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## **REVIEW OF MICROBIAL MODELING TECHNIQUES FOR MEAT INDUSTRY**

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***Abstract** Government policies developed after seeing a series of food borne outbreaks caused by pathogenic microorganisms require the meat industry to ensure reduction or elimination of those organisms in meat products. For this, Industry needs to take up an extensive amount of work to generate and accumulate data on behavior of microorganisms in the food product. It is expensive, time consuming and laborious. Predictive microbial modeling is a rapidly emerging food safety engineering technique to model the effects of various environmental conditions in growth or survival of microorganisms during manufacturing of food. This technique can serve better in meat industry for ensuring food safety. In this paper, literature on microbial modeling techniques for meat industry is reviewed and the origin, history and development of various models; the superior performance, advantages and disadvantages of artificial neural network (ANN) based models; necessity of modeling with sufficient data; and necessity of developing tertiary models for industrial applications are outlined. **Keywords:** microbial modeling, predictive microbiology, food safety engineering.*

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## Introduction

About 30 – 40 percent of world food production is wasted because of improper storage, affecting food quality and safety. Microorganisms play a major role in reducing quality; spoiling and toxifying of the stored foods and rendering them unsuitable for consumption. Food scientists and engineers have developed methods for controlling the growth of microorganisms physically, chemically and biologically. To assist in activities to prevent losses due to spoilage there also exists the need of predicting the growth and/or inactivation of microorganisms in foods during processing, storing and preservation. To ensure the stability and safety of food products until consumption, the growth or activity of microorganisms should be below the levels defined in the food safety objectives (Jay et al. 2005) for specific foods.

Most countries and the Food and Agriculture Organization reaffirmed at the World Food Summit that it is the right of everyone to have access to **safe and nutritious food**, consistent with the right to adequate food and the fundamental right of everyone to be free from hunger. This is the Rome declaration of world food security (FAO, 1996). This declaration emphasizes every country to concentrate on ensuring secured supply of food to its citizens with quality and safety. Several food policies of every country require its food industries to ensure food safety on delivery of products. For example, a series of food borne illness outbreaks caused by *E.coli* O157:H7 in fermented sausages (Anon, 1995; WHO, 1997) led the Canadian government to adopt new U.S. food safety regulations which require sausage manufacturers to protect these products from this pathogen (Reed, 1995) by ensuring at least a 5 log reduction. Ground beef is a common vehicle for spread of contamination by *E.coli* O157:H7 and raw or undercooked meat products prepared using ground beef have been implicated (Health Canada, 2000).

The growth of microorganisms has high biological variability in relation to the intrinsic and extrinsic factors of food. Their physiological responses to these factors are very complex and poorly understood (Geeraerd et al., 1998a). The primary objective of food microbiologists is to identify and quantify the microorganisms that have both beneficial and deleterious effects on the safety and quality of raw or processed foods. An extensive amount of work is needed to generate and accumulate the data on behavior of microorganisms in food. It is expensive too. Unfortunately, these data provide little insight in explaining the relationship between physiological processes and the growth or survival of microorganisms. The models developed based on these data are helpful in solving this limitation and in predicting unknown values through interpolation.

Predictive modeling is a rapidly emerging food safety engineering technique with microbiological applications. It has attained separate sub discipline status in the field of food microbiology. It has potential to be a powerful tool for development and evaluation of methods to improve the stability and safety of food products in meat industry.

### Why predictive models?

Why do we need a model while the accumulated knowledge of the response pattern itself can be sufficient? To answer this question in a practical sense, it is clear that the additional efforts made to develop and validate a model will, if properly done, lead to formulation of a general rule for describing the effect of environmental factors on microbial activity. Whereas the response pattern itself, if no model is developed, is more likely to describe only the output of limited experimental trials. Its applicability will again need challenge testing often under slightly changed conditions. Challenge testing is also expensive and time consuming. To answer the question in a philosophical sense that lie in nature of science itself. "When you can measure what you are speaking about and express it in numbers, you have something about it; but when you cannot

measure it, when you cannot express it in numbers your knowledge is of a meager and unsatisfactory kind” (sic)-Lord Kelvin (McMeekin 2004). So modeling is nothing but a quantitative science which is inherently more useful than the qualitative description of a phenomenon.

