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Status of Microbial Modeling in Food Process Models

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ABSTRACT: Food process models are typically aimed at improving process design or operation by optimizing some physical or chemical outcome, such as maximizing processing yield, minimizing energy usage, or maximizing nutrient retention. However, in seeking to achieve these objectives, one of the critical constraints is usually microbiological. For example, growth of pathogens or spoilage organisms must be held below a certain level, or pathogen reduction for a kill step must achieve a certain target. Therefore, mathematical models for microbial populations subjected to food processing operations are essential elements of the broader field of food process modeling. However, the complexity of the underlying biological phenomena presents special challenges in formulating, validating, and applying microbial models to real-world applications. In that context, the narrow purpose of this article is to (1) outline the general terminology and constructs of microbial models, (2) evaluate the state of knowledge/state of the art in application of these models, and (3) offer observations about current limitations and future opportunities in the area of predictive microbiology for food process modeling.

Introduction

The previous 2 articles described the status of food process modeling from 1st-principle and observational perspectives. If the complexity of a given system or process is currently beyond the reach of 1st-principle models, various observational modeling techniques can be applied. Nevertheless, in the case of purely data-driven, observational models, the relationships between input and output parameters are still governed by fundamental laws of physics and chemistry, even if the model form does not directly reflect those laws.

However, when trying to model a biological system, the complexity can be orders of magnitude greater than for a purely physical system, reflecting a diverse set of cellular processes. This implies special challenges in developing knowledge, model forms, and application tools, with respect to extendibility, robustness, and validity across the domains of interest.

Given those special challenges, it is necessary to recognize the importance and unique role that microbial models play in food process modeling. The ultimate purpose of most process models (such as heat and mass transport) is to optimize system performance. In those cases, the objective function reflects a physical or chemical outcome such as maximizing process yield, minimizing energy usage, or maximizing nutrient retention. In contrast, the microbial outcome is typically a constraint in the system/process model; for example, a processor must ensure that microbial growth does not exceed a certain level or that pathogen reduction achieves a certain target.

Those constraints might derive from government regulations or internal specifications for a given food product. However, given that the target of interest is often a pathogenic organism, it is typically impractical or impossible to directly and experimentally verify that a given commercial process is achieving the target, because pathogens cannot be introduced into commercial operations. Therefore, mathematical models for microbial growth, survival, and inactivation are essential elements in food process models, and understanding the impact of model form, the domain of validity, and the underlying uncertainty is critical to proper use of such models.

A comprehensive review of previous work in predictive microbiology for food processing is beyond the scope of this article. For that degree of depth, the reader is directed to 3 different books in this field. McMeekin and others (1993) cover the wide range of model forms in predictive microbiology, with particular attention to comparing Bělehrádek-type (power-law) and Arrhenius-type secondary models. McKellar and Lu (2004a) is a more recent coverage of the field, extended to introduce developments in software/databases and risk assessment. Finally, Peleg (2006) provides a thorough thesis aimed at challenging many of the traditional practices in the field and proposing alternative approaches to microbial modeling. All 3 books are valuable contributions, and the reader is encouraged to explore them accordingly.

Given those previous, in-depth summaries and analyses of the field, the narrow purpose of this article is to assess the current status of predictive microbiology, *with respect to applications to food process modeling*. Specifically, this article will (1) outline the

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general terminology and constructs of microbial models, (2) evaluate the state of practice in applications of these models, and (3) offer observations about current limitations and future opportunities in the area of predictive microbiology for food process modeling.

What Are Microbial Models?

For the purposes of this article, microbial models are mathematical expressions that describe the number of microorganisms in a given food product or system, as a function of relevant intrinsic or extrinsic variables, generally on a macroscopic scale. As such, most microbial models quantify populations of organisms, or probabilities of the presence of organisms, but rarely model (yet) the behavior or cellular level functions of single organisms.

Microbial models can be classified as primary, secondary, or tertiary (Whiting 1995). Primary models describe how the number of microorganisms in a population changes with time under specific conditions. Secondary models relate the primary model parameters to environmental or intrinsic variables such as temperature or pH. Tertiary models combine primary and secondary models with a computer interface, providing a complete prediction tool. Most of this article is organized around this classification scheme; however, the section "What Are the Key Limitations" will address how separating primary and secondary model fitting can impact the error and uncertainty for microbial models.

