

Chapter 2

Experimental Design and Data Generation

Abstract One of the most critical steps when generating a predictive model is to correctly design an experiment and collect suitable microbial data. Experimental design will influence model structure and validation conditions. The survival and growth of microorganisms in foods is affected not only by the chemical composition of the food and its storage conditions but also by the food matrix. In this sense, a better quantification of the food structure effect has been studied throughout these years. Regarding the method of data collection, although plating count has been widely used (and still is used), there are rapid methods to obtain reliable and cost-effective data. These achievements were primarily based on turbidimetry, although other methods (microscopy, image analysis, flow cytometry, etc.) have arisen as novel approaches in the predictive microbiology field. These aspects are further discussed in this chapter.

Keywords Experimental design • Food matrix • Challenge testing • Data generation • Absorbance • Turbidimetry • Flow cytometry • Microscopic methods

2.1 Experimental Design

In predictive microbiology, as with other scientific disciplines, collection of high-quality data is an essential part of exploitation of results. Both the selection of an appropriate model structure and the identification of accurate model parameters are data-driven processes; that is, the efficiency and accuracy of these procedures are determined by the quality of the experimental data.

The experimental design of a predictive model will mainly depend on its final application into a real case scenario. This process is completely different when estimating growth kinetic parameters as a function of certain environmental factors than when one qualitatively estimate the probability that a given microorganism may or may not grow under a specific set of conditions. Similarly, when performing a validation study in a food matrix, design will be accomplished according to the

representativeness of data to real conditions or to the time invested in analytical experimentation. For this purpose, it is very useful to previously screen the main factors affecting microbial behavior through different assays. Implicitly, when increasing the number and levels of involved factors, the experimental design will be more complex.

Devlieghere (2000) already described the main factors to be considered to plan an adequate experimental design. Some relevant questions are these:

- What is the main objective of the predictive model?
- Which are the main factors to be controlled, so that this objective will be achieved?
- Which are the factor levels to be used?
- Which is the inoculum state to be employed? This refers to physiological state, use of cocktail strains, inoculation form, etc.
- Which are the dependent variables of the proposed model?
- Which is the substrate or medium used?
- How many combinations of environmental factors will be finally included in the model (from those previously identified)?
- How will data be collected?

Some of this information is discussed in the following sections. In the meanwhile, referring to the experimental design, a two-step procedure is often applied: (1) screening experiments are performed at an extended range of the factors, followed by (2) an extensive data collection study within the region of interest (Gysemans et al. 2007). This latter point is referred to the inclusion of additional levels of identified factors to obtain a more refined model and consequently, more accurate microbial predictions.

In modeling microbial responses in foods, traditionally, full experimental designs are chosen (Tassou et al. 2009). This approach considers all combinations of the different explanatory variables. The main advantages are its ease of implementation together with its data processing. Further, one is sure that all information is gained from the experiment, as all combinations are explored. However, this experimental design is often labor intensive and costly.

Alternatives are being adopted by carefully selecting experimental conditions (implementing an efficient design-of-experiment, or DOE). The number of experiments required for it is calculated as $(N) \sim 2^k$, where (k) is the number of variables.

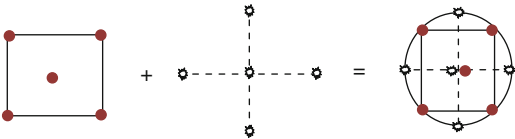
If the number of variables is large, the fractional factorial design can be more indicative.

Fractional factorial designs are reduced versions of full factorial designs, and some of these have been published (McKellar and Lu 2001; Valero et al. 2009). These designs are based on 'a priori' knowledge or assumptions on the most important factors or expected interactions. A particular class of fractional factorial designs called the Box–Benhken designs has been used for modeling microbial growth or inactivation. Combining two-level factorial designs with balanced incomplete blocks forms this experimental design. The repeatability of the model

Fig. 2.1 Schematic representation of a four-factor level Latin-Square design

A	B	C	D
C	D	A	B
D	C	B	A
B	A	D	C

Fig. 2.2 Schematic representation of a two-factor Central Composite design



is normally tested through the performance of additional experiments at the central points of the experimental design.

