Journal of Applied Microbiology, 2011, Vol 110, issue 1, pp61-68

Title:

A new model for the effect of pH on microbial growth: an extension of the Gamma hypothesis

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Running title: Gamma modelling of pH

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Abstract

Aims: To investigate the appropriateness of the Extended Lambert-Pearson model (ELPM) to model the effect of pH (as hydrogen and hydroxyl ions) over the whole biokinetic pH range in comparison to other available models.

Methods and Results: Data for the effect of pH on microbial growth was obtained from the literature or in-house. Data were examined using several models for pH. Models were compared using the residual mean of squares. Using the ELPM, pH was modelled as hydrogen ions and hydroxyl ions, hence the model was monotonic in each. The ELPM was able to model data more successfully than the Cardinal pH Model (CPM) and other models in the majority of cases.

Conclusions: Examining the effect of pH as hydrogen and hydroxyl ions has the advantage that the basic form of the ELPM can be retained as each is treated as a distinct antimicrobial effect. With the ELPM each inhibitor is described by two parameters, from these parameters the pHmin, pHopt and pHmax can be obtained. Further the idea of a dose response, absent from other models becomes important.

Significance and Impact of the study: The CPM is an excellent model for certain situations – where there is a high degree of symmetry between the suboptimal pH and superoptimal pH response and where there are few data points available. The ELPM is more amenable to highly asymmetric behaviour, especially where plateaus of effect around the pH optimum are observed and where the number of data points is not restrictive.

Keywords: Predictive modelling, hurdles, cardinal parameters

Introduction

The history of models describing the effect of pH on microbial growth has followed the same pattern as those models describing the effect of temperature: initially exponential or square root models followed by a move to Cardinal polynomial models.

Presser *et al.* (1997) suggested the following function for the suboptimal pH range for the ratio of the growth rates with respect to the optimal growth rate (μ_{opt}) :

$$\frac{\mu_{\max}}{\mu_{opt}} = \gamma_{pH} = \left(1 - 10^{pH_{\min} - pH}\right) \quad (1)$$

Whereas Tienungoon *et al.* (2000) quoted a model for the full biokinetic range, which we have termed the Extended Presser Model (n.b, a publication error put the superoptimal pH range under a second square root).

$$\frac{\mu_{\max}}{\mu_{opt}} = \gamma_{pH} = \left(1 - 10^{pH_{\min} - pH}\right)\left(1 - 10^{pH - pH_{\max}}\right) \quad (2)$$

Many microorganisms of concern in foods have pH optima between 6 and 7, although there are some notable exceptions (see for example Fig 1 of Zwietering *et al.*1993). Although, perhaps, not obvious, equation (2) imposes symmetry between the pH_{min} and pH_{max}, i.e. it assumes the pH optimum occurs exactly half-way between the two growth extremes.

Other models for pH used in the literature, which make use of Cardinal pH values, are the square–root type model (but with an extra fitting parameter – c_2, Zwietering *et al.* 1992, 1993)

$$\gamma_{pH} = \left[\frac{(pH - pH_{\min})(1 - \exp\{c_2(pH - pH_{\max})\})}{(pH_{opt} - pH_{\min})(1 - \exp\{c_2(pH_{opt} - pH_{\max})\})} \right]^2 \quad (3)$$

, the simple Cardinal pH model,

$$\gamma_{pH} = \frac{(pH - pH_{\min})(pH_{\max} - pH)}{(pH_{opt} - pH_{\min})(pH_{\max} - pH_{opt})} \quad (4)$$

and the expanded Cardinal pH model (CPM), (Rosso *et al.* 1995).

$$\gamma_{pH} = \frac{(pH - pH_{\min})(pH - pH_{\max})}{(pH - pH_{\min})(pH - pH_{\max}) - (pH - pH_{opt})^2} \quad (5)$$

Models 1 to 5 are given in their Gamma form, which is the relative effect of a given pH to that at the optimal pH value; multiplication by, for example, μ_{opt} gives an absolute value. Figure 1 shows the fit of the Extended Presser model (2) and the CPM (5) to published Cardinal parameter data for *Listeria monocytogenes* (cardinal parameters from Rosso *et al.* 1995). The symmetry of the Presser model is obvious; the pH optimum value allows the CPM to model the majority of non-symmetrical (as well as symmetric) behaviour. The figure also shows that (2) allows for a plateau of growth rate, whereas the CPM insists on a particular optimum value.

One particular problem with pH models is that they are not 1:1 - two values of pH give the same growth rate. If we consider the definition of pH, that pH = -log[H^+], then at face value both a high and low concentration of hydrogen ions give the same growth rate effect. It is probably inherently understood that we really mean acid pH and alkali pH, but our models do not distinguish this. Cole *et al.* (1990) showed that the pH inhibition of *Listeria* *monocytogenes* was better modelled using the hydrogen ion concentration suggesting that the inhibition was linearly related to the hydrogen ion concentration. Indeed model (2) can be rewritten as

$$\gamma_{pH} = \left(1 - k_1[H^+]\right)\left(1 - k_2[OH^-]\right) \qquad (6)$$

For the analysis of pH on the time to detection (TTD) of growth of bacterial cultures, we have used the hydrogen ion concentration directly (Lambert and Bidlas 2007). At superoptimal pH values the model used in these previous studies fails – giving a simple plateau of maximum growth for the given environmental conditions for all pH > pHopt. A simple rationalisation of the approximate bell-shaped or quadratic-like structure of many observed pH/growth rate profiles leads to the supposition that the hydroxyl ion is now in control of the growth rate when pH > pHopt. Using the model published previously (Lambert and Bidlas 2007), which employs the Gamma hypothesis as a base (Zwietering *et al.*1992), a model was constructed using hydrogen ions and hydroxyl ions directly, instead of pH, and examined for its utility and is described herein.