A 2nd layer of classification can be applied across microbial models. Peleg (2006) noted that quantitative models can be classified as either empirical, phenomenological, fundamental, probabilistic, or population dynamic models. Selection of model type depends highly on the quality of knowledge about the system and the quality and quantity of data available from that system. Currently, insufficient knowledge and data are available to implement truly fundamental/mechanistic models for microbial growth or inactivation, so most of the models being used are either purely empirical or some type of phenomenological model.

Finally, microbial models can be classified as growth, inactivation, survival, or combined models. Growth models describe an increase in population over time. Inactivation models describe a decrease over time, resulting from the application of some lethal treatment (such as heat or radiation). Although the term "survival" is often used interchangeably with "inactivation," some survival models describe a decrease over time, or probability of survival over time, for the special case when the environmental conditions are neither clearly lethal nor supporting of growth (McKeller and others 2002; Yu and others 2006). A few combined models have been constructed to describe changes in a microbial population subjected to conditions that vary from the growth to inactivation ranges (Whiting and Cygnarowicz-Provost 1992; Jones and others 1994; Ross and others 2005; Corradini and Peleg 2006). Membre and others (1997) applied a purely empirical model to describe growth, survival, and death of *Listeria monocytogenes* at low temperatures with high concentrations of phenol and NaCl. For combined models, if separate semimechanistic or phenomenological modeling forms are applied for each physiological state, then this type of model requires special consideration to avoid discontinuities at the interface between growth, survival, and inactivation. Overall, getting 1 mathematical function to describe the complete range of microbial responses is a significant task that is probably not necessary for most food process models, which typically are designed to simulate a single unit operation that is affecting growth or survival, but not both. Additionally, in the case of modeling growth for spoilage or pathogenic organisms, if the population reaches the mortality phase, then the product has likely spoiled beyond utility, and the decreasing population of viable organisms is not relevant.

What Is the State of Knowledge/State of the Art?

Primary models

Growth models. Microbial growth is generally assumed to follow a pattern consisting of a lag phase, an exponential growth phase, a stationary phase, and a death phase (Figure 1). Nearly all models for microbial growth ignore the death phase and are based on the assumption of a sigmoidal growth function. The various forms of the sigmoidal functions are a mixture of empirical and semimechanistic equations (McKellar and Lu 2004b). The simplest form deconstructs the sigmoidal response into a 3-part (or 2-part, if ignoring the stationary phase) linear function (Buchanan and others 1997). Zwietering and others (1990) reparameterized 5 different forms of sigmoidal curves in order to relate the model parameters to biologically meaningful terms (that is, lag period, maximum specific growth rate, and asymptotic value). However, many published studies report growth model parameters that do not necessarily have this direct linkage to the biological phenomenon.

The most frequently used primary growth models are the modified Gompertz and Baranyi (Baranyi and others 1993) equations, the first being a sigmoidal relationship and the second being based in part on the concept that the rate of bacterial growth is controlled by the rate of a "bottleneck" biochemical reaction. Several researchers have compared the performance of these 2 models plus some other models applied to different microorganisms (Buchanan and others 1997; Juneja and others 1999; Baty and Delignette-Muller 2004). For example, Baty and Delignette-Muller (2004) found that the Gompertz model seems to be influenced more by the quality of the data set than is the Baranyi model. They also concluded that the Baranyi model provided the best fit for the majority of their data and gave reasonably precise estimates of the lag time. Another study also found that the Gompertz equation can overestimate the model parameters, which could bias the comparison with a different model (Membre and others 2004). In comparing the performance of 4 different growth models when fitting a *L. monocytogenes* data set, McKellar and Lu (2004b) showed that the nonlinear regression procedure had as much, or more, influence on the goodness of fit as did the model form. They illustrated an extremely important point—that the data, model form, and fitting procedures all influence the quality of any primary model for a given application.

