. The *Listeria* predictions for all the models (except the Arrhenius model) overestimate the lag time at the lowest temperature, and underestimate the lag time at the third and second lowest temperature for *L. innocua* and *L. monocytogenes* respectively. The Arrhenius model underestimates the data at the lowest three and two temperatures for *L. innocua* and *L. monocytogenes*, respectively. The Arrhenius model also underestimates the actual lag time for the longest *Y. enterocolitica* datum point as well. The square root model over predicts the value for this same point.

The poor fits for any of the models are troubling, especially when one would like to have more accurate predictions for those conditions where one would expect to find most food products (e.g. low temperatures). Part of the reason for the poor fits at lower temperatures is the natural logarithm transformation used for all the models compared here. As noted in the Material and Methods section above, this transformation is the one typically used with the WLF equation. This transformation reduces magnitude of the large lag times more than the magnitude of the small lag times, so the differences between the untransformed data and the prediction are greater at longer lag times. It has been shown that a natural logarithm transformation is approximately the same as weighting by the inverse of

the square of the response variable (Alber and Schaffner, 1992). If this is the case, this problem can be remedied by weighting during non-linear regression by the square of lag time to counteract the effect of the transformation. When this is done, the fits are better at lower temperatures, but not as good at higher temperatures (results not shown).

#### 4. Conclusions

All of the models tested were able to explain most of the variability (> 94%) in all three data sets. The Arrhenius model did not fit as well as the other models when used with the *Listeria* data sets. The square root model did not perform as well as the other models when fit to the *Y. enterocolitica* data set. Three of the models (Arrhenius, Davey and response surface) had a very high degree of parameter correlation.

The WLF model performed as well as any of the other models tested. It also has three other advantages: Its parameter estimates are not as highly correlated as some other models, it is closer to being derived from first principles (since it indicates a possible mechanism) and gives better fits than the other similarly classified model (Arrhenius) tested.

A model based on mechanism or first principles may point the way toward more fundamental modeling research which might elucidate a key biochemical reaction or family of reactions that are responsible for the relationship between lag time and incubation temperature for psychrotrophic pathogens.

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