Data collection and capture systems for microbial modeling

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SUMMARY

Microbial modeling experiments require an integrated and efficient design to overcome constraints on time and human resources. The choice of an experimental system is effected by first determining the goals and scope of the model to be constructed. Kinetic studies, for example, require a different approach from single end-point models, such as time to toxin detection or growth probability. Studies have been conducted in liquid broth tubes or batch culture, agar plates, and food matrices. These traditional systems are labor intensive, however, which constrains experimental size, and thus, a model's scope and validity. To maximize experimental size, experimental systems should be automated and linked to electronic data manipulation, analysis, and presentation. Microbial modelers should also consider the relationship between the experimental environmental factors, such as pH, a_w, or temperature, and their impact on growth, virulence or toxigenesis determinants. Attaining these goals will increase the probability that the model will accurately predict microbial responses in food systems.

INTRODUCTION

Data collection is the most tedious aspect of the microbial modeling process. Limits on time and resources require experimental designs that permit interpolation from incomplete factorial studies to formulate predictive models. Consequently, predictive power of the models may be reduced. To overcome this obstacle experimental techniques need to be employed, that promote efficiency. In doing so, investigators will conserve valuable human resources and increase sample numbers to those that approach the full factorial design. The result of this effort would improve the predictive power of models.

Laboratory automation and electronic data capture are key technologies which will permit laboratories performing predictive modeling to fully integrate experimental procedures and analysis. Laboratory information management systems (LIMS) have revolutionized the collection of data for analytical chemistry. Chemical analyses are now automated and nearly continuous using autosamplers on chromatographs or spectrometers. In most automated laboratories, sample preparation is the limiting step. The widespread use of laboratory robots, however, has hastened these procedures. Microbial modeling laboratories could benefit from using this approach as well. This paper will review current and potential strategies to automate microbiology laboratories performing modeling studies.

Generally, collection of modeling data today employs classical bacteriological techniques such as sampling from liquid or solid menstrua, diluting, and enumerating onto agar plates. This labor-intensive system could be analyzed and streamlined by using a systems approach to divide the problem into unit operations, and to identify those techniques which would enhance efficiencies. The steps involved in microbial modeling include: define aims, identify the data collection system, design the experiment, set up the experiment, collect data, analyze data, formulate model, validate model, and finally, use the model to predict unknowns. The key steps affecting date collection and capture are discussed below.

Defining aims

The experimental system should reflect the goals and scope of the model to be determined. There are two general types, kinetic and single end-point modeling. Each has its unique experimental system requirements. Kinetic models predict the growth or death of an organism with respect to time, i.e. a fundamental rate determination. The sampling increments are a function of the rate of growth or death. Thus, for thermal death time (D-value) studies, data must be collected rapidly (in seconds) because logarithmic population declines occur in tenths of minutes. Alternatively, only hourly readings are necessitated in kinetic modeling studies where permissive growth conditions exist. If the rate of change is rapid, precision requires continuous data acquisition. In this regard, Gibson et al. [7] discussed the relationship between data point numbrs and curve fitting quality using logistic or Gompertz equations. They found that insufficient data at particular parts of the growth curve sometimes resulted in curve-fitting program failure. This is usually not a problem for non-thermal death studies, where, for example, only a 10-fold drop in population density may occur within a month. Single end-point, in contrast to kinetic

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modeling, is used to predict a single phenomenon. Examples include modeling of lag phase duration, time required to reach a predetermined population density, or toxin production; the probability of these events occurring may be modeled as well. Data collection frequency depends on the occurrence of the phenomenon. Thus, modeling aims bear heavily on the choice of the experimental system, and sampling frequency is a crucial element to determine automation needs.

Linkage of experimental design to data collection systems

We often identify constraints on experimental design by the initial set-up time required, sampling interval frequency, and sample preparation and analysis. The competence of the analyst to analyze the vast amount of data generated by modeling experiments is frequently an afterthought, which often delays completion of the project. The true challenge, therefore, is to plan accordingly to determine the most reasonable experimental size that can be analyzed, that produces the most accurate end product, and that minimize human resources. Experimental systems which are simple to set up and automatically collect data would largely eliminate restrictions on experiment size. Futhermore, automated systems offer the advantage of permitting additional variable combinations to be investigated, which may be prohibitive for manual operations. Rather than partial factorial studies, full factorial experiments could be planned, adding statistical power and rigor.