In general sense, a combination of descriptions, mathematical functions or equations, and specific starting conditions is used in the model for describing a system mathematically.

## **Origin and History**

The canning industry in 1920 developed the methods for calculating the thermal death time. The modeling of behavior of microorganisms with respect to controlling parameters like temperature began from then. This predictive modeling resurged again from 1980 with the growing number of refrigerated foods developed, and the development of hurdle technology and of computer technology. Predictive microbiology is centered on the assumption that the growth or inactivation of microorganisms due to varying levels of controlling parameters can be predicted within the biological variability.

### ***Inactivation models for thermal processing of foods***

Among the earliest work on predictive modeling of microbial activity, Scott (1937) did a study on the effect of temperature on microbial growth on meat. He emphasized the importance of the knowledge of microbial growth rates at different temperatures while studying meat spoilage. The relative influence of spoilage caused by various microbes at each storage temperature and their changes in population could be predicted using these data. He also did a study on the effect of water content on microbial growth and spoilage (Scott 1936) and the accumulated knowledge from both the studies, at a time when there was no explicit model, allowed the shipment of non-frozen meat from Australia to Europe.

### ***Models of mathematical expressions***

A mathematical equation can describe the effect of integrated functions of controlling parameters on microbial growth in food. Models should be validated with a number of selected tests. Despite the mathematical modeling was popularly used in fermentation industry (Monod, 1949), this technique did not receive attention in modeling the growth and survival of microorganisms in foods until 1980.

The spoilage of highly perishable foods depends on the temperature of storage. According to Olley and Ratkowsky (1973) spoilage can be predicted by a spoilage rate curve; the general spoilage curve was incorporated into the circuitry of a temperature function integrator that read out the equivalent days of storage at 0° C. So, it could predict the remaining shelf life at 0° C.

Use of the Arrhenius equation is a basic approach for finding the relationship between the reaction rate and temperature in published studies, but it is applicable for chemistry reactions. Instead of using this equation, the following model was developed to describe the relationship between the growth rate of microorganisms and minimum and optimum temperature (Ratkowsky et al., 1982).

$$\sqrt{r} = b (T - T_0) \quad (1)$$

where,

$r$  = growth rate,

$b$  = slope of the regression line drawn between growth and time.

$T_0$  = conceptual temperature of no metabolic significance.

T = Temperature of food

Using this formula there is a linear relationship between the growth of spoilage bacteria in foods or in broth media utilizing amino acids.

The model has been further developed (Wijtzes et al., 2001) to include the effects of other factors like pH and Aw on growth rate:

$$\sqrt{r} = b * \sqrt{(Aw - Aw \text{ min})(pH - pH \text{ min})(pH - pH \text{ max})(T - T \text{ min})} \quad (2)$$

where,

Aw, pH and T - water activity, pH and temperature of given food

Aw min, pH min and T min - lower or minimum limits of above factors

pH max – Maximum limits of pH of food

This model is popularly known as the square root model.

## Development of Predictive Model Studies

Davey (1992) pointed out that food process engineers faced difficulties in understanding the effect of various environmental factors influencing the growth or survival of microorganisms during production. Davey modified and introduced an arrhenius-type model to account the effect of temperature and water activity:

$$\ln k = C_0 + \frac{C_1}{T} + \frac{C_2}{T^2} + C_3 Aw + C_4 Aw^2 \quad (3)$$

where,

*k*, *T*, and *Aw* are population, temperature and water activity

*C*<sub>0</sub>, *C*<sub>1</sub>, *C*<sub>2</sub>, *C*<sub>3</sub>, and *C*<sub>4</sub> are coefficients to be determined.