Inactivation models. The relationship between microbial populations and time, when subjected to a lethal treatment (assuming a constant temperature or concentration of the lethal agent), has been reported to follow a variety of patterns (Figure 2). It is presumed here that these inactivation patterns are due to biological responses and do not reflect experimental artifacts, even though the latter is clearly a possibility in many studies. Some

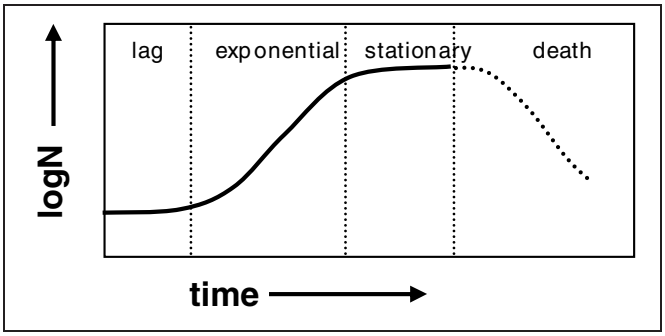

Figure 1—The sigmoidal (plus death) pattern of a typical microbial growth curve.

inactivation data exhibit (A) a tailing pattern, (B) an increase in population, due to spore activation, before inactivation, (C) a shoulder or lag prior to inactivation, or (D) a sigmoidal pattern with both a lag and a tail. However, the vast majority of inactivation data that are published are presumed to follow a log-linear pattern with time, and nearly all inactivation modeling *applications* use the log-linear form. The appropriateness of any particular model form is a question clearly without a consensus answer in the field of predictive microbiology, and there is no particular reason to assume that one model form would be universally valid for all organisms, substrates, and processes.

Among the choices, the log-linear models are derived from a mathematical analogy to 1st-order reaction kinetics, such that:

$$dN/dt = -kN \quad (1)$$

and therefore

$$\ln(N/N_0) = -kt \quad (2)$$

where N is the number of surviving bacteria, k is the inactivation rate constant, and t is time. From this log-linear relationship, the commonly used D-value (time required to achieve a 1-log reduction in the population) can be computed as $\ln(10)/k$. This log-linear relationship has been used since the beginning of predictive microbiology, when the focus was on inactivation of *Clostridium botulinum* spores in low-acid canned foods. In nearly all food microbiology textbooks, it is the only inactivation model that is described.

However, in recent years, significant evidence and work have addressed observed nonlinearities in inactivation data. There are nearly as many alternative model forms as there are publications addressing this subject. Some are purely empirical, while others seek to advance a mechanistic or phenomenological explanation.

Among those, perhaps the most prominent has been the use of the Weibull model, as proposed by Peleg (2006). An underlying premise is that every cell in a microbial population has its own resistance to the lethal agent, and that resistance can be expressed as the time of exposure until that cell is no longer viable. The Weibull population function is then one choice for describing the distribution of resistance within a population. The cumulative distribution of the Weibull function can be expressed in a variety of forms (Peleg 2006), including:

$$\log\left(\frac{N_t}{N_0}\right) = -bt^n \quad (3)$$

where b is a nonlinear rate parameter, and n is a shape factor. When $n < 1$, this function appears as a concave, upward, semilogarithmic inactivation curve (Figure 2A). When $n > 1$, it appears as a concave downward curve (Figure 2C). When $n = 1$, the Weibull-based model reduces to a log-linear model consistent with the previously described 1st-order kinetic. Consequently, the Weibull model is capable of describing a wider range of inactivation phenomena, where the log-linear result is just a special case. In applying the Weibull model to 55 different inactivation data sets, van Boekel (2002) showed $n > 1$ for 39 cases, $n < 1$ for 14 cases, and $n = 1$ for only 1 data set.

Given such compelling evidence that microbial survival curves are rarely log-linear (and that the analogy to chemical kinetics is, at best, imperfect), one might wonder why use of the simple log-linear model has persisted for nearly a century in food microbiology (and remains the dominant model used in food microbiology publications). Certainly, there are a variety of reasons. Traditional, while not a compelling logical argument, is no less a strong force in the scientific community than in society at large. As generations of microbiologists were trained using these techniques, and subsequently wrote the monographs, textbooks, and government regulations in this field, perpetuation of log-linear models is not a surprising outcome.

