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Review Paper

Modelling the growth, survival and death of microorganisms in foods: the UK Food Micromodel approach

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Abstract

Techniques for the development of mathematical models in the area of predictive microbiology have greatly improved recently, allowing better and more accurate descriptions of microbial responses to particular environmental conditions, thus enabling predictions of those responses to be made with greater confidence. Recognising the potential value of applying these techniques in the food industry, the Ministry of Agriculture, Fisheries and Food (MAFF) initiated a nationally coordinated five-year programme of research into the growth and survival of microorganisms in foods, with the aim of developing a computerised Predictive Microbiology Database in the UK. This initiative has resulted in the systematic generation of data, through protocols which ensure consistency of methodology, so that data in the database are truly comparable and compatible, and lead to reliable predictive models. The approaches taken by scientists involved in this programme are described and the various stages in the development of mathematical models summarized. It is hoped that this initiative and others being developed in the USA, Australia, Canada and other countries, will encourage a more integrated approach to food safety which will influence all stages of

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food production and, eventually, result in the development of an International Predictive Microbiology Database.

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1. Introduction

Predictive microbiology has emerged as a discipline in its own right in recent years, and the usefulness of the approach is becoming widely accepted. This has resulted in considerable international interest particularly in Western Europe, USA, Canada, and Australia. The true power of this approach is that, unlike the traditional storage trial, models, once validated, can be used swiftly to predict with confidence the responses of organisms under a variety of conditions. Such a tool is invaluable for the modern food microbiologist in day-to-day decision making.

Following a review of research priorities in 1987-88, the Ministry of Agriculture, Fisheries and Food (MAFF) identified predictive microbiology as one approach to help ensure food safety. Initially, the use of literature data was considered for modelling purposes but was not deemed suitable for a number of reasons. The published data on some bacteria, particularly newly emerging pathogens, were scarce and experiments had rarely been designed to generate sufficient data to develop models; it was difficult to combine data from different studies as they were often not complementary; many of the important factors and interactions affecting growth, survival and death of bacteria in foods had been poorly quantified. Consequently, a UK coordinated programme of research on the growth, survival (in conditions that did not support growth), and thermal inactivation of food-poisoning bacteria in conditions relevant to food was initiated by MAFF. The aim was to build on the new modelling expertise becoming available and to generate a computerised Predictive Microbiology Database (Gould, 1989).

A five-year coordinated programme of practical research was developed involving microbiologists and mathematicians from the Norwich and Reading laboratories of the Institute of Food Research, Campden Food and Drink Research Association (CFDRA), the Flour Milling and Baking Research Association, Leatherhead Food Research Association and Torry Research Station, along with Unilever Research and some input from the Universities of Surrey, Bath and Wales College of Cardiff. The data produced from all the participating laboratories are submitted to the UK Predictive Food Microbiology Database. This has resulted in a large database comprising data produced to a high standard using common protocols, ensuring that data from different laboratories could be combined and modelled successfully. Once accepted by the Models Selection Group, models are submitted to the UK models base. The database and models base are located at CFDRA. This was launched in October 1992 as a commercial service – Food Micromodel. The system is available for use by the food industry. A personal computer (PC) based version is also being developed.

A multidisciplinary group of food microbiologists (Model Selection Group,

MSG) with experience in the field of predictive microbiology was set up to help to co-ordinate technical activities within the programme, and to consider the appropriateness and validity of models generated. This paper is intended to give an insight into the approaches this group has taken to evaluate the performance and applicability of the models.

2. Experimental design

The intended use of a model is an important consideration when designing experiments to describe the effects and interactions of environmental factors on the growth, survival or death of a population of microorganisms. Although a wide range of factors may affect microorganisms in foods, the major determinants of microbial growth are generally temperature, pH and water activity (a_w). The experiments within the programme have been designed primarily to examine the effects of these three main controlling factors, although the effects of a number of additional factors known to be important in particular circumstances e.g. atmosphere and preservatives, have also been studied. Once it had been established that the salmonellae model developed from experiments in broth mimicked growth responses in foods it was decided to generate the data for model construction using laboratory media. Liquid media are homogeneous, easily and accurately adjusted and controlled, allowing similar or more rapid growth than that observed in foods. The resultant models will therefore be independent of food-type, and give predictions tending to err on the safe side i.e. they will generally predict faster growth than will be observed in a food.