Materials and methods

Effect of pH on the growth of *E. coli*

Escherichia coli ATCC 25922 was grown overnight in a flask containing 80ml tryptone soya broth (TSB, Oxoid CM 129), with shaking at 30^{o}C. The cells were harvested, centrifuged to a pellet, washed and re-suspended in peptone water (0.1%). A standard inoculum was produced by diluting the culture to an OD of 0.5 at 600nm. The pH of thirty TSB solutions was adjusted with HCl to give a pH range from 7 to 3. These solutions were placed into a Bioscreen plate in triplicate. Diluted standard inoculum (pH adjusted) was added (50μl) to all wells except the negative control wells. The plate was then incubated in a Bioscreen Microbiological Analyser (Labsystems Helsinki, Finland) for 5 days at 30^{o}C, with shaking, with OD readings taken every ten minutes.

Model Fitting

The models used in these studies were developed from the Lambert-Pearson model (LPM) and the Extended Lambert-Pearson model (ELPM, Lambert and Pearson 2000; Lambert and Lambert 2003). These models were used to examine time to detection (TTD) data from optical density experiments. It was hypothesised that the general form of these equations would be applicable to growth rate data as obtained from traditional growth curve measurements (Equation 7).

for a given hurdle or hurdles $$\gamma = \begin{cases} if \quad \sum_{i=1}^{n}[x_i]=0, \quad 1 \\ else \text{ if} \\ \qquad EffC<1 \\ then \\ \qquad \exp(-EffC) \\ else \text{ if} \\ \qquad EffC>e, \quad 0 \\ Else \\ \qquad \frac{1}{e}(1-\ln[EffC]) \end{cases} \qquad (7)$$

where γ = gamma factor (the ratio of the observed growth rate to the optimal growth rate), $[x_i]$ is the concentration of the i^{th} inhibitor, the effective concentration (*EffC*) is defined as

$$EffC = \sum_{i=1}^{n}\left(\frac{[x_i]}{P_{2i-1}}\right)^{P_{2i}} \qquad (8)$$

P_{2i-1} is the concentration of the i^{th} inhibitor giving a relative inhibition of 1/e (approx. 0.37), P_{2i} is a slope parameter which has been defined as the dose response due its similarity with the Hill model. For combined inhibitors (where n > 1), the model is applicable, in this form, only if each individual $P_{2i} \approx 1$. Equation (7) was used to study the effect of pH in terms of $[H^+]$ and $[OH^-]$ on published data sets, where the effective concentration is therefore given by

$$EffC = \left(\frac{[H^+]}{P_1}\right)^{P_2} + \left(\frac{[OH^-]}{P_3}\right)^{P_4} \qquad (9)$$

A constrained variant of (7) where the parameters $P_{2i} = 1$ was termed the constrained extended Lambert-Pearson model, (ELPMc).

For the effect of hydrogen ions alone against a microbe, the effect of pH (as hydrogen ions) on the rate to visible detection of a growing culture where $pH \le pH_{opt}$ is given by the following function

$$\gamma_{pH} = (\text{RTDobs}/\text{RTDopt}) = \begin{cases} \textit{if} & [H^+] < P_1 \\ \textit{then} & \\ & \exp\left(-\left(\dfrac{[H^+]}{P_1}\right)^{P_2}\right) \\ \textit{else}\ \text{if} & \\ & [H^+] \geq P_1 \exp\left(\dfrac{1}{P_2}\right), \quad 0 \\ \textit{else} & \dfrac{1}{e}\left(1 - P_2\left(\ln\left(\dfrac{[H^+]}{P_1}\right)\right)\right) \end{cases} \qquad (10)$$

Where RTD (the rate to detection) is the reciprocal of the observed time to detection (TTD) and RTD_{opt} is the reciprocal of the optimal TTD value (least inhibitory condition), and where all other parameters are defined as in (8).

In previous inhibition studies the MIC of a given antimicrobial has been defined by the expression

$$MIC = P_1 \exp\left(\frac{1}{P_2}\right) \qquad (11)$$

This is equivalent to defining $\gamma = 0$. Therefore, using the definition of pH, the minimum pH for growth is given by

$$pH_{\min} = -\log_{10}[P_1] - \frac{0.4343}{P_2} \qquad (12)$$

Data Analyses

Experimental data or literature growth-rate data where growth rates were obtained as a function of pH normally over the whole biokinetic pH range were modelled using non-linear regression with the minimised sum of squares as the search criterion. Analyses were done using the *Mathematica* 7.0 package (Wolfram Research Inc, Champaign, Il, USA) or the JMP Statistical Software (SAS Institute Cary NC USA). Comparison between models was based on the mean square of the error (MSE), which is a criterion that takes into account differences in the number of parameters (degrees of freedom). Monte-Carlo (MC) analyses were carried out using *Mathematica*: the “NonlinearModelFit” procedure of *Mathematica* was used to obtain a fit to the data, estimates of the parameters and the standard error of the fit (RMSE). Random error with distribution N(0, RMSE) was added to the modelled data and the “NonlinearModelFit” procedure carried out on this virtual set of data. This was repeated 11000 times per set of original data. From each run the new set of modelled parameters were obtained, the mean and the 95% quantiles were obtained for each of them. To obtain the pH optimum value, equation (7) was differentiated with respect to pH by redefining (9) in terms of pH, equation (13).

$$\frac{d\gamma}{dpH} = 2.303\left\{ P_2\left(\frac{10^{-pH}}{P_1}\right)^{P_2} - P_4\left(\frac{10^{pH-14}}{P_3}\right)^{P_4} \right\}\exp\left(-\left(\frac{10^{-pH}}{P_1}\right)^{P_2} + \left(\frac{10^{pH-14}}{P_3}\right)^{P_4} \right) \qquad (13)$$

For a given data set, equating (13) to zero gave the pH optimum. This procedure was carried out automatically within the MC analyses and therefore confidence intervals were also found. The *Mathematica* code used is available from the author.