However, there are other, more pragmatic reasons. Certainly, the computational simplicity of the log-linear model, requiring only linear regression of survivor data, was important in earlier days, but is a less compelling justification today, given the availability of software and computing power for nonlinear regression (even of large data sets). However, the log-linear model is a single parameter model, whereas the Weibull (or other alternative models) require 2 or more parameters to be estimated. As trivial as that might seem at first glance, models with more parameters inherently require more data in order to achieve satisfactory parameter estimates via nonlinear regression of the survivor data, and quality microbial inactivation data are neither trivial nor inexpensive to generate.

Lastly, if we accept that most microbial inactivation curves are truly nonlinear, it can be argued that this could have a significant negative impact on product safety, particularly if tailing exists and is ignored by using and extrapolating log-linear models. We might wonder, then, why the practitioners and users of microbial models, and regulatory agencies, have not rushed to revise the paradigm for modeling microbial food safety. The answer, perhaps, is that compelling *evidence* has not yet been presented that using the "erroneous" log-linear model form is significantly impacting product safety or quality. This is not meant to be an argument for the log-linear assumption, but only an assessment for why its use has persisted in spite of significant scientific evidence to the contrary.

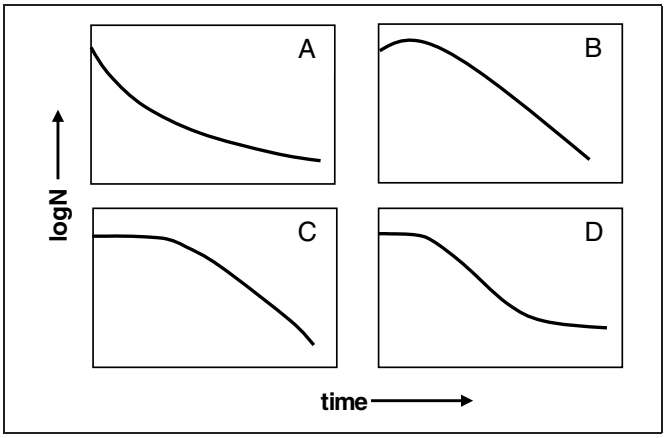

Figure 2—Various published forms of microbial inactivation functions, describing (A) tailing, (B) spore activation and inactivation, (C) a shoulder/lag, and (D) a sigmoidal response.

Secondary models

There is significant information about the impact of individual variables (particularly temperature) on the growth and inactivation of bacteria. For example, several reviews have summarized significant heat resistance literature for various pathogens, as influenced by food material, temperature, pH, and so on (Doyle and Mazzotta 2000; Doyle and others 2001). Unfortunately, much of that information is specific to a certain product, organism, and methodology, and is not integrated into validated secondary models that can be used to predict future outcomes.

Ideally, the fundamental mechanisms relating to the treatment variables and the microbial response would be known, and an appropriate secondary model form could be adopted. However, in reality, little is known about most of these responses, so there is a wide variety of secondary models that are used in both growth and inactivation models.

Along with the traditional, log-linear primary inactivation model, many published studies utilize an Arrhenius-type model, so that ln(k) is linearly related to the inverse absolute temperature. McMeekin and others (1993) extensively compared the Arrhenius-type model to Bělehrádek-type (power-law) models. The Arrhenius-type model, again deriving from an analogy to chemical kinetics, has several flaws, including the implicit property of an "activation energy" of inactivation that is not a function of temperature.

Peleg (2006) argues instead for a log-logistic model form. While the log-logistic secondary model is not mechanistic, it does have the advantage of exhibiting properties that are more consistent with the observed biological phenomenon. When applied to an inactivation rate constant, this includes predicting a zero value at temperatures below the lethal range, and an increase in the inactivation rate that is somewhat linear with temperature.