The following section summarises the approach the MAFF initiative has used to design experiments for the above purpose. A more detailed guide to experimental design for predictive modelling can be found elsewhere in the literature (Davies, 1993; Ratkowsky, 1993; McMeekin et al., 1993).

2.1. Range of controlling factors

Prior to undertaking the experimental work, the range of conditions over which the model is to be used should be defined carefully as empirical models should not be applied beyond the range of factors used in their construction. The ranges may be based on prior knowledge of the likely microbial responses. These are usually well characterised for factors acting independently, but less well for factors acting in combination. The amount of experimental work needed can usually be reduced considerably by the use of suitable screening experiments which may make use of automation (e.g. turbidimetric techniques using multi-well plates).

2.2. Choice of acidulant and humectant

Hydrochloric acid was used as the acidulant in most of the work since it is generally less inhibitory than organic acids. The effects of pH quantified by the

resulting models are, therefore, not significantly influenced by specific effects relating to either undissociated or anionic species of organic acids, and will more accurately reflect the effect of hydrogen-ion concentration alone and tend to predict faster growth. Where organic acids have been used, known amounts have been added so that the concentration of undissociated acid (generally the inhibitory agent) is easily determined. Sodium chloride was used because it is the most commonly used humectant in foods. The effects of other humectants, such as sugars and glycerol, on bacterial growth, survival and thermal death are being investigated, because of their relevance to particular foods (e.g. bakery products), by comparing the effects at selected a_w values across arange of other environmental conditions.

2.3. Choice of strain and size of inoculum

Since it is not feasible to screen large numbers of strains against numerous combinations of factors, mixtures (cocktails) of strains have been used for the growth models within the programme, in order to determine the 'leading edge' of growth i.e. the growth response will be determined by the fastest growing strain within the cocktail. Using a mixture of strains in thermal inactivation studies would, however, produce thermal inactivation kinetics data that may be difficult to interpret. Consequently, single strains with greater, but not abnormally high, heat resistance were used in studies of heat resistance.

The inoculum levels were chosen to ensure that the expected microbial response could be measured, and not to reflect the numbers commonly present in foods. Generally, the following levels were used: 10^2–10^3 cfu/ml for growth conditions; 10^6–10^7 cfu/ml for survival; and 10^8–10^9 cfu/ml for thermal inactivation.

2.4. Number and position of data points

With respect to kinetic responses, although there are no clear rules for the number of estimates of viable numbers with time, it is recommended that at least 10 points are generated for each growth or inactivation curve. More importantly, the usefulness of data can be related, often, to the number of determinations in the region of rapid change e.g. end of lag phase. Hence, sampling times are an important consideration. With survival and thermal inactivation studies, geometric-scale time points may be more appropriate than arithmetic-scale time points, because of this reason.

When considering the effects of independent variables on kinetic responses (e.g. the effect of temperature on generation time) the areas of most interest and concern to the food microbiologist are often those closest to the conditions needed to prevent bacterial growth. The variance of the measured response in these areas tends to be greater than in other, less inhibitory, regions. To have the same confidence in the predictions over the whole experimental matrix, it is necessary to have more datasets in these areas than in areas where the response is more

reproducible. Although knowledge from previous experiments (e.g. screening studies or data from the literature) can often help in deciding where data sets are required, in the absence of a precise definition of the boundaries for growth and, in order to position data in the correct areas, modification of the experimental plan may be necessary during the course of the study.

2.5. Standardising the protocols for recording data

A protocols document has been produced to standardise the production and recording of data within the predictive modelling programme (Walker and Jones, 1993). The document ensures that all data are captured, calculated and recorded in a standard way for ease of incorporation into the database via a spreadsheet.