Algorithm used for the analysis of the data

1. Fit the CPM to the data and obtain parameters and the mean square of the error (MSE)
2. Fit Eqn. 7 with $P_{2i} = 1$, obtain the three parameters: the two P_{2i-1} and the RTD_{opt} (or the μ_{opt}) along with the MSE.
3. Using the parameters from 2., fit the ELPM (relax the P_{2i} restriction).
4. Confidence intervals calculated using Monte-Carlo analysis where required

Results

The observed and modelled data for the effect of hydrogen ions against *E. coli* in a broth system at 30°C is shown in Figure 2; RTD_{opt} = 1/314 min^{-1} , P_1 =3.78 x 10^{-5} mol l^{-1}, P_2= 0.74, pH_{min} = 3.84 (95% CI 3.76 – 3.90). The optimum time to detection for this particular experiment was 314 minutes.

It should be noted (in accordance with the suggestion of Cole *et al.* 1990) that plotting TTD against the hydrogen ion concentration gave a good straight line fit down to pH 4.22, below which the linear model failed. The variance of the data, however, increased with decreasing pH and a weighting regime should be used to reduce the bias. The reciprocal transformation of the TTD data gave homogeneous variance.

The CPM model (5) was applied to the data and gave a pHmin of 3.90 (3.85 – 3.94) and a pH opt = 6.61 (6.49 – 6.80). The MSE value of the fit of the CPM (0.00113) was smaller than that of the LPM (0.00121), however, it showed a high degree of correlation between the parameters unlike the LPM. This was due to fitting an inappropriate model (the CPM) to the particular data set. Although the data were only obtained to a maximum pH of 7.2, the CPM was able to provide a fit, indeed predicting a pH_{max} of 10.20 (9.27 – 11.77).

A model for the full range of growth pH

Data taken from the literature for the effect of the full pH range on microbial growth were used to compare the effectiveness of the ELPM with either a simple quadratic or the CPM models. In these cases the measured growth rates were used directly, where $\gamma = \mu_{max}/\mu_{opt}$.

Listeria innocua: Le Marc et al 2002 : The growth rate over the pH range 4 – 10 was obtained for *Listeria innocua* (ATCC 33090) by Le Marc *et al.* (2002). The CPM model was fitted to the data and the three Cardinal pH values obtained. Four other models were also fitted: a simple quadratic, the Presser model, the ELPMc and the ELPM. The fit of the latter two and the CPM model to the observed are shown in Figure 3. Table 1 compares cardinal values obtained from the various models used. In general, the model with the lowest MSE fits the observed data best. In this case the Presser model has the greatest MSE and is the poorest fit, the quadratic model has a lower MSE than the CPM, but not to the ELPM, which has the lowest MSE of the five models tested. The latter model's main drawback (as is the case with the simple quadratic model), is that the three Cardinal pH values have to be calculated unlike the CPM where the cardinal parameters are explicit. The pHmin and pHmax are relatively easy to calculate from a standard equation (12), however, to obtain the optimum pH requires differentiating the model with respect to pH and finding the root (eqn. 13). This can be quickly accomplished using *Mathematica*.

Butyrivibrio fibrisolvens: Rosso et al (1995) used the pH data of Kistner et al (1979) to describe the utility of the CPM. Several such data sets were used, some containing only few data leading to limited degrees of freedom in the fitting of a model. The data for *B. fibrisolvens* were obtained and analysed using the models described. The pHmin, pHmax and pHopt cardinal values obtained by the CPM were 5.42 (5.32 – 5.61), 7.56 (7.41 – 7.83), and 6.54 (6.44 – 6.63) respectively and for the ELPMc were 5.37 (5.31 -5.44), 7.68 (7.59 – 7.77), and 6.52 (6.49 – 6.57) respectively. In this case all models fitted the data well, including the basic quadratic, Table 2. The symmetric nature of the pH profile lends itself well to the ELPMc (Figure 4). From the MSE value, this model was considered to give the best fit to the observed data, the simple quadratic also gave a better fit, in this case, than the CPM.

Bacillus thermoamylovorans: a moderately thermophilic, non-spore forming bacterium isolated from palm wine. Data from Combet-Blanc *et al.* (1995) were used to construct the pH/growth profile, shown in Figure 5 along with the fitted CPM and ELPM. Essentially, both the CPM and the ELPM fit the data well. The pHmin, pHmax and pHopt cardinal values obtained by the CPM were 5.41 (5.34– 5.47), 8.46 (8.41 – 8.51), and 6.92 (6.86 – 6.98) respectively and for the ELPM were 5.40 (5.34 -5.46), 8.42 (8.36 – 8.47), and 6.94 (6.88 – 7.00) respectively. An analysis of the MSE, Table 2, shows that the ELPM fitted the data to a better degree.