Simple primary statistical models were successfully developed to model the bacterial growth rate using one or two environmental factors (Ratkowsky et al., 1991; McMeekin et al., 1993). The lab generated data from studies on the effect of cultural parameters on microbial growth rate at different pH, Aw, temperature and preservative concentrations was used to develop several types of mathematical models. Computers are used for rapid analysis of this multi-factorial data. The traditional viable count method is still dominating the modeling studies. This method is standard where other methods need validation. Though it is laborious and time consuming it can be done with good lab practice, sensitivity, accuracy, precision, reproducibility and repeatability.

### **Sigmoidal model**

A kinetic based statistical model which is capable of indicating the growth rate for both lag and exponential phases is discussed below. The growth curves were statistically fitted by using non linear regression analysis in conjunction with Gompertz functions. The results were then analyzed to develop this sigmoidal model. This model was introduced by Gibson et al. (1987). This model has been recognized and also developed by United States Department of Agriculture for predicting the microbial growth in food environments containing many control parameters (Ray 2004). The expression of this asymmetric sigmoidal model is:

$$N = A + C \times e^{-e^{-B(t-M)}} \quad (4)$$

where,

A =  $\log_{10}$  CFU per ml at initial time (asymptotic count)  
N =  $\log_{10}$  CFU per ml at time 't'  
C = the difference in value of the upper and lower asymptote  
M = time at which maximum growth rate occurs;  
B = relative growth rate at time M

This model gives us the lag time, the maximum growth rate constant, and the maximum microbial load directly from non linear regression between the growth numbers and time data.

### ***Polynomial and multiple linear regression models***

Polynomial models are most common models in predictive microbiology especially for modeling the survival of microorganisms against the environmental conditions. Pond et al. (2001) developed four polynomial models to describe the survival of *E.coli* O157:H7 by using published data on inactivation of *E.coli* O157:H7 in uncooked fermented salami. Over prediction of reduction of *E.coli* O157:H7 was given by these models which is fail dangerous. They concluded that modeling can be a useful tool in assessing manufacturing practices for uncooked fermented sausage processes. However they failed to include all relevant variables such as drying temperature and relative humidity.

Tsujihata et al. (1998) successfully developed a quadratic polynomial model to predict the bactericidal effect of processed vinegar on *E.coli* O157:H7. Polynomial models are considered simple and relatively easy to fit to experimental data by multiple linear regression, which is available in most statistical packages (Ross and Dalgaard, 2004). The application software packages identified as the "Pathogen Modeling Program" and "Food Micro Model" rely primarily on the use of polynomial models (Buchanan, 1993; McClure et al., 1994a). Logistic regression is comparatively good against linear polynomial regression for modeling the percentage data that are "bounded" and may be considered as rescaled probability values (Zhao et al., 2001). Lebert et al., (2000) developed polynomial model that satisfactorily predicted the growth of *Pseudomonas* spp. in meat.

Polynomial models allow virtually any input variable and its interaction to be taken into account. It is easy to use the estimated coefficients when the model is included in application software. However, polynomial models also have some limitations. For many coefficients there is no biological interpretation. This makes difficulty in comparing the polynomial model with other secondary predictive models. Baranyi et al. (1996) recommended that the interpolation region of a polynomial model is within the minimum convex polyhedron (MCP) defined by the ranges of environmental conditions used for developing the model.

### ***E.coli O157:H7 survival models in salami industry***

Tomicka et al. (1997) explored the survival of *E.coli* O157:H7 in a model representing fermented salami production. They determined the combined effect of starter culture ( $10^7$  CFU of lactic acid bacteria per ml), dextrose (0.8%), sodium chloride (2%), nitrite (200 ppm), plus temperature (37 and 22° C) and concluded that the lower temperature and longer fermentation time ("European style") was better for elimination of *E.coli* O157:H7 from a model system than fermentation at high temperature and short time ("American style"). The model assisted in choosing the optimal levels of ingredients and environment factors in processed meat manufacturing.