3. Modelling

Predictive models have been developed describing growth, survival and death, and thermal inactivation of foodborne pathogens. For most models a two-step approach has been followed. In the first step, curves have been fitted to data derived experimentally, and in the second step, kinetic parameters derived from the curve fitting exercise have been modelled against the controlling factors (pH, a_w, temperature).

3.1. Curve-fitting

In general, growth curves were fitted with a modified-Gompertz function as described by Gibson et al. (1988). In addition, alternative approaches have been considered such as those described by Baranyi et al. (1992) and Jones and Walker (1993). The former uses differential equations to describe growth, whilst the latter describes growth and survival by a single mathematical function.

Thermal inactivation data demonstrating near log-linear death kinetics have been fitted using traditional decimal reduction times (Peck et al., 1993). However, where significant deviations from log linearity were seen the log-logistic approach (Cole et al., 1993) has been used. Both these approaches may be used to determine the time to a specified decrease in cell numbers.

3.2. Modelling kinetic parameters

To develop models, kinetic parameters derived from the curve-fitting exercise have been fitted to the environmental factors (e.g. pH, temperature, a_w etc). This relationship is described by the deterministic part of the model. The extent to which the predicted response deviates from the observed (stochastic or error term), however, is equally important. To obtain the best fit of the model to the data, the error in the estimate of the selected response must be independent of the value of the response and if not, then a suitable transformation should be used to normalise

this variance (alternatively, a weighted fitting procedure may be used). This is particularly relevant when comparing the performance of different models (McMeekin et al., 1993). Taking the logarithm of the response was found to be a suitable transformation for normalising the variance associated with these data.

In most cases a sequential fit to a quadratic response surface was used (McClure et al., 1993). However, the fit to some data sets was improved by using a surface fit (one stage procedure) as described by Jones and Walker (1993). Models should use the minimum number or set of terms which describe the response adequately (ie be parsimonious), where an adequate fit is defined by certain criteria (see Section 4.1., Mathematical testing).

4. Acceptance

Once a model of the experimental data has been developed, it is discussed at the MSG. Before a model is accepted for inclusion in the database, it is analyzed to ensure that it describes the data well, and checked that it makes biological sense. The fit of the model to the data is checked visually by plotting fitted responses against observed responses to determine potential problem areas.

The importance of working within the limits of the experimental matrix (i.e. without extrapolating) of empirical models has already been mentioned. In these experiments, not all of the combinations of factors will allow growth, hence, simple maximum and minimum values of factors used are not adequate to define the domain over which the model is valid. Instead, the domain must be defined by the actual combinations of conditions used to generate the model or a polygon describing these. Food Micromodel applies an algorithm which uses the experimental matrix to describe this domain of validity. Acceptance of a model is therefore also subject to the submission of the complete experimental matrix of factors used in the construction of the model.

4.1. Mathematical testing

The adequacy of a model to fit data was assessed on the basis of root mean square error (RMSE) and percentage goodness of fit (percentage fit).

RMSE is a measure of the variability remaining after fitting a model, that is not accounted for by the deliberate changes in factors such as temperature, pH, a_w and NaCl. This error may come from several sources including natural variability, systematic errors and bias. Natural variability, may be due to variability inherent in the microorganism, systematic errors may be due to analytical/laboratory methods; and bias may be due to model mis-specification e.g. fitting a linear equation where the underlying data would be better described by a quadratic equation.

Providing an appropriate modelling technique is used, the contribution of the bias term is negligible, and the RMSE is a measure of the reproducibility of the measurements. The acceptability is related to the natural variation so that a higher RMSE is acceptable from a naturally more variable system. For example, it has

been noted that measuring rates of bacterial growth from spore inocula is more variable than growth from vegetative cells, therefore the acceptable RMSEs associated with spore inocula are larger than those associated with vegetative cell inocula.

The percentage fit is a measure of how well the model describes the data. Generally, the higher the fit, the better the model describes the true underlying relationship.