Some of the other data sets used by Rosso *et al.* (1995) were further analysed (Table 2) and showed that the CPM model fitted data to a better degree than other models when the number of data (degrees of freedom) were small. This suggests that where data is sparse then the CPM offers the best alternative and the ELPM be used when data is not limiting.

Lactobacillus plantarum: The growth rate of *L. plantarum* was analysed by Cuppers and Smelt (1993) as a function of pH at two temperatures: 21 and 15^{o}C. They showed that the growth rate dropped by approximately 50% at the lower temperature, but that the minimum and optimum pH did not appear to change. Table 3 describes the results obtained from fitting the models to the published data. Data obtained at 15^{o}C were not amenable to a fitting by the models described – there were sparse data beyond the expected pHopt value. The CPM returns a very large confidence interval, the ELPM refused to compute one. Applying the LPM for the effect of hydrogen ion only (eqn. 10), gave the parameters P_0 = 0.0812 (0.078 – 0.084), P_1 = 0.000132 (0.00103 – 0.00018), P_2 = 0.889 (0.647 – 1.177), MSE = 1.00x10^{-5}. A Monte Carlo analysis (10,000 iterations) gave the pHmin = 3.45 (3.31 – 3.55).

One of the consequences of the Gamma hypothesis is that it suggests that different antimicrobial hurdles act independently. The ELPM was modified to take account of the change in the maximum growth rate with temperature (through the addition of a simple linear model between 21 and 15^{o}C). The combined data set was then re-modelled. Figure 6 shows the ELPM applied to the data set taking into account the temperature change.

The fit of the model suggests that the Gamma hypothesis is valid: pH and temperature are independent factors affecting the growth of this organism over the ranges studied. Using the F-test method described by Pin and Baranyi (1998) it was shown that there was no significant difference ($F = 1.753$, $P=0.15$), between the two separated models and the combined model, which might have been expected if temperature had an influence on the fitting parameters. A comparison of the CPM with the ELPM showed that the latter model gave the better fit to the data available. The cardinal pH values were obtained and a comparison made to the CPM, Table 4.

Discussion

pH is a major hurdle used by the food industry to stabilise products from microbiological growth. In general the minimum pH for pathogen growth is known (less frequently for spoilage organisms) and can be used to set limits for the pH of foods. One of the recurring problems with Cardinal values is a lack of knowledge of how the effect of other hurdles such as temperature or the addition of weak acid preservatives interact or combine with pH. For example the pH minimum for growth of *E. coli* is considered to be approximately 4.0, but if at pH 4.0 the product cannot be sold for taste reasons, can a higher pH be used in conjunction with another hurdle? In essence this is the idea behind the 'Cole cliff face', where increased knowledge about combined hurdles can allow greater flexibility in formulation, whilst retaining safety or shelf-life.

The ELPM, developed from the original Lambert & Lambert (2003) model, allows different antimicrobial hurdles to be analysed separately and then to be assembled together to form a quantitative multiple-hurdle system.

Within the open literature the pH model developed by Rosso *et al.* in the mid-90's has become the standard model for the effect of pH and is known as the Cardinal pH model (CPM). If the three cardinal pH values are known – pH minimum, optimum and maximum then the CPM can be used directly, else data are obtained over the full pH growth range and these cardinal values estimated. One interesting aspect of this fitting method is that both the pH minimum and pH maximum are extrapolated values since a value of 'no growth' cannot be used in the fitting process. Since the CPM model is a polynomial quotient function, such extrapolation is usually not permitted. But since the model 'works' this mathematical discrepancy is usually overlooked. The CPM is well suited to certain types of pH profiles, but not to those with a flatter region which encompasses the pH optimum. This occurs when the range between the pHmin and pHmax is greater than approx. 5.5 pH units and the dose response (as described by the P_{2i} parameters in the ELPM) is high (a P_{2i} value greater than approx 0.85), and under these conditions there is a clear difference between these two models. The CPM is at its best when the pH profile is close to symmetric about the pH_{opt}, but a simple quadratic may also provide a good fit to the data.

Some of the reasons put forth by Rosso for the adoption of the CPM by the modelling community were that it gave biologically relevant parameters and did not give structural correlations between parameters (which caused problems with the parameter estimates especially with the calculation of confidence intervals). The CPM also had ‘parsimony’ – a minimum number of parameters and was also convenient to use for biologists. The one thing that was missing from the list of advantages was whether the model created an advance in the discipline itself or was just a simple (but elegantly constructed) empirical tool for estimating values previously defined, e.g. pH_{min}.

The simple idea that the pH-growth profile is due to hydrogen ions and hydroxyl ions is, of course, not ground-breaking, but few people question the dichotomy of using pH which is defined using the hydrogen ion concentration when the pH profile is not monotonic. The model developed herein splits the contribution of pH into its two constituent parts and attempts to model on that basis. This model is generally more suitable to pH data than is the CPM. Furthermore the parameters used to describe the model are those found from experimental data unlike the CPM which relies on extrapolation to define the cardinal values used in its own fitting. In some cases using a constrained model, by forcing P_{2i} = 1, (the ELPMc) improves the fit over the CPM, in these cases the pH profile was found to be symmetric and the dose responses are approximately 1.

Unlike the CPM, the ELPM requires the pH minimum, pH maximum and pH optimum to be calculated. This is a relatively simple and elementary process and a model should not be discarded, as Rosso *et al.* suggested, simply because it is not immediately amenable to those without the required background in mathematics. The advances in mathematical software (e.g. *Mathematica*, Math-Lab) and in the robustness of statistical packages such as JMP or Statistica, places in the hands of microbiologists a very comprehensive toolbox with which to investigate large amounts of data and/ or to provide very sophisticated analyses.