## ***Effect of full and partial experimental data on the models***

McClure et al. (1994a) demonstrated that there is a potential for combining data sets from different groups and extending the useful range of resulting models in order to generalize the model for all kinds of variables and all kinds of food. Bratchell et al. (1989) attempted to determine the result of systematic removal of data from a model and its consequences. Their study illustrated the consequences of using insufficient data and demonstrated the risk of the erroneous model. A model with insufficient sampling will become less important with increasing use of automated sampling. Also, from this study, we could understand that the robustness of a model against the outliers, other unusual observations and the scarcity of data is affected due to reduction of data for developing the model.

## ***Model categories***

Primary vs. Secondary: Whiting and Buchanan (1993) have classified the models into primary and secondary types. The models which describe the response of microorganisms with change in a small set of parameters, for example, temperature over time are called primary models. Whereas, the models which describe the effect of environmental conditions, say, physical, chemical, and biotic features, like storage atmosphere, water activity, pH, food preservatives and additives on the values of the parameters of primary models are called as secondary models. The various primary and secondary models are detailed by McKellar and Lu (2004).

Growth vs. Survival: Growth models and survival models are respectively describing the growth and inactivation of microorganism during the process.

Descriptive vs. Explanatory: Descriptive models are data driven. They are observational, empirical, “block box” or inductive approaches such as polynomial functions, artificial neural nets, and principal component analysis. As these models cannot be extrapolated beyond the data used to build them, true predictions are difficult to make. In spite of that, these have been widely used with considerable success in predictive microbiology. Explanatory models are mechanistic; ‘white box’; or deductive models which will relate the given data with fundamental scientific principles, or at least to measurable physiological processes. These are composed of analytical and numerical models.

Kinetic vs. Probability models: Kinetic models predict the extent or rate of growth whereas probability models are the ones that can recognize and account the uncertainty or variability in experimental system i.e. predict the likelihood of say spore germination or toxin formation in a given period of time.

Tertiary models are the models incorporated with environmental values of interest continuously entered into the secondary models to obtain the values of the predicted variable. Application software, risk assessment simulations and expert or decision support systems are the main examples of tertiary model systems. They make the modeling technology and data bases readily available for application in industries. This paves way for deciding the type of new data collection in such a way that they could be merged with the existing data bases.

The rules (parsimony, parameter estimation properties, and range of variables, stochastic assumption, and interpretability of parameters) for model selection were formulated by Ratkowsky (1993). Selection of an appropriate experimental design consistent with the purpose of the study should be an important pre-requisite. Despite the researchers have been targeting

towards mechanistic models, No model is mechanistic in predictive microbiology so far. Predictions can be done by interpolation only. The interpolation region only defines the applicability of model. Interpolation region is affected not only by the range of individual variables but also by the experimental design (Ross et al., 2000)

Moving from an empirical or phenomenological description to a mechanistic or deterministic description of a process indicates advancement in 'good science' hierarchy. The former stochastic models give just mathematical relationship whereas the latter have theoretical basis i.e., interpreting the observed biological response on the basis of underlying theory. None of the secondary models is truly mechanistic. So Arrhenius type dependence models have a greater mechanistic basis than the square root or Ratkowsky type models. But Belehradek(1930) did not support use of these because the Arrhenius models are based on chemical kinetics which cannot be used for biological reactions. The recently secondary models like square root type or Ratkowsky type (1982) were developed based on Belehradek type.

## **Use of Artificial Neural Networks for Predictive Modeling**

The use of artificial neural networks (ANN) in predictive modeling remains limited. ANNs are a data driven, black box approach to predictive modeling in contrast to other secondary models that can be written as an equation with coefficients and parameters. Neural network models are empirical ones and many methodological issues remain to be resolved. ANNs are robust and are able to handle high biological variability and non-linear data.