There must be a balance sought to design the proper experimental system for the specific type of model being developed. A Most Probable Number (MPN) type system, for example, is more appropriate for a single end-point determination than a kinetic model, because the MPN most accurately indicates the presence or absence of growth. It is less accurate for population density quantification. Broth tubes or food samples subjected to various environmental conditions, such as temperature, salt level, water activity, etc., and enumerated on agar plates, conversely, are quite appropriate for population density studies. Agar plates seeded with a specific number of organisms can be subjected to different conditions, then quantified. Gradient agar plates, moreover, can be used to save considerable time and supplies.

Data collection and detection systems

At the USDA, ARS, Eastern Regional Research Center we have developed a variety of experimental systems for dealing with specific problems associated with data collection. Buchanan et al. [3] used sealed trypsinizing flasks to maintain anaerobiosis during kinetic modeling studies. Anaerobic sampling is facilitated by withdrawing samples through a rubber septum using a sterile syringe. In another example, we conducted single end-point (botulinal toxin) and kinetic oxygen-free studies in an anaerobic chamber equipped with sample processing and culturing materials [9]. Whiting and Naftulin [12] employed a fermenter to perform rigorously controlled modified atmosphere studies. Systems used by others to facilitate unique problems or to greatly improve

data acquisition efficiency include: pH and NaCl gradient agar plates, temperature gradient slabs, programmable incubators to permit predetermined temperature abuse, and use of multi-well plates for colorimetric or turbidimetric monitoring.

Where possible, automated and sensitive systems for detection of growth or other end points should be incorporated into the experimental scheme. Even where classical microbiological approaches to data collection are employed, such as enumeration on agar plates, automation and electronic capture can be employed. In our laboratory, for example, we use agar plates for most kinetic modeling experiments. We have, however, integrated modern technology to the greatest extent possible. Our modeling efforts require about 2500 Petri dishes per week. They are inoculated by seven Spiral Systems platers (Cincinnati, OH). Food samples are blended in a Stomacher bag, equipped with a nylon mesh insert. The insert is required to keep particulates out, which could foul the Spiral Plater's 26–27 gauge tubing. Since the system dilutes approximately 1000-fold as it applies samples, two 1:100 dilutions are usually sufficient for samples containing $\leq 10^9$ organisms per gram. Sensitivity is 21 CFU g^{-1}. Enumeration is automated using the Spiral Systems Laser Colony Counter. All data are maintained on a personal computer, and reports contain averages of replicate plates as well as quality assurance data pertaining to the 'countability' of the colonies. Most important is the capacity to electronically transfer the data to other information management systems, such as personal computer spreadsheets or minicomputers. This electronic 'handshake' is a critical linkage in a pursuit toward complete automation, since the captured information can be edited and manipulated without any further data entry. The Spiral System plating and counting instruments were evaluated and shown to be comparable to classical bacterial enumeration and counting [4,8].

In addition to agar plates, other detection systems have been used by others for data collection. These include: turbidimetry, biomass detection, conductance, and specific rapid methods including toxin assays. Advanced technologies which may have potential for measurement of bacterial growth or other end-points include: nuclear magnetic resonance (NMR) spectroscopy, fourier-transform infrared (FT-IR) spectroscopy, UV resonance Raman spectroscopy, and flow cytometry. Most of these techniques lack the sensitivity, however, of the more classical methods. The most advanced system available is the Bioscreen (Labsystems, Helsinki, Finland), a computer controlled dispenser-shaker-incubator-reader-analyzer for 200-well microtiter plates. The system uses optical density as the detection system, which lacks the sensitivity of plate enumeration, however. Nevertheless, Bioscreen is clearly the most integrated and labor saving system available to modelers to date.