The major importance of the use of the ELPM is that it shows that growth across the entire pH range can be modelled by a simple, general, equation. Indeed the model used has not been modified in any way from its normal appearance – we have simply considered hydroxyl ion as a separate antimicrobial factor to hydrogen ion. When data is sparse, e.g. when experiments have been conducted under acid conditions only, then the ELPM can be reduced to the more simple LPM (i.e. the ELPM with n = 1 (eqn. 10))

The ELPM contains all the features that Rosso *et al.* suggested make a good model, but it also introduces the idea of the dose response – a phenomenon reflected in the P_{2i} parameters, which is absent from the CPM. In some cases the dose response of hydrogen ions and hydroxyl ions are similar and approximately equal to 1 (hence the ELPMc equation fits the data well) at other times they are different. Zwietering *et al.* 1993 gives the pH range for a group of organisms: many are symmetric, but notably *Pseudomonas* and *Listeria* are asymmetric. Are the latter observations a reflection of different metabolic strategies used to maintain homeostasis in different pH environments whereas a symmetric pH response shows a conservative metabolic response? With the advent of systems microbiology beginning to be applied to food microbiology (Brul et al 2007), that we can ask the question is a step in the right direction.

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Tables

Table 1. Estimations and 95% confidence intervals of the cardinal pH values for *Listeria innocua* ATCC 33090

Model	pHmin	pHmax	pHopt	MSE
CPM	**4.17 [3.91- 4.33]**	**10.08 [9.70 - 10.85]**	**7.20 [6.88 - 7.53]**	0.00431
ELPM	**4.17 [3.72 - 4.54]***	**9.85 [9.53 - 10.64]***	**7.40 [7.10 - 7.82]**†	0.00343
Quadratic*	**4.19 [4.05 - 4.30]**	**10.15 [9.95 – 10.40]**	**7.17 [7.08 – 7.27]**	0.00398
ELPM$_c$	**4.29 [4.20 – 4.38]***	**9.91 [9.78 – 10.08]***	**7.10 [7.04 – 7.18]**†	0.00452
Extended Presser	**4.43[4.35 – 4.50]**	**9.66 [9.46 – 9.85]**	**7.05 [6.96 – 7.16]***	0.01045

*Confidence interval found through Monte-Carlo simulation (11000 trials)

†Parameter and confidence interval found through MC analyses (11000), differentiating each of the resulting models and finding the root.

Table 2. Comparison of models by MSE

Organism	Quadratic (P=3)	CPM (P=4)	ELPMc (P=3)	ELPM (P=5)	n
Butyrivibrio fibrisolvens	**0.002977**	**0.003151**	**0.002399**	**0.002678**	**16**
Bacillus thermoamylovorans	**0.000252**	**0.000288**	**0.000381**	**0.000191**	**10**
Streptococcus bovis	**0.03015**	**0.009904**	**0.01432**	**0.00858**	**19**
Selenomonas ruminantium	**4.779E-04**	**1.103E-05**	**4.767E-04**	**2.205E-05**	**6**
Brucella melitensis	**9.364E-05**	**5.493E-05**	**2.571E-04**	**8.404E-05**	**8**

P = no of parameters of each model; n = number of observations.

Table 3. Estimations and 95% confidence intervals of the cardinal pH values for *Lacobacillus plantarum*

Estimate	pHmin	pHmax	pHopt	MSE (n=23)
CPM T=21oC	3.31 [3.19- 3.40]	9.81 [9.31 – 10.53]	5.78 [5.64 – 5.92]	5.75E-05
CPM T=15oC	3.49 [2.96- 3.77]	10.9 [8.36– 21.97]	6.00 [5.79 – 6.63]	9.05E-06
ELPM T=21oC	3.25 [3.09 – 3.40]	8.98 [8.61 – 9.71]	5.87 [5.63 – 6.22]	3.66E-05
ELPM T=15oC*	3.29	8.20	6.08	9.43E-06

*** At 15^{o}C the ELPM converged but could not provide 95% confidence intervals for the parameters (although providing standard errors)**

Table 4. Estimations and 95% confidence intervals of the cardinal pH values for *Lacobacillus plantarum* for the combined dataset

Model	pHmin	pHmax	pHopt	MSE (n = 42)
CPM	3.30 [3.22 - 3.37]	9.72 [9.35 – 10.20]	5.82 [5.72 – 5.92]	3.65E-05
ELPM	3.24 [3.10 – 3.36]	9.02 [8.70 – 9.58]	5.90 [5.70 – 6.14]	2.64E-05

Legend to Figures

Figure 1. Effect of pH on the relative growth rate of *Listeria monocytogenes* (pHmin = 4.6, pH max = 9.4, pH opt = 7.1) as predicted by two pH models using the same cardinal values: CPM (filled symbols)and the Extended Presser model (open symbols).

Figure 2. Effect of hydrogen ions on the relative growth of *Escherichia coli* (ATCC 25922) at 30°C in TSB, where γ_{pH} =RTD_{obs}/RTD_{opt}: (○) observed γ_{pH} and modelled γ_{pH} (solid line).

Figure 3. Effect of pH on the growth rate of *L. innocua* at 30°C: comparison of three models with observed values: (●) observed; solid line, CPM; dashed line $ELPM_c$; dash-dot line, ELPM.