ANNs have been used to generate complex statistical models using predictor and response variables. The ANNs learn and remember the underlying implicitly non linear relationship between the input and output variables following 'back propagation' techniques. Secondary models have also been developed using ANNs for estimating microbial growth rates (Garcia-Gimeno et al., 2003; Geeraerd et al., 1998a; Jeyamkondan et al., 2001; Lou and Nakai, 2001; Najjar et al., 1997), under fluctuating environmental conditions (Cheroutre-Vialette and Lebert, 2000; Geeraerd et al., 1998a), and have also been used to predict microbial inactivation (Geeraerd et al., 1998b). ANNs have been suggested as an alternative to polynomial regression modeling techniques (Tu, 1996).

Lou and Nakai (2001) developed an ANN model for predicting the thermal inactivation of *E.coli* due to combined effect of temperature, pH and water activity and compared with other two models using root-mean-squares-error and  $R^2$ . They showed ANN's prediction performance 0.144, 0.949 was superior than that of response surface methodology model 0.232, 0.868 and Cerf's model 0.234, 0.815 (Cerf et al., 2006).

Cheroutre-Vialette and Lebert (2002) have developed a dynamic model based on recurrent neural networks (RNN) and concluded that the complex effects of environmental variable conditions on microorganism behavior can be represented by this kind of model.

Two neural networks were developed by Mittal and Zhang (2002) to predict thermal process evaluation parameters like  $g$  (retort temperature – temperature of food at slow heating location) and found that ANN models closely followed the observed values.

Hajmeer and Basheer (2003) developed a hybrid model by integrating ANN and Bayes' statistical theorem for computing the probabilities of bacterial growth for probabilistic modeling of

the bacterial growth / no growth interface. It has outperformed the other approaches in its accuracy as well as flexibility to extract the implicit interrelationships between the various parameters.

Yu et al. (2006) developed and compared an ANN model of a three-layer back-propagation neural network trained using the survival and growth interfacing data-set and its model of McKellar et al. (2002). ANN's accuracy was more than 99 % for training data; 90% on classification accuracy for additional literature data-sets used for validation; and 100 % on all observed growth. ANN model has been recommended as an alternative tool for classification of survival and growth conditions in predictive microbiology.

The effect of temperature, pH and NaCl on the heat resistance of *Bacillus stearothermophilus* spores was described using low-complexity, black box models based on ANN by Esnoz et al. (2006). Published data were used to build and train the neural network. The ANN models gave better predictions than the classical quadratic response surface model in all the experiments tried. Good predictions were also obtained when the neural networks were evaluated using new experimental data, providing fail-safe predictions of *D* values in all cases.

### **Comparing ANN model with Statistical model**

By using published data, Jeyamkondan et al. (2001) used Generalized Regression Neural Networks (GRNN) to develop model for predicting the lag phase period and generation time of *Brochothrix thermosphacta*, *Aeromonas hydrophila*, and *Shigella flexneri*. GRNN perform regression rather than classification tasks (Specht, 1991). The GRNN copies the training cases into the network for estimating the response on new points (Statsoft, 2006). They compared the predictions of GRNN and of the published statistical models with the observed data. Six statistical indices, namely graphical plot, mean relative percentage residual (MRPR), bias factor, mean absolute relative residual (MARR), accuracy factor, and root mean square residual (RMSR) were used for model evaluation. GRNN predictions were found to be far superior to statistical modeling for the training data set, whereas they were similar or slightly worse than statistical models for test data (Jeyamkondan et al. 2001).