Concern for the effect of experimental procedures on the tester strains

Bacteria exhibit remarkable survival strategies in response to adverse environments, including changes in their pheno-

type or genotype. An important challenge to microbial modelers therefore, is to consider and account for the relationship between the modeling environment and its impact on biological responses, especially alterations in those factors most likely to affect spoilage or public health, including growth, virulence or toxigenesis determinants. Therefore, investigators need to relate gene expression in a model system to foods stored under identical conditions.

There are a host of stress-related proteins which have a significant bearing on this issue. Examples of these gene products include proteins affected by temperature, pH, culture phase, osmotic pressure, and oxygen tension. Each is briefly discussed below.

Temperature. Bacterial genes respond to temperature, and conducting experiments over a temperature range can affect those gene products which influence virulence or growth potential. For example, *Yersinia enterocolitica* expresses a virulence plasmid at 25 °C that is cured when the organism is grown at 37 °C. Elevating the experimental temperature to 37 °C will yield avirulent organisms, which behave differently from those virulent organisms at the lower temperature [2].

pH. pH is important for regulation of gene expression in enteric bacteria. For example *Vibrio cholera* virulence is mediated by *toxR*, a pH sensitive gene [10]. *Salmonella* acid shock genes allow its survival in the host. Foster and Hall [6] showed that *S. typhimurium* could be induced to survive more acid conditions than expected. They found that growth of the organism at pH 5.5–6 allowed for expression of genes whose products enabled cells to retain viability when the culture was transferred to pH < 4. They were able to recover and grow the cells after adjustment to a neutral pH. In addition, Farber and Pagotto [5] demonstrated that HCl acidification increased thermal resistance of *Listeria monocytogenes.*

Culture phase. Culture phase influences resistance to external challenges. Many bacteria are more resistant in the stationary phase of the growth cycle than in the logarithmic phase. Tormo et al. [11] demonstrated the presence of a survival gene (*sur*) in *Escherichia coli* which becomes expressed in stationary phase. Gene expression confers increased survivability during prolonged starvation. While this situation is unlikely to occur in foods, the expression of the gene in other stationary culture conditions may be of significance.

Osmotically modulated proteins. Werner Goebels' group at the University of Wurzburg showed that high salt conditions inhibited a 60 000-Da (p60) protein in *L. monocytogenes.* This protein is responsible for forming septa and its repression causes the formation of long uninterrupted cells (Personal communication). Work conducted by Zaika and Kim [13] showed that phosphates have an identical effect on cells.

Anaerobiosis. Aliabadi et al. [1] showed that oxygen tension controlled the expression of virulence genes in *Salmonella.*

These examples point to the generalization that the modeling environment has a significant bearing on the growth and virulence of the common foodborne bacterial pathogens. It is critical that microbial physiology and virulence factors be accounted for in the planning stages and tested for during the conduct of modeling experiments. Rapid screening techniques for specific gene markers may be used to alert the investigator to phenotypic or genotypic changes. Commercially available biochemical test strips which are frequently used for bacterial identification can ensure that 20–30 markers remain consistent throughout the experiment. Also, simple classical virulence tests can be employed, such as stabs onto blood agar to test for retention of hemolysis. More complex analyses could be undertaken as well, such as protein profiles using SDS–PAGE or plasmid or genomic DNA profiles using agarose gel electrophoresis. While we endeavor to automate experimental systems, concurrently we need to exhibit prudence to avoid the pitfall of using these systems as 'black boxes' and run the risk of inaccurate predictions of the natural world by artificial biases of the test system.

CONCLUSION

The ideal system for collecting modeling data would encompass a non-destructive autosampler which is sensitive and selective for bacterial growth, death, and other endpoints. Data would be collected in a manner that would allow for direct analysis and model formulation. Without further file transfers, user-friendly application software would be created. Unfortunately, this is not the world we live in. At present we frequently require use of manual experimental operations, hand data recording, and sometimes incompatible computer programs. We need to be continuously striving toward the integration of our data collection and capture systems to permit design and conduct of larger experiments. Achieving this goal will facilitate more rigor and power in the models we generate.

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