Figure 4. The effect of pH on the growth rate of *Butyrivibrio fibrisolvens*: observed values and fitted models: (●) observed; solid line, CPM ; dashed line, $ELPM_c$.

Figure 5. The effect of pH on the growth rate of *Bacillus thermoamylovorans*: observed values and fitted models: (●) Observed; solid line, CPM; dash-dot line, ELPM.

Figure 6. Observed (symbols;) and fitted ELPM (solid lines) growth rate of *Lactobacillus plantarum* at (●) 21°C and (□)15°C.

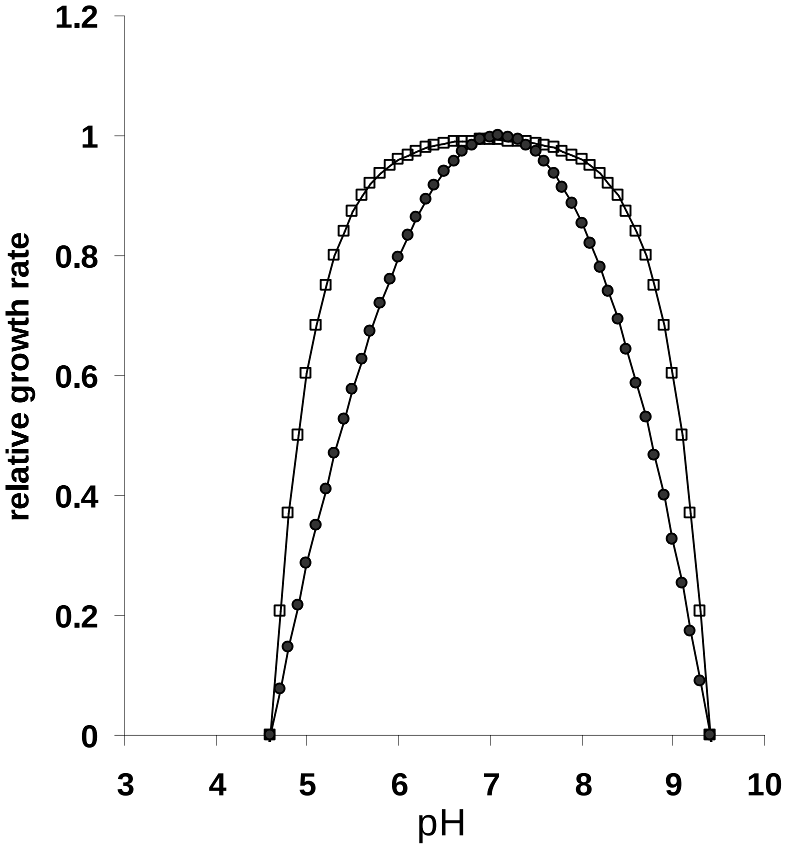
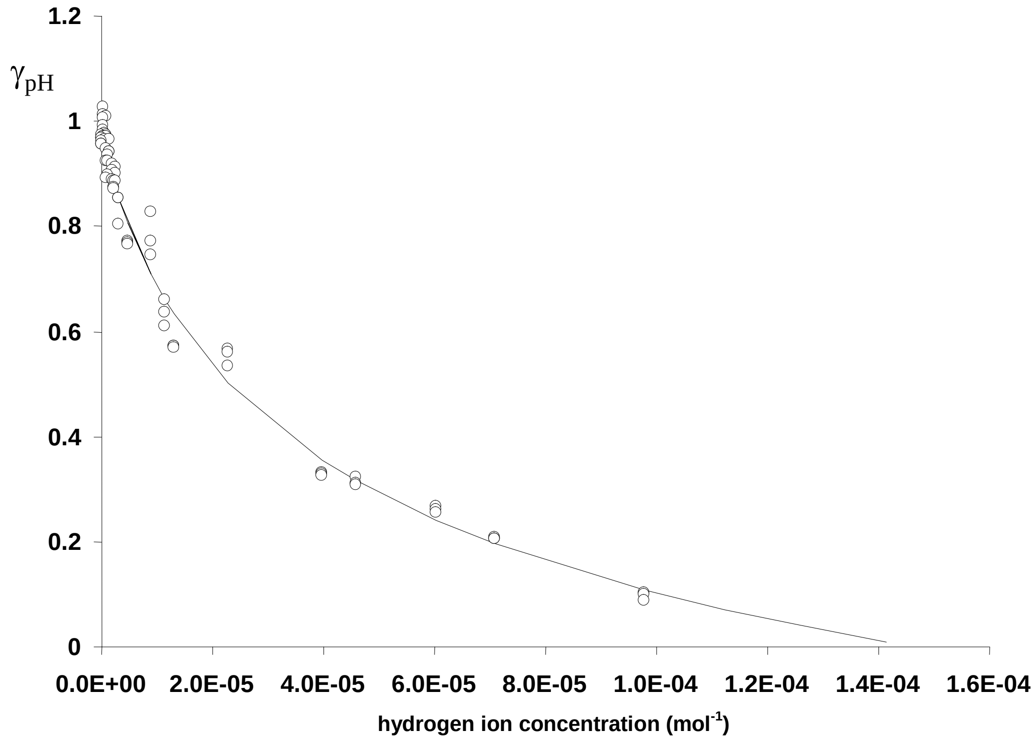


Figure 1.



