Application of mathematical modelling techniques for predicting mould growth

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Abstract

Predictive modelling of microbial growth involves the development of mathematical models describing microbial growth. Such models can be used as the basis for expert systems that predict the rate or amount of growth and whether particular microorganisms will survive, die or produce toxins in foods under a wide range of conditions. Potential benefits for the food industry are large, as such systems may be used to develop new products and processes and to optimise and control existing processes with a greater certainty that foods will be safe. Food-borne bacterial pathogens were used initially as the basis for modelling, but similar modelling techniques have now been applied to moulds. The growth responses of *Aspergillus flavus* and closely related species have been successfully modelled to predict the growth rate (increase in diameter) of a mould colony, or the time to reach a 3mm diameter colony. The model covers a range of water activity (a_w) levels and three storage temperatures. In most foods a_w and temperature are the main controlling factors for mould growth. Such models may be used in the food industry to predict whether a particular mould is capable of growth when a product is held under given conditions. These models should be readily applicable to grain storage where control of humidity and temperature is of vital importance.

Introduction

Mathematical models which describe microbial growth may be used to predict the rate or amount of growth and whether particular microbes will survive, die or produce toxins in foods under a wide range of conditions. Potential benefits of such systems for the food industry are large, as they may be used to develop new products and processes and optimise and control existing processes.

Most models have been developed for bacteria (McMeekin et al. 1993). Techniques used for predicting bacterial growth are now being applied to moulds (Gibson et al. 1994). Moulds cause substantial spoilage losses in the food industry. Some moulds are also capable of producing mycotoxins, including some known carcinogens, in foods. Therefore, growth of spoilage and toxigenic fungi in foods must be avoided. The growth responses of *Aspergillus flavus* and related species have been successfully modelled to enable the prediction of mould growth rate over a range of water activity (a_w) levels. Work to extend the models is continuing. These models should be readily applicable to grain storage, where control of humidity and temperature is of vital importance.

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Rationale for Predictive Modelling

To make predictions about microbial growth in foods we first need to establish which factors are controlling growth and then study, in detail, how those factors, and their interactions, influence growth of the microbes of concern. This process requires the generation of a large amount of information on the responses of microorganisms to several physicochemical variables at a range of levels. To produce a reasonable model, mathematical analysis of the data produced and subsequent validation of that model using other published data or specific experimentation are necessary.

In foods, where nutrient limitation is rarely of concern, microbial growth is predominantly controlled by storage temperature and water activity. Within specific food types other factors such as pH level, gaseous atmosphere, presence of preservatives, thermal processing etc. can be manipulated to further control microbial growth. Traditional research on the growth of microorganisms has focused on specific foods and the findings have not been readily applicable to other products.

Research on pasteurised cured meats illustrates the development and application of predictive modelling. Canned cured meats are preserved by a complex, interacting group of antimicrobial agents. Factors including nitrite, salt (NaCl), nitrate, iso-ascorbate and polyphosphate all contribute to the control of microbial growth in the mildly heated product. The detection of carcinogenic 'nitrosamines' in some cured meat products during the 1970s prompted demands to reduce to a minimum the quantities of nitrite used. Such demands resulted in substantial research into the microbiological safety of cured meats. These products can support growth of the toxin-producing bacterium *C. botulinum* if the preservative system is inadequate.

Large-scale, multifactorial experiments were then carried out (frequently using experimental cured meat systems), in order to answer questions such as 'what is the minimum level of nitrite required to prevent toxin production by *C. botulinum* in pasteurised cured meats?' (see reviews by Sofos et al. 1979; Roberts et al. 1981a; Hauschild 1982; Tompkin 1983; Gibson et al. 1984; Roberts and Gibson 1986). Such experimentation was very costly to conduct and also time-consuming, individual experiments often taking up to 6 months. At the end of the work the data were relevant to pasteurised cured meats only. However, that type of work illustrated the complexity of the effects and interactions between the various factors which combine to control microbial growth in pasteurised cured meats. An experienced microbiologist might be able to predict the outcome should one of those many factors be changed but would be unable to predict the outcome should several factors be changed simultaneously.

In order to extract meaningful conclusions from the copious data of the cured meat experiments, computerised data

handling was used. From that analysis a model was produced which enabled the prediction of the probability of toxin production in a model pasteurised cured meat system at a variety of combinations of temperature, level of pasteurisation, inoculum, nitrite and NaCl, and presence or absence of isoascorbate, polyphosphate and nitrate (Roberts et al. 1981b).

From that initial model, the concept of mathematical modelling of microbial growth continued to be developed. The first *C. botulinum* model predicted toxin production (hence growth) of *C. botulinum*. Models were developed later which predicted the whole bacterial growth curve, including lag phase and growth rate, from which generation or doubling time and time to reach a particular population density (level of concern) could be calculated (Gibson et al. 1988; Zwietering et al. 1990; Buchanan 1993a.) McMeekin et al. (1993) present a detailed review of the various models currently being used for predicting bacterial growth.