Several secondary ANN models have been developed so far for *Aeromonas hydrophila*, *Brochothrix thermosphacta*, *Shigella flexneri*, *E.coli*, *Listeria monocytogenes*, and lactic acid bacteria (Jeyamkondan et al. 2001; Garcia-Gimeno et al., 2003; Cheroutre-Vialette and Lebert 2000; and Lou and Nakai 2001). They were compared with polynomial, square root type models and reported that ANN's may provide slightly improved predictions. Now a days, the development of ANN model has become relatively easy with the use of available commercial neural network software. But it cannot provide classical secondary models (equation with coefficients and parameters), which is essential to be incorporated with user friendly application software in industry, teaching and research. This issue has to be sorted out by the future researchers of predictive microbiology (Ross et al., 2004).

### **Advantages and disadvantages of ANN models**

Tu (1996) compared the advantages and disadvantages of ANN approach with those of statistical regression modeling.

### Advantages:

- i. Require less formal statistical training to develop
- ii. Detect implicitly the non linear relationship between predictor and response variable
- iii. Detect the possible interactions between predictor variable
- iv. Empirical and many methodological issues remain to be resolved.
- v. Possibility of adding the influence of new parameter with existing model by simply adding more neurons or layers (Geeraerd et al., 1998a) exists.

### Disadvantages:

- i. black box approach and limited ability to specifically identify casual relationship
- ii. Require greater computational resources.
- iii. prone to over fitting.

## Limitations

Some researchers contend that because predictive models are often developed based on the data generated from well controlled laboratory conditions there are possibilities for the model to fail in predicting the behavior of target organisms in real food and environments which represent actual situations that occur during production, processing, and storage. Brocklehurst (2004) directs our attention towards the effects of food structure, including emulsions and surfaces which may significantly affect microbial behavior.

The predictive power of a model will always be constrained by the complexities of interactions of food microbes. Most of the models are developed based on only one targeted microorganism or at most a few strains in a homogeneous broth. But a variety of heterogeneous strains of the same organism may be present in the food and behave differently (Barbosa et al. 1994).

A model could be satisfactory only if it overestimates the observed growth and the models over predicting the generation time or under estimating the growth rate are always fail dangerous (Ratkowsky 2004). The model should be used to perform only for within the applicable boundary conditions beyond which the prediction should not be made. Ross (1999) characterized the performance and limits of models under the headings of Model Applicability and Model Accuracy.

## Industrial Applications

Presently, the predictive modeling technique is not at all being used by the meat industry. Even though, they may be willing to use the one or two predictive models so far developed, there is no simple and easy way for the meat industry to incorporate these models into their online processing systems. An ANN model with the data informatics of salami processing variables would be very useful in 'online processing' for choosing the best combination of environmental variables for salami processing or predicting the residual population of *E.coli O157:H7* under any environmental conditions within a minute by a single person. Little time, labour or laboratory equipment is needed to complete the ANN analysis. The plate count requirement is eliminated.

The growth and inactivation of microorganisms during fluctuating temperature condition was modeled by combining the heat transfer theories (Amezquita et al. 2005). Devices that monitor environmental conditions, a tertiary model (spreadsheet program) that converts the temperature history into estimates of microbial growth, a decision support expert system includes software packages: bioinformatics data bases and biomathematics models, set up together in the food industry will support the on line processing systems (McKellar and Lu 2004).

## Conclusion

Predictive microbial modeling could be widely used as a food safety management tool in the area of food microbiology. International level integration is needed to accumulate all the experimental data to create 'data informatics' and to develop generalized model for all microorganisms under all kind of environmental conditions. Future researches should focus on bridging the gap in estimating the microbial growth in between lab-controlled and real industrial environments. Heat transfer models could be coupled with predictive models in estimating the dynamic growth during fluctuating temperatures.

While looking at the industrial application part, tertiary models, applications software, expert systems should be given attention to make this technology readily available to provide solutions to industrial problems. Potential users of this concept should adopt predictive modeling as a food safety management tool in the short term (McMeekin 2004). A suitable model should be developed using numerical techniques for modeling the lag phase of bacteria.

Moving from an empirical or phenomenological description to a mechanistic or deterministic description of a process indicates advancement in good science development. This is still an ideal goal and needs to be realized in the forthcoming researches.

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