

Abstract.—The problems that arise when applying predictive microbiological methods to the industrial food supply chain, include unreliable sampling, uncertain growth characteristics and other perturbations operating at different time scales, the result of uncontrollable environmental effects are discussed. Methods of dealing with these issues, including QA procedures, improved statistical techniques and model updating methods are proposed. The utility of applying variance and covariance component analysis to the industrial food supply chain to improve aspects of the management of food spoilage associated with cost benefit analysis is discussed.

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I. INTRODUCTION

The modelling of microbial population dynamics is a substantial element of predictive microbiology. The goal of such modelling is to predict the state of a future population of microbes subject to the initial population size, the substrate and the environmental conditions. There are a number of semi-empirical models, which with a proper parameterisation can provide a suitable description of microbial growth. The majority of cited below works were based on the Baranyi model [1], which has a number of structural advantages over Gompertz or logistic type models. Environmental effects are generally introduced into these models by modifying the parameters governing the population growth. Most commonly such parameters as specific growth rates or bacterial lag time are considered as known functions of environmental variables. A number of examples are given in [4]. The work of many authors has attested to the efficacy of these empirical models to describe microbial population growth quite

accurately in the laboratory. However, the industrial food supply chain introduces further aspects that require scientific attention if food spoilage is to be managed.

II. RESULTS AND DISCUSSION

The application of models based on microbial population dynamics, in the industrial situation must consider the following problems:

- Initial population sizes are unknown, and sampling protocols are generally inadequate to provide reliable estimates. The costs of adequate sampling are prohibitive.
 - Control of the environmental variables affecting microbe growth along the supply chain is limited.
 - Uncertainties in the microbial population growth characteristics may occur, perhaps due to interactions with other microbe species, seasonal characteristics etc. This causes deviations from model growth curves, which are essentially unpredictable.
 - Acceptance sampling at stages along the supply chain provide unreliable estimates of microbial population size.
- We have developed methods to address each of these problems

Determining the Initial Microbial Population Size

Typically industrial laboratories use measurements of the population size of microbes that are subject to a detection limit. A zero measurement does not necessarily mean there are no microbes present, merely that the number are below the detection limit. Using a sample measurement of zero to calculate the statistics of an initial population size introduces a bias. For example, the average population size will be underestimated. This problem has been dealt with in [5] by considering a frequency distribution truncated at the detection limit and then using maximum likelihood methods to find estimates of the population size.

The second problem is the poor sampling coverage, which is generally inadequate to estimate the microbe initial population size with suitable accuracy. The

approach taken to this problem is to assume that the packing plant microbial population is in equilibrium, so that the frequency distribution of initial microbial population size is stationary. That is, the size of the microbial population has the same frequency distribution from one period of time to the next. A gamma probability density has been found to be a suitable quantitative description of this initial population distribution in the cases we have considered, but other probability densities may also be considered.

As samples are collected at the packing plant over time they can be used to test the hypothesis that the initial microbial population size remains within the stationary distribution, using Quality Assurance (QA) procedures. A Cusum method proposed in [3] is one way of doing this. Sequential sample results are plotted on a cumulative sum chart, and if the QA statistical tests signal a rejection of the current stationary initial population size distribution, a new stationary distribution can be calculated using recent measurements. In this manner a continuous adjustment can be made to a new regime, if conditions that affect microbial load change.

Uncertain measurements of environmental variables along the supply chain. It is common practice to include measurement loggers in a meat shipment to obtain a continuous recording of environmental variables. Temperature is the environmental variable usually recorded.

However, because these loggers record temperatures at discrete times, and because the way temperature affects microbe growth is generally not known exactly, the relationship between actual changes in environmental conditions and microbial growth is uncertain. This phenomenon is known as process variance. Over a period of 60 days of shipment this uncertainty may increase to be significant. This issue has been addressed by using stochastic differential equations where the stochastic disturbance term represents the uncertainty in the relationship between temperature changes and microbial growth [7].

Changes in microbial growth characteristics Microbes on meat consist of heterogeneous interactive sub-populations. As environmental conditions change the nature of these interactions also change. For example,

the introduction of new hygiene protocols at a packing plant may trigger a change in the relative population sizes and a change in the growth dynamics. Seasonal effects on the frequency of microbial types in packing plants are well known, but difficult to predict. These effects may drive an unpredictable change in the population dynamics over time, so that the population growth models used become inaccurate.

Our approach to this problem has been based on a technique from control engineering known as updating models. When the models describing the outcome of interest are linear the Kalman Filter is generally applied. However, when the relationships are nonlinear, as they are in microbial population growth, other techniques must be applied. The Particle Filter based on Bayesian probability methods has proved to be useful in this respect [2], but other nonlinear model updating methods could also be used. The technique changes the model parameters to conform to the new situation in an optimal way taking into account correlations between parameters.

That is, a probabilistic basis for predictive microbiology models is essential for operating model updating. Starting with the probability density for the initial conditions estimated, as described above, and data from temperature loggers, a dynamic model calculates a probability density for the microbial population size some future time. At this time an acceptance sampling of microbe population size is taken and this sample compared objectively with the predicted probability density. If the acceptance sample is significantly different from the predicted probability density, a model update, or reassessment of the model parameters may be required. This is an objective procedure which adjusts nominated model parameters according to a joint probability density of those parameters. Typical parameters chosen for updating are those describing how temperature affects the relative microbe population growth rate, and the supply chain process variance.