For many growth models, such as those used in the USDA Pathogen Modeling Program (USDA 2003), the secondary models for lag phase duration and maximum growth rate are purely empirical response surface models. Baty and Delignette-Muller (2004) compared a variety of models for growth lag phase duration and reported that differences among formulations (including those derived from different biological explanations) resulted in estimates that were quite close, and that the uncertainty of the estimates was typically larger than the differences among estimates. Also, as expected, the quality of the original data set is particularly important in generating the estimates. In any case, great caution should be exercised to avoid extrapolation when using purely empirical secondary models; in particular, response surface models can yield nonsensical results if applied outside the domain of the data from which the parameters were estimated.

In the area of inactivation, several researchers have reported D- and z-values for various microorganisms in different types of meat (such as Veeramuthu and others 1998; Juneja 2000; Juneja and others 2001, 2003; Smith and others 2001; Murphy and others 2003, 2004). However, for the same type of meat and microorganism, there are notable differences in the estimated parameters. Among these studies, the D-values for *Salmonella* in turkey meat at 60 °C ranged from 4.6 to 13.2 min, given different serovars, fat contents, heating methods, and recovery methods. This illustrates how the variation in methodologies has influenced the body of knowledge in this arena, and ultimately influences the secondary model parameters.

Tertiary models (tools)

If a tertiary model is defined as the integration of a primary and secondary models with a user-friendly interface (Whiting 1995), then there are very few true tertiary models available to the end users who need these tools. Two tertiary modeling tools are freely available and widely used in the United States—the AMI Process Lethality Spreadsheet (AMI-PLS; www.amif.org), available from the American Meat Inst. (AMI 2002), and the Pathogen Modeling Program (PMP, v.7.0) developed by the USDA—Agricultural Research Service (USDA 2003). Additionally, the latest version of ComBase (www.combase.cc) now includes a modeling tool that utilizes its database to generate growth or inactivation curves (Baranyi and Tamplin 2004).

The AMI-PLS spreadsheet uses log-linear kinetics to calculate the number of surviving organisms, given transient product temperature data supplied by the user. However, this tool does not account for any product effects, and relies on the user to supply thermal resistance data (which is particularly problematic for small or very small processors) or to use the "default" values without consideration of their validity for the user's specific product case. Therefore, although this tool technically implements primary (D-value) and secondary (z-value) models, it requires user-supplied model parameters. As such, it is really just a calculator designed for a targeted user group (processors of ready-to-eat meat and poultry products). Nevertheless, it is broadly used within this industry, because it is (1) freely downloadable from the web, (2) relatively simple to use, (3) meets a specific industry need, and (4) is recognized and utilized by regulatory personnel.

In contrast, the PMP is an example of a tertiary model that includes greater functionality, calculating pathogen growth, survival, or inactivation as a function of temperature, pH, sodium chloride, and sodium pyrophosphate concentration, with a very straightforward user interface. However, one problem is that almost all of the models within the program were parameterized using broth-based data, so that applicability to a specific food system is not assured. The growth models are modified Gompertz equations, and the inactivation models are log-linear. The secondary models are 2nd-order response surface equations. Given 4 independent environmental variables, this results in single secondary models with as many as 15 parameters. In the PMP, the Gompertz equations are implemented with a presumed (fixed) asymptotic value; therefore, with secondary models for each of the 2 remaining Gompertz parameters, that results in 30 parameters for each growth model. In some of these cases, the individual terms of the response surface models are not statistically significant; however, the models include all of the terms (Martino 2006). Although the original, broth-based data sets were large (hundreds of curves), so that 30 parameters could be realistically estimated from the data, there should be some concern of overfitting when a model includes so many parameters. Nevertheless, several studies have tested the validity of the PMP growth models against independent data in food products and have shown reasonably good predictions (Campos and others 2005; Martino and others 2005).

Additionally, the existing thermal inactivation models in the PMP do not account for nonisothermal conditions, limiting their application to real-world food processing applications. Nevertheless, the PMP is an excellent example of what is required for predictive microbial models to achieve broad acceptance and utilization: ease of acquisition (meaning a free web download) and a simple user interface.