1.2
γpH
1
0.8
0.6
0.4
0.2
0
0.0E+00
2.0E-05
4.0E-05
6.0E-05
8.0E-05
1.0E-04
1.2E-04
1.4E-04
1.6E-04
hydrogen ion concentration (mol-1)

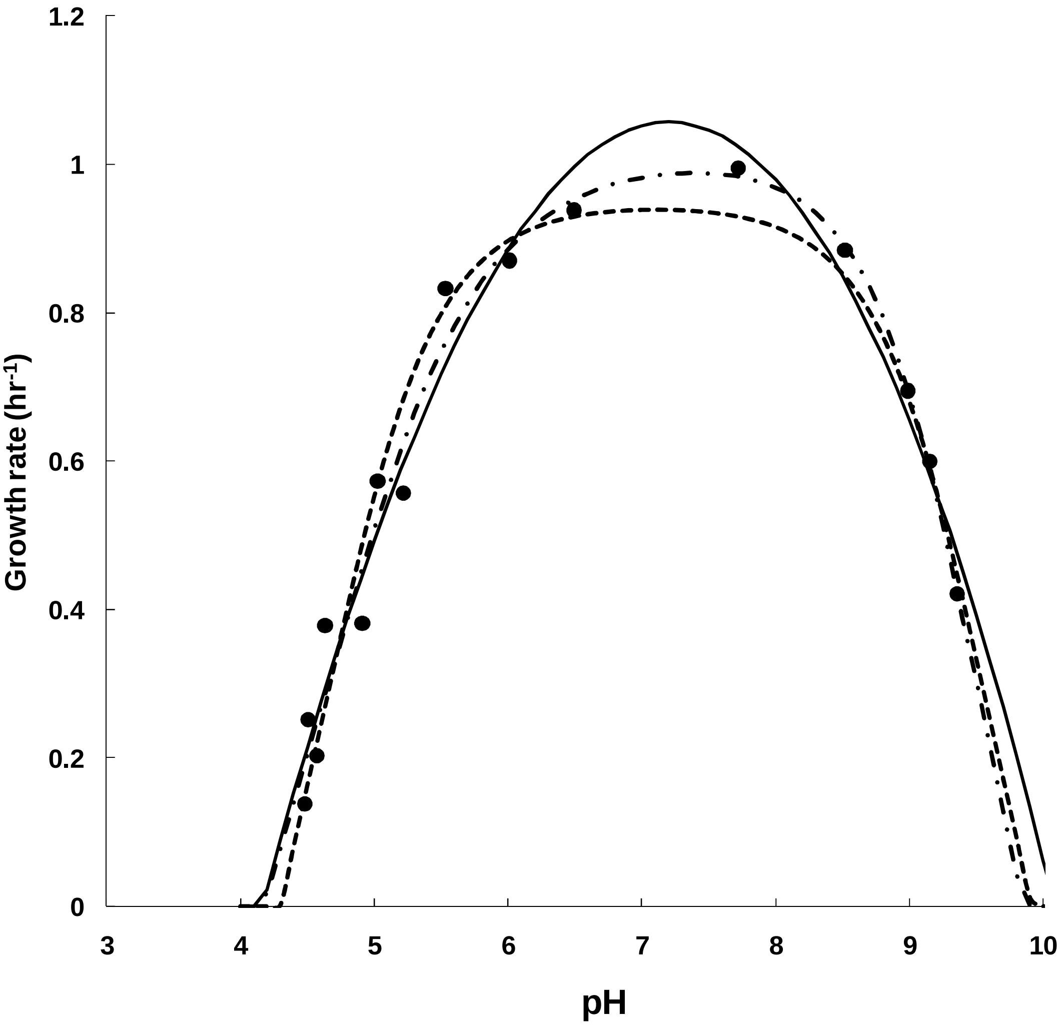


Figure 3.

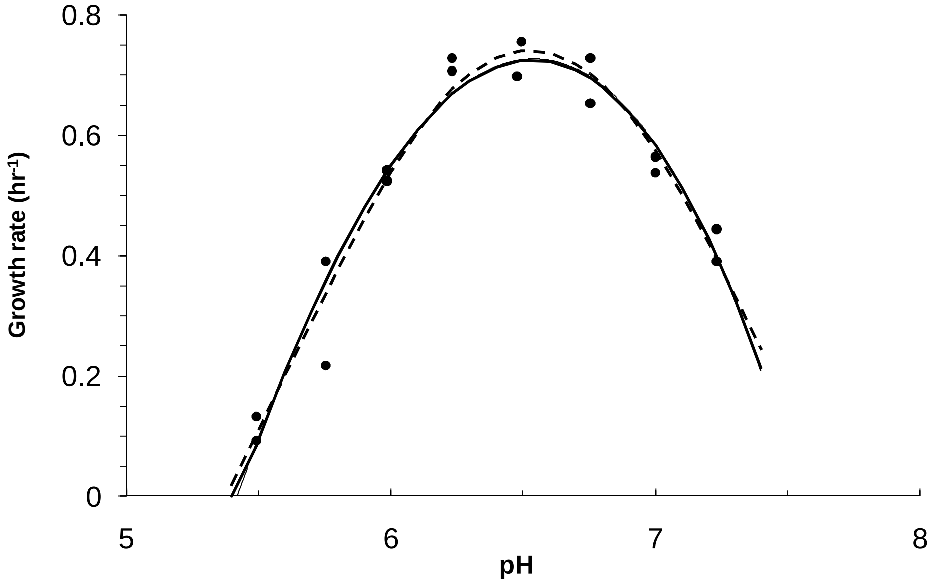


Figure 4.