4.2. Biological sense

Predictions from models are examined to ensure that the response is similar to that expected by experienced microbiologists. With poorly designed experiments (e.g. more data under optimal conditions for growth than under inhibitory conditions), or over-parameterized models where the principle of parsimony has not been observed, experience has shown that although the fit to the data is acceptable, the predicted response can be erroneous for conditions not originally tested.

5. Validation

A key stage in the production of Food Micromodel is validation of the models for use in foods. This has been achieved by comparing the behaviour predicted for each organism by a model against its behaviour observed in foods using data from the literature generated by others and, where the literature data are insufficient, in experiments conducted specifically for this purpose.

Before a model is accepted for inclusion in the database, it is used to predict the response of a microorganism in a wide range of foodstuffs, varying in such factors as pH, a_w, NaCl and temperature. The performance of the model in the prediction of growth in food is judged according to two criteria (i) generation time and (ii) time for a specified change in numbers (taking account of both generation time and lag time). For growth, the models are not judged on lag time alone because the determination of this parameter from experimental data is greatly influenced by the choice of times at which counts are taken, the definition of lag, the way the curve is fitted, and the 'pre-history' of the inoculum. Experience has shown that the estimation of lag is generally less repeatable than generation time.

In food validation studies, for each food tested, the pH and NaCl concentration (or a_w) were determined. The food was inoculated with a known concentration of the microorganism and stored at a known temperature. The change in viable numbers was estimated at suitable intervals and used to determine kinetic parameters of growth, survival and death.

A different approach was taken for data extracted from the literature, allowing a wider validation exercise to be carried out than could be done within the resource constraints of the programme. The literature validation exercise revealed marked deficiencies in the literature itself, many authors giving incomplete information about their foods, experimental designs and/or methods, and rarely