However, any model parameters can be nominated to be updating parameters. Unreliable acceptance sampling Acceptance sampling procedures suffer from the same problems as sampling for initial conditions, low sample numbers giving unreliable estimates. Therefore, a single comparison between an acceptance sample and a predicted probability size density is

unlikely to identify any major changes in the process. Thus, both the assessment of a model update and the update itself needs to take account of previous comparisons between the predicted probability density and the acceptance samples. Typically, an update may be tuned to assess a time trend in these comparisons, using the ensemble of measurements to test a divergence between model predictions and the actual population size at acceptance. In addition, it would be unwise to calculate a model update using the difference based on a single acceptance sample. Rather the updating procedure uses previous acceptance samples as well as the current sample.

Improving the management of the industrial food supply chain An improvement in the quality management of a supply chain generally means a reduction in either or both of the mean and the variance of microbial population size. Achieving this requires knowledge about how the mean and variance of microbial population size develop. The methods discussed above generate a wealth of data that can be used to address these issues and thus to improve methods to control food spoilage.

It is expected that after a period of operation different supply chains (e.g. European or North American) would have different model parameters characteristic of the supply chain concerned, and hopefully optimised for the supply chain concerned. These differences and the changes in both the initial population frequency distribution and the model parameters over time can be used to improve knowledge about the performance of the supply chain as it relates to food spoilage. Such knowledge can be used to design improvements to increase product shelf life.

The changes in the initial population size distribution for each microbe type measured can be analysed to find relationships between changes in the stationary distribution and packing plant conditions. Generally, several microbe types will be monitored and the co-dependence between different types can be assessed. Such an analysis can be very useful in establishing how differences in plant operations, seasonal effects and livestock sources change the initial microbial population sizes in terms of changes in the probability density – i.e. not just changes in the mean microbial population size.

Similar analyses can be performed by associating changes in the model updating parameters with observed conditions in the supply chain. For example, some shipping routes may be advantageous, or some packing technologies better than others.

A more sophisticated analysis would use the changes in the model parameters for different supply chains to infer packing plant attributes. For example, to try and locate a problem that is either internal or external to the packing plant.

There is scope for work to improve the estimation of the starting microbial population sizes at the beginning of the supply chain at the packing plant. A packing plant routinely measures more than 1 microbe species, and if conditions change it would be expected that such a change might affect all the microbe species in some manner. That is, the starting population size frequency distribution is really a joint frequency distribution, potentially with some associated dependence. If this is the case then there is information in the changed frequency of one microbe species about the frequency of another microbe species. This information can be used to improve the accuracy of the size of the starting microbial population size. FoodQSMTM stores data from microbial measurements of samples at the packing plant into a database that makes retrieval for such analysis easy.

The industrial food supply chain is uncontrolled in comparison to a laboratory environment. Important variables such as temperature and pH are not closely monitored as they are in a laboratory, even in a reasonably controlled environment, temperature and pH profiles over time are subject to random fluctuations and are known with some degree of uncertainty.

The analysis of variance components is a technique developed for such circumstances. It is an essential tool in animal breeding, which deals with similar situations to the management of a food supply chain, but to date it has received little attention in predictive microbiology. The idea is that in the food chain there are no preferred temperature levels, but rather an ensemble of levels characterised by temperature being a random variable. In such a situation it is the variance of the temperature fluctuations in the supply chain that is of interest, and this is measured by the variance

component of the variable of interest associated with temperature fluctuations. Clearly the (time dependent) value of such a variance component in a supply chain can be used to decide the value of actions to control spoilage, and also where in the supply chain to place the effort to achieve this. Covariance components between the model parameters associated with an effect like temperature are also important in the management of the supply chain to reduce spoilage. The methodology is presented in [6].

One application has been presented in [6] for calculation of the variance and covariance components for the growth of *Erwinia Carotovora* subject to changing temperatures. In a relatively controlled experiment it was shown that the intra – class correlation was 0.8, meaning that temperature variation controlled 64% of the variation in the growth of this microbe.

The utility of variance components is that they can be applied to calculate the value of taking action to control variation in variables affecting microbial growth in the industrial supply chain. For example, the question of to what degree the uncertainty in microbial population size can be reduced by reducing the temperature variation by a nominated amount can be addressed. This provides a basis for a cost benefit analysis of supply chain strategies aimed at managing food spoilage by allocating resources to better control some variables that can be controlled during food processing or storage. Variance component analysis provides an important link in applying laboratory results to the industrial food supply chain.

III. CONCLUSION

The industrial food supply chain imposes many challenges associated with variation and uncertainty that operate at different time scales. Fast variation is identified with rapid changes in variables like

temperature, and in the application of an empirical model to quantitatively describe microbial population growth. Slow variation is associated with changing relationships among the microbial species involved, perhaps driven by seasonal effects, but also other factors that are typically unknown.

This paper proposes a methodology for dealing with these issues, based on a probabilistic description that incorporates methods from QA procedures, and control engineering techniques based on model updating. These methods are currently incorporated into the software package FoodQSMTM for application in the meat industry.

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