Finally, more mathematically sophisticated, spreadsheet-based tools have been deployed via the web (www-unix.oit.umass.edu/~aew2000) by Peleg (2006). These tools utilize a Weibull primary model for inactivation and log-logistic secondary models, so they account for a broader range of inactivation behavior and can be applied to nonisothermal temperature profiles. However, there are 2 limitations to these tools being broadly utilized as tertiary models. First, the major limitation is that the user must supply a full set of model parameters for the Weibull/log-logistic functions, which necessitates that sufficient organism/substrate-specific tests have been previously conducted to generate estimates of those parameters for the users. Second, although relatively simple to use for an expert in the field, the tools are not "street ready," in terms of having user interfaces that make them accessible to practitioners in the field (such as typical food processors or government inspectors). However, they are still an important step in making these models available in a useable form.

Ultimately, the "correctness" of a tertiary model is not going to be the determining factor affecting its utilization and impact. A tertiary model will have real impact on the food safety system only when it is readily available, meets a specific industry need, is easy

to use by the relevant practitioners, relates product characteristics to microbial outcomes, and is validated for the case of interest.

Risk models

Risk modeling is a subject that requires a much broader discussion than is possible here. However, it is important to recognize that microbial models are a critical piece of risk models, but not the only piece. A risk model encompassing the entire food system for a given pathogen or food product must also include process/systems models for the critical operations within the process, and often "expert knowledge/estimates" are utilized in filling in knowledge/data gaps in the system model. In that context, van Gerwen and Zwietering (1998) described the importance of comparing process variation against microbial model variation when determining which model to use; given the complexity of most food systems, it is desirable to use the simplest model possible. Ross and McMeekin (2003) also noted that the underlying uncertainty in microbial growth models (and assumptions made in the applications of those models) can translate into large errors in risk estimates; therefore, it appears that highly reliable/accurate/precise estimates of microbial risk are not yet feasible, and risk models are still relatively qualitative tools for ranking risks and making broad policy or regulatory decisions. To date, the USDA and FDA have, to varying degrees, completed risk assessments for *L. monocytogenes*, *Salmonella*, and *Clostridium perfringens* in ready-to-eat meat and poultry products, and provided extensive documentation of the assumptions, sensitivities, and conclusions of the risk assessments.

What Are the Key Limitations?

Clearly, utilization of microbial models in food process modeling is limited by a variety of factors. This section attempts to outline those factors in terms of the data domain, the experimental methodologies, model uncertainty, and the degree to which state-of-the-art microbial modeling techniques are (or are not) currently being integrated into food process models.

Data domain

Microbial model parameters are rarely estimated using a data set that covers the entire domain of interest, and are even more rarely validated using *independent* data across the domain of interest (which is why using phenomenological or semimechanistic models is preferred, as they behave better when interpolated and extrapolated). Broth-based models are widely used and then applied to real food systems; however, assumptions that broth-based growth models are conservative, because of the ideal growth conditions, might not be valid (Tamplin and others 2005). For inactivation, it is widely known that bacterial pathogens tend to be more resistant to heat in real food products than in broth-based media; however, there are relatively limited data available from tests conducted in actual food products. Additionally, more complex models mean more parameters, therefore requiring more data. However, although microbial growth and inactivation data are being published regularly, the global data set will always fall short of covering the entire domain of interest for the broad food safety system. Quite simply, generating microbial response data in food systems is nontrivial and costly research.

ComBase (Baranyi and Tamplin 2004) is a significant and important initiative that is compiling thousands of data records for microbial responses in food systems. ComBase is making available, for the first time, a unified assemblage of diverse data, which will enable an entirely new line of investigations. As the database expands, researchers will be able to conduct systematic, comparative analyses of the available data for a given organism or substrate, and evaluate the quality and scope of those data. However, the power of the database is, of course, limited by the quality of the original data and information published in the studies generating those data.

Methodologies

One of the largest problems affecting the quality and utility of microbial response data and models is the lack of standard methods for conducting a given type of study. Standard methods exist for various laboratory methods, such as sampling and enumerating various types of bacteria. However, there are not standard protocols for conducting, for example, thermal inactivation studies for *Salmonella* in meat or poultry products. Therefore, given the challenge of limited data noted above, the problem is compounded by the fact that data generated for a given organism in a given substrate across 2 studies (from 2 different research groups) are likely to have been generated using different treatment protocols (and probably different serovars or cocktails of serovars of the organism of interest). Additionally, many published studies fail to report some of the critical experimental parameters, such as the minimum limit of detection, which can have a major impact on the interpretation of model fitting and validation.