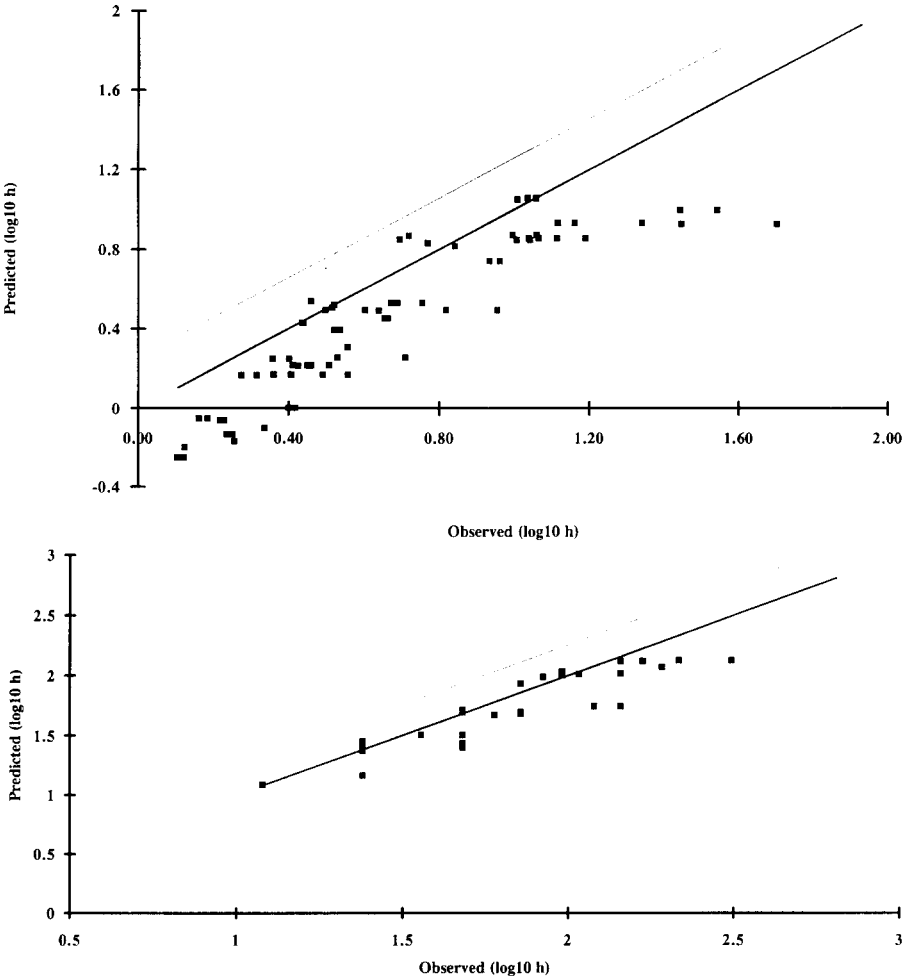

Fig. 1. A comparison of the predicted generation times (a) and time to 1000-fold increase (b) of salmonellae from Food Micromodel with data taken from inoculated food studies, generated within the UK Predictive Microbiology Programme. Foods include a range of meat, vegetable, dairy, egg, and bakery products. The prediction is accurate close to the continuous line. The dashed line is the upper 90% confidence interval for the predicted value (McClure et al., 1993).

generating data suitable for curve-fitting and deriving estimates of kinetic parameters such as lag times and doubling times.

Values of generation time and time for a 1000-fold increase from the literature or inoculated food studies were plotted against predicted values from the model for the same conditions (Figs. 1 and 2). A $\log_{10}$ scale was used in this comparison as this transformation was found to normalise the variance (discussed earlier). Thus the upper error of the prediction can be represented by parallel line above the line of perfect agreement. Where points fall below the line the model predicts