The value of such models is now well accepted and one group of models is being made available commercially in the United Kingdom as the Food Micromodel service offered by the Ministry of Agriculture, Fisheries and Food. The models are in a mainframe computer and can be interrogated by accredited organisations. With the ready availability of sophisticated personal computers it is also possible to condense databases and simple interrogation programs onto a single computer disc. The Pathogen Modelling Program (Buchanan 1993b) is an example. This approach enables direct use by food technologies in industry of databases for particular microbes. The physical and chemical parameters of a food of concern are entered into the program by following a series of on-screen prompts. The predicted growth curve or predicted curve parameters for that particular microbe are obtained within a few seconds.

Model Building

In the first step of the early models, a sigmoid curve was fitted to the microbial growth data. In the second step, a polynomial model was fitted to describe the variation of the curve parameters as a function of the growth conditions (Gibson et al. 1988). Subsequently, a revised model that improves the fit to the data was developed (Baranyi et al. 1993). It also allows for the absence of data in the stationary phase, a useful advantage in some circumstances.

Until recently these predictive models were generally restricted to growth of bacteria, particularly pathogenic bacteria. Baranyi's models have now been adapted to allow the prediction of growth of *A. flavus* and closely related species as affected by a_w and temperature.

Modelling of Growth of *Aspergillus* Species

Unpublished data of J.I. Pitt and B.F. Miscamble were used as the basis for the modelling. They had studied the effect of a_w level (within the range 0.81–0.995) on the rate of increase in colony diameter of 3 isolates of each of 4 species of *Aspergillus* at 25, 30 and 37°C. *Aspergillus flavus* FRR 3084, 2807 and 2755, *A. oryzae* FRR 2336, 1675 and 1677, *A. parasiticus*, FRR 2806, 2752 and 3385, and *A. nomius* FRR 3545, 3543 and 4546 were used in that study.

Pitt and Miscamble's data, collected at 30°C, were modelled in a similar fashion to the bacterial data (Gibson et al. 1994). Using colony diameter (mm) as the basis for a 'growth curve', colony diameters were plotted against time. Maximum colony growth rates for each a_w were then calculated and modelled as a function of a_w. To assist the modelling process, novel transformations of a_w and colony growth rate were introduced which allowed a quadratic function to be fitted separately to data for each strain studied. From that quadratic function, colony growth rate can be predicted for any a_w within the range studied (Gibson et al. 1994). Ongoing work allows for the inclusion of temperature within the range 25–37°C into the model.

Step 1 - curve fitting

Figure 1 gives examples of growth curves obtained, in this instance for *A. flavus* (FRR 2755) at 30°C and a_w 0.93, 0.913, 0.88 and 0.848, plotted against time. Actual data points and the fitted curve are shown.

Step 2 - modelling of growth rate against a_w

The form of the model for growth rate against a_w is:

$$y(t) = y_0 + gA_n(t) - \frac{1}{m}\ln\left(1 + \frac{e^{mgA_n(t)} - 1}{e^{m(y_{\max} - y_0)}}\right) \qquad (1)$$

where

$$A_n(t) = \int_0^t \frac{s^n}{\lambda^n + s^n}\, ds \qquad (2)$$

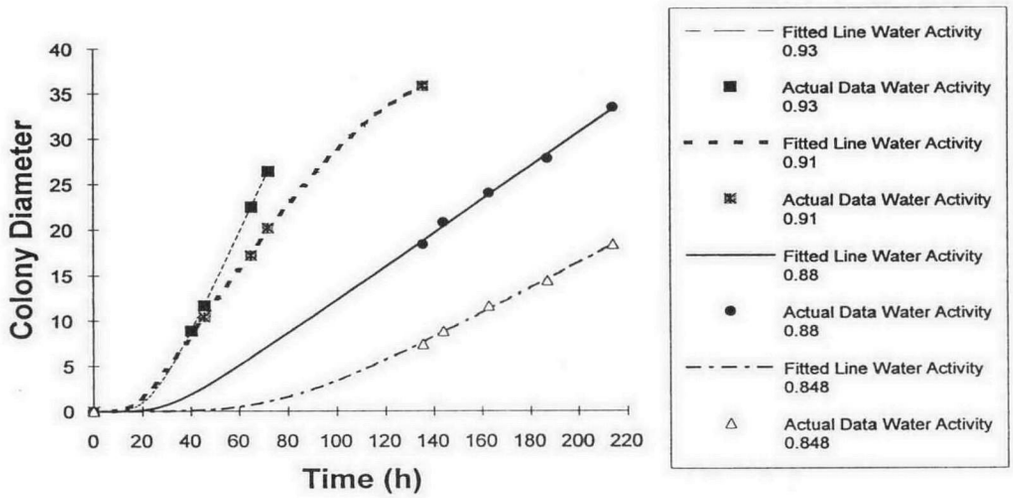

Fig. 1. *Aspergillus flavus* 2755: Colony diameter (mm) against time at 4 water activity levels

The parameters are as follows:
y_0 = diameter of the colony at the time $t = 0$
g = maximum colony growth rate
y_{max} = maximum diameter of colony
λ = lag phase

The curvature constants m and n are fixed empirically at 0.1 and 4, respectively. A full description of the model and its parameters is given in Gibson et al. (1994).