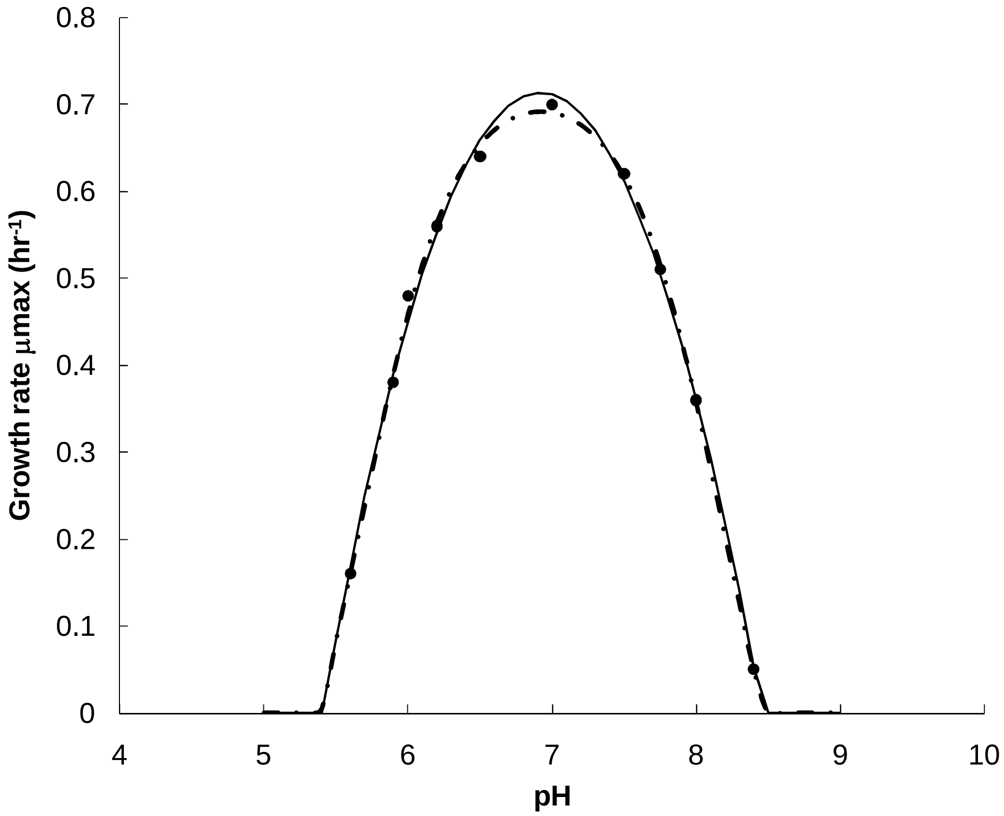


Figure 5.

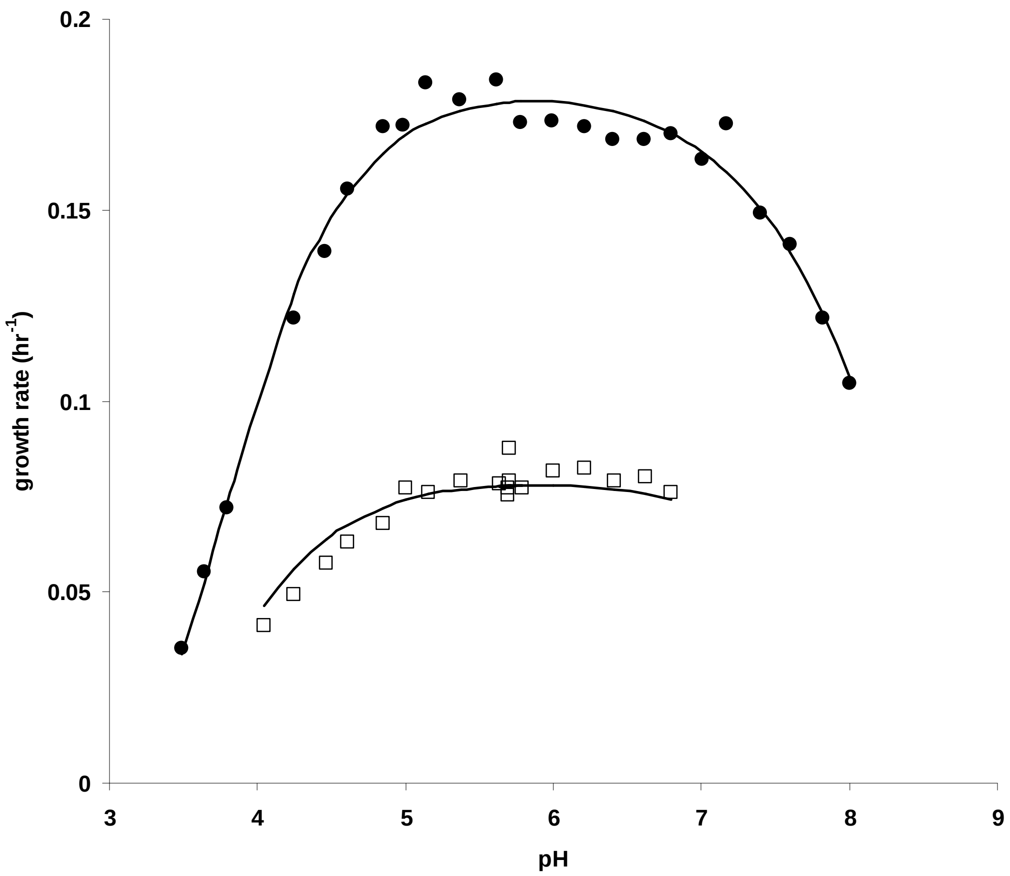


Figure 6.