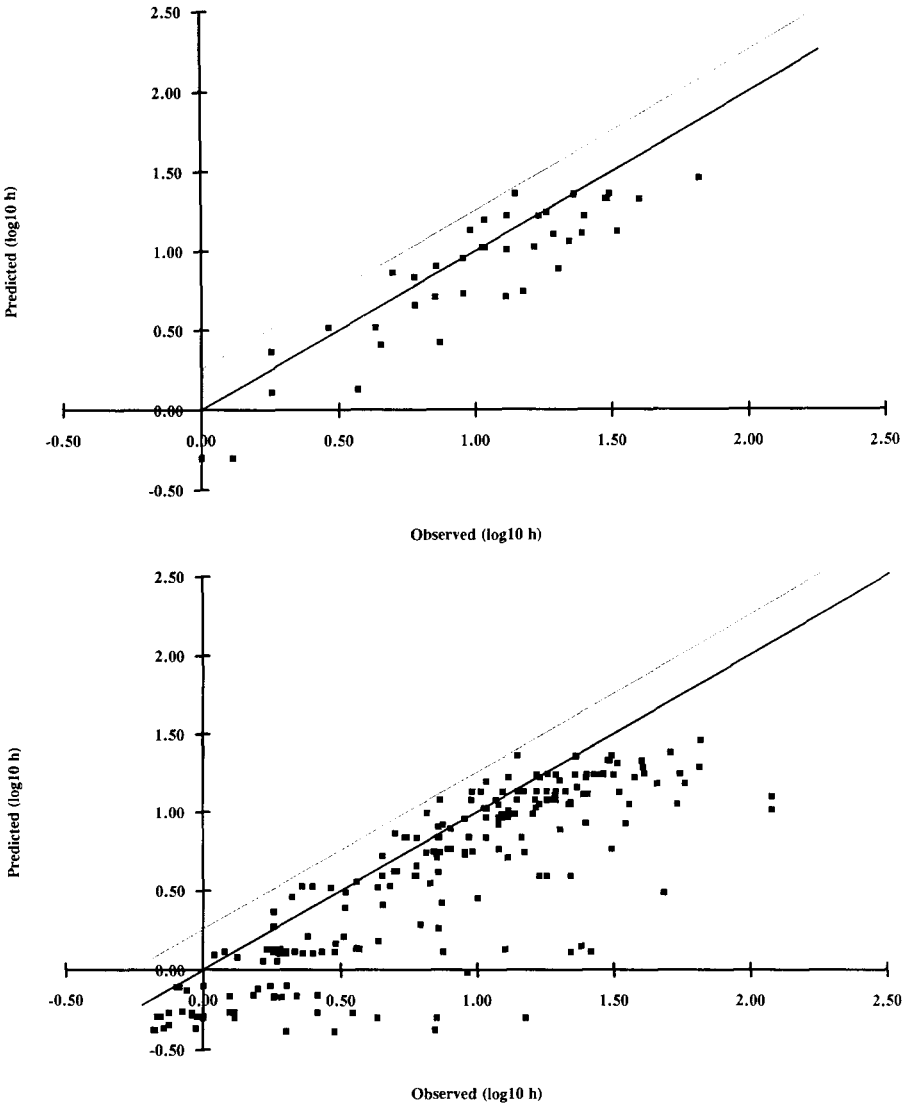

Fig. 2. A comparison of predicted generation times of *L. monocytogenes* from Food Micromodel with data taken from the literature, in all products (a) and meat and poultry products (b). The prediction is accurate close to the continuous line. The dashed line is the upper 90% confidence interval for the predicted value (McClure et al., 1993).

faster growth than that observed in the food, and predictions err on the safe side. For points above the line, however, the model predicts slower growth than that observed in the food and the prediction would 'fail-unsafe'. These plots may be used to compare different product groups, and identify foods where the agreement is poor. In general, the models have generated predictions relevant to most food

groups, showing excellent agreement, and deviations from the model are usually explicable by other preservative factors. Although predictions outside the range of the model should never be used to advise on new or modified formulations, comparisons made with literature data outside the domain of validity (as shown in Figs. 1 and 2) serve to highlight areas where more data should be added to the original dataset to make the model more applicable to a wider range of products.

After accepting a model, it is incorporated into Food Micromodel and may then be used to produce predictions in foods where the model has been validated. Data sets used to construct models are continually being extended, to improve their robustness and to increase the domain of validity, sometimes by increasing the range of conditions tested or by adding another controlling factor, such as CO_2. Models derived from these enlarged data sets are scrutinized in a similar manner as above, and once validated, will supersede models already stored within Food Micromodel.

6. Future aspects

The MAFF database and models-base held at the CFDRA in the UK represent the culmination of work by laboratories participating in the UK modelling project. These laboratories have participated in a research programme to develop a database and models for the pathogens *Salmonella, Listeria monocytogenes, Staphylococcus aureus, Yersinia enterocolitica, Escherichia coli* O157:H7, *Bacillus cereus, Bacillus subtilis*, psychrotropic strains of *Clostridium botulinum, Campylobacter jejuni, Clostridium perfringens* and *Aeromonas hydrophila*. The resulting models have been fully validated in foods using published literature or, where there is a paucity of published data, by extensive challenge tests in representative foods. Food Micromodel is fully operational and accessible by the Food Industry and operates as a commercial service.

With experience, techniques for data acquisition are improving, and modelling can be performed on personal computers. Modelling techniques with a better mathematical and biochemical basis will result in improved models so that it is an appropriate time to plan the organisation of computerised predictive databases for use by food industries, regulatory bodies and other relevant groups. This will help to provide a safer food supply for consumers and improved control, shelf-life and stability of food products for manufacturers, retailers and consumers.

Research in predictive microbiology and the development of computerised databases is being pursued by a number of groups in several countries. This non-integrated approach inevitably leads to some unnecessary repetition. In addition, results from experiments which may be incompatible in design cannot be successfully combined and modelled. In the future, it is important for all countries involved in predictive modelling to collaborate to avoid unnecessary duplication. Opportunities for collaboration exist and in some cases are ongoing e.g. the Food-Linked Agro-Industrial Research (FLAIR) Concerted Action (No. 5) "Predictive Modelling of Microbial Growth and Survival in Foods" in the European

Community. Collaboration will make research more cost-effective by avoiding the duplication of models encompassing a number of different factors which are important in controlling the growth of microorganisms in an ever-widening range of foods.

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