Similarly, the statistical methodologies vary widely from study to study. Logarithmic transformation of microbial counts before fitting models can result in important differences in parameter estimates, when compared to models fit via nonlinear regression of the model against the untransformed data. Also, the nonlinear regression procedures, and initial estimates, can significantly affect the parameter estimates and goodness of fit of a model. Similarly, the criteria by which a given model or model terms are accepted can impact the robustness of the model, particularly if the model is overfitted to a given data set.

Additionally, if the primary model regression and the secondary model regressions are conducted sequentially (a 2-step regression), important contributions to overall model uncertainty can be ignored, and the overall model error can increase. Martino and Marks (2007) showed that a 1-step, global regression (including the secondary models in the primary model and conducting a single regression) reduced the model error by 15%-20% when compared to a 2-step regression for *L. monocytogenes* growth.

Ultimately, experimental microbial response data are needed for fitting and validating microbial models. Given that the measure of interest, microbial counts, is a biological variable, repeatability and reliability of experimental measures are critical issues. Unfortunately, unlike physical measures, such as temperature and pressure, there is no simple point-sensor for measuring bacterial populations, so that measurements are still generally generated by plating the substrate and counting colonies (or some indirect measure, such as turbidity or optical density of a liquid substrate). Such measurements will always entail a relatively large variability. The long-term goal should be to move toward some standard methods for common experiments, such as growth and thermal inactivation studies. The short-term goal should be to require a standard battery of parameters to be reported with any published study in this field, and to ideally require that the microbial data be deposited in ComBase to advance the overall scientific effort in this field.

Variability and uncertainty

Many models are reported and used as if they are deterministic; however, realistically, all microbial models have some degree of underlying uncertainty. That uncertainty includes the original experimental error, uncertainty in the primary model form and regression, and uncertainty in the secondary model and fitting procedures. Often, when confidence intervals are reported for a given model, they neglect significant portions of the overall model uncertainty.

For example, the USDA PMP reports 95% confidence intervals with computed growth curves; however, these confidence intervals are computed based solely on the error in the secondary (response surface model) fit, thereby neglecting the error in the original experimental data and the error in the primary model fit, which can actually be greater than the secondary model uncertainty. In testing this specific case, for modeling the growth of *L. monocytogenes*, Martino (2006) showed that the relative contributions of the primary model regression, secondary model regression, and experimental replications to the overall model error were 1.02, 1.48, and 0.26 log(CFU/mL), respectively.

Additionally, the reported confidence intervals reflect the range in which the *mean value* is 95% likely to fall. In applications, the intervals of interest should be the much broader *prediction intervals*, which capture 95% of the *individual future outcomes*. This is necessary, if the model is to be used to relate the probability of bad outcomes to the acceptable limits. Unfortunately, a great portion of published values for microbial models fail to include any statistical measures of uncertainty, and this creates a real limitation in drawing meaningful conclusions when applying those models to real applications.

Integration into process models

All of the analyses discussed previously focused on microbial models as independent tools. In fact, most existing microbial modeling tools are "stand-alone" tools that are not integrated with process models. The user is either inputting fixed environmental variables or plugging in experimental time–temperature data. None of the previously mentioned tertiary models is integrated into process models. This is one of the great gaps in applying microbial modeling tools to broader efforts in process modeling and improvement.

With respect to integration of microbial models into process models, Lebert and Lebert (2006) presented a conceptual structure comprising 3 elements: (1) process models for heat and mass transfer, (2) predictive microbial models, and (3) the thermodynamics of material properties. In their analysis of 5 previous studies that have reported such integrated models, only two actually included all 3 elements, and both of them were applied only to model food products (that is, broth or gel systems).