From this model, growth rates can be predicted for any a_w within the range tested. Table 1 gives examples of some of the predictions obtained from the models. Predicted growth rates at optimum a_w indicate the fastest growth rate that was obtained for each isolate. The growth rate at a_w 0.85 is also provided. The time taken for a mould colony to reach a diameter of 3 mm is of practical significance since a colony of that size would be clearly visible to the naked eye and the product therefore considered spoiled. Such a time can be calculated from the model for any a_w studied and time to reach a 3 mm diameter colony at optimum a_w and a_w 0.85 is tabulated as an example.

Comparison of Predictions with Published Data

Many investigators have studied the effect of a_w on the growth of a variety of moulds including *A. flavus*. However, their experiments were carried out at a variety of temperatures, using different humectants to control a_w, using different strains and employing different methods of measuring a_w. Therefore, there is wide variability between published growth rates at some a_w levels. Predictions from this model were found to compare well with data published by other investigators at a_w levels between 0.85 and 0.98, although predictions from the model showed higher growth rates at a_w 0.99. Predictions close to the limits of the model are always subject to greater error than those well within the range of the model, and those at high a_w particularly so because of the inherent problems in accurately measuring a_w at those levels. A comparison of predictions from the model and data from other workers is given in Table 2.

Table 2. Growth rates for *Aspergillus flavus* predicted by the model compared with published growth rates. Data from several publications summarized by ICMSF (1993).

a_w	Predicted colony growth rates (mm/hour)	Published colony growth rates (mm/hour)
0.99	0.85-0.92	0.24-0.52
0.98	0.85-0.90	0.26-1.00
0.95	0.60-0.64	0.23-0.86
0.90	0.31-0.33	0.04-0.44
0.87	0.20-0.22	0.01-0.22
0.85	0.14-0.17	0.04-0.13

Conclusion

The ultimate objective of this type of research is the development of computer software, based on mathematical models, that can reliably predict the behaviour of moulds in stored products. That objective will not be attained for several years. The research performed so far has shown that models used for bacterial growth are appropriate tools for the interpretation of mould growth data. Within limits, the model produced by Gibson et al. (1994) is able to predict the effect of a_w on the colony growth rate of moulds. Further work is necessary to improve the precision of the model, because the data used in model development were collected originally for purposes other than for modelling and have important limitations. Nevertheless, that investigation has provided an insight into the type and quantity of data needed. Modifications to experimental methodology would increase the stability of the models and provide greater reliability in their predictions. Further

Table 1. Examples of predictions from the models for growth rate (mm/hours) and time (hours) to reach a 3 mm colony diameter.

	Growth rate model[a]			Time to 3 mm colony model[b]	
	a_w(opt)	g(opt)	g(0.85)	t_3(opt)	t_3(0.85)
Aspergillus flavus					
FRR 3084	0.9951	0.926	0.166	12.08	93.77
FRR 2755	0.9917	0.928	0.142	13.39	94.52
FRR 2807	0.9947	0.880	0.165	13.21	115.71
Aspergillus nomius					
FRR 3543	0.9737	1.028	0.087	3.21	135.66
FRR 3545	0.9742	0.880	0.113	9.76	107.10
FRR 4546	0.9771	1.061	0.118	11.33	69.15
Aspergillus oryzae					
FRR 1675	0.9877	0.920	0.134	12.97	154.04
FRR 2336	0.9865	0.967	0.109	11.42	108.91
FRR 1677	0.9846	0.725	0.111	8.86	79.34
Aspergillus parasiticus					
FRR 2752	0.9850	0.826	0.132	12.66	90.62
FRR 3385	0.9875	0.895	0.134	12.89	103.08
FRR 2806	0.9933	0.722	0.079	12.90	113.79

[a] a_w(opt) predicted optimum a_w for maximum colony growth rate; g(opt)predicted colony growth rate at optimum a_w; g(0.85)predicted colony growth rate at a_w 0.85; [b] t_3(opt)predicted time to reach 3mm colony at optimum a_w; t_3(0.85)predicted time to reach 3mm colony at a_w 0.85

work, including investigation of storage temperature as a variable, is in progress. Additional factors such as pH and type of humectant must also be taken into account.

Although data on the effect of a_w on mould growth are copious, comparison of results between investigators is often difficult because of differences in methodology or isolates used. Standardisation of methodology across combinations of factors affecting growth can help to produce an empirical model to describe growth under any combination of conditions within the range tested. If researchers could be encouraged to collect and report their data more systematically, modelling those accumulated data would give increased confidence in the predictions.

The amount of work required to develop reliable models for important fungi is greater than can be performed readily by one group of workers. Collaboration between groups of workers and industry is therefore necessary to produce the extensive databases required. Such collaboration could produce meaningful and useful models to predict minimum or optimum conditions for growth, rate of growth or time to reach a particular population density or size.

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