In a specific application to the real-world case of cooling cooked hams, Amézquita and others (2005) integrated a growth model for *Clostridium perfringens* growth into a heat transfer model for product cooling. In this case, the predicted temperature history of the product was the only input into the microbial growth model; nevertheless, validation experiments indicated excellent agreement between predicted and observed microbial growth, with root mean squared errors $\leq$ 0.4 log(CFU/g) in 6 out of 7 of their test scenarios.

Similarly, Pradhan and others (2007) and Watkins (2004) both integrated a log-linear model for thermal inactivation of pathogens into finite element solutions for combined heat and mass transport during moist-air impingement cooking of chicken breasts and meat patties, respectively. In both cases, good agreement was reported in validations for both the physical and biological outcomes. However, in both of these studies, the thermal inactivation model was a simple log-linear model that did not account for changing substrate properties or other factors affecting instantaneous inactivation rates during the process. Generally similar approaches also have been applied to single-sided frying of beef patties (Pan and others 2000; Ou and Mittal 2007).

As part of the multinational BUGDEATH project (James and Evans 2006), Valdramidis and others (2006) went 1 step further. They started with a sigmoidal-type primary model for thermal inactivation of *L. monocytogenes* during surface pasteurization and notably tested 3 different secondary models (response surface, Arrhenius-type, and Bigelow-type) that included the effects of both temperature and water activity, which can be particularly important in processes where surface moisture conditions are dynamic.

In a very interesting recent article, Mackey and others (2006) reported on a study in which they heated a finite cylinder of agar, with *Salmonella* Typhimurium embedded in the cylinder, and subjected the cylinder to heating. They predicted and measured the temperature profile in the cylinder and predicted the aggregate inactivation (given *D* and *z* from isothermal laboratory tests). They then plated slices of the cylinder (using differential components in the medium for the surviving *Salmonella*), to visualize the "ring of survivors" in the cylinder as an experimental validation of the temporal and spatial change in microbial population. Although the substrate was a simplified model food, and the microbial model was only log-linear, the overall methodology was novel and the results quite useful in assessing the integrated model performance.

However, when researchers are focusing on developing and testing process models (incorporating elements such as heat transfer, mass transfer, viscoelastic mechanics), microbial models that get included tend to be relatively simple. Therefore, the microbial piece of the process model very rarely accounts for the effect of changing product factors (beyond temperature) or adaptive microbial populations. As such, there remains a gap between the state of the art in microbial modeling and the state of the art in process modeling that needs to be bridged in order to fully realize the potential of both modeling fields. There is a real need for research that directly links and validates both the physical and microbiological components of process models.

What Are the Future Opportunities?

A positive spin on the limitations described previously results in a list of opportunities for improved application of microbial models in food process modeling and process improvement efforts.

- While most existing models treat microbial cells as either alive or dead (a binary function), the reality is that there is a full spectrum of cell states ranging from healthy to nonviable, with the population between those extremes exhibiting characteristics of sublethal injury. There is significant opportunity to improve population-based modeling of microbial inactivation by incorporating functions that account for this degree of injury within the population. Sublethally injured cells can be significantly more resistant to subsequent lethal treatment, and may also have greater virulence (for pathogens).
- The introduction of ComBase has created a significant opportunity for improved synthesis and utilization of data sets within a given domain of interest, and for analysis of the factors affecting variability in those data sets.
- Given that there will never be sufficient experimental data to develop and validate microbial models across all the important application domains, there is great opportunity to apply advanced data synthesis and sampling methods (boot-strapping, Monte Carlo simulations, and others) in order to generate improved estimates of model parameters and the uncertainty of resulting model predictions.
- As quantitative information about cellular functions and genomics continues to explode, there will be unique opportunities to connect that field of study (focused on the molecular level) with the existing field of predictive microbiology (focused on the population level) to continue development of microbial growth and inactivation models that better reflect mechanistic understanding of the relationship between relevant variables and the cellular and population responses.

- Finally, there is immediate opportunity to bridge the gap between the knowledge domain about microbial responses to environmental variables and the knowledge domain advancing food process modeling efforts. The knowledge and tools exist on both sides of this gap, so that microbial modeling knowledge can result in more significant and direct impact in process improvement efforts. This will occur when process models incorporate more sophisticated microbial models accounting for varying effects of product properties and process dynamics.

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