Latin-Square designs are special types of fractional factorial designs. According to its definition, a Latin square of order x is an arrangement of x letters in an x -by- x array so that each letter appears exactly once in each row and exactly once in each column. In the context of experiment design in predictive microbiology, one primary (treatment) factor (represented by the letters) is typically studied in the presence of several blocking (nuisance) factors, although the approach is not limited to one principal factor of interest. Latin-Square designs can be extended to more individual factors, such as the Graeco-Latin Square or the hyper Graeco-Latin Square designs. A Latin-Square design of order 4 is presented in Fig. 2.1.

Another experimental design commonly used in the field of predictive microbiology, the Central Composite Design (Cheroutre-Vialette and Lebert 2002; Arroyo-López et al. 2009), contains an embedded factorial or fractional factorial design with center points that is augmented with a group of ‘star points’ which allow estimation of curvature. If the distance from the center of the design space to a factorial point is ± 1 unit for each factor, the distance from the center of the design space to a star point is $\pm \alpha$ with $\alpha > 1$. The precise value of α depends on certain properties desired for the design and on the number of factors involved. If two factors are included, the Central Composite design will appear as shown in Fig. 2.2.

The Doehlert matrix describes a spherical experimental domain and stresses uniformity in space filling. For two variables it consists of one central point and six points forming a hexagon, situated on a circle. The formula used for calculation of the number (N) of experiments required is $(N \sim k^2 + k + C_0)$, where (k) is the number of variables and (C_0) is the number of center points. Replicates at the central level of the variables are performed to validate the model by means of an estimate of experimental variance.

For rather simple model structures and a limited number of levels per environmental factor, full factorial designs are preferable because these designs guarantee accurate and reliable model parameters (Mertens et al. 2012). However, for more complex cases, a Latin-Square design can be considered as an attractive alternative as it does

not require a priori knowledge of the model structure (as is the case for the reduced full factorial design), while keeping the experimental workload and cost low. In contrast, central composite designs should be avoided because of the high degree of uncertainty on the parameter estimates.

2.2 Growth Matrix: Food Versus Artificial Medium

For a long time, the ability of specific foods to support microbial growth of pathogenic as well as spoilage microorganisms has been evaluated by inoculating the target organism and monitoring its growth and survival over a certain period of time. This methodology, traditionally named challenge testing, is still being used in the field of predictive microbiology because it is sometimes necessary to gain information about the microbial stability of a novel product formulation or to assess the behavior of a specific microorganism (not previously tested). These experimental tests are useful to determine microbial shelf life and growth/survival kinetics parameters, such as maximum growth rate or lag phase. However, although this approach was considered the gold standard, it is also time consuming and costly. Thanks to the development of predictive microbiology, microbial behavior is explained with only a few significant environmental factors (mainly temperature, pH, or a_w), thus yielding accurate predictions in the majority of foods.

Most published studies of predictive microbiology (at least the earlier ones) used artificial media, that is, culture media with a chemical composition that is intended to mimic the food environment, which allowed a reduced variability in the results (mainly because chemical composition can be more accurately adjusted). Also, it is recognized that artificial liquid media provides a more homogeneous distribution of microorganisms, leading to obtaining similar kinetics under the same environmental conditions.

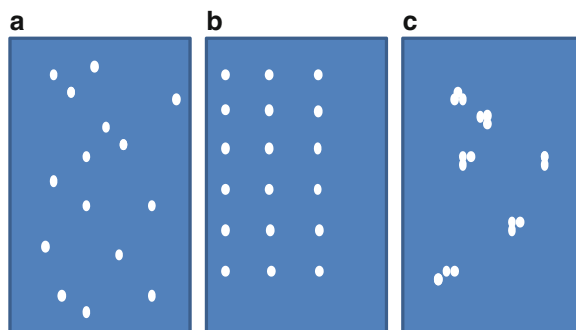
In principle, observed data can be easily fitted to mathematical models because they tend to be more robust and replications do not vary greatly.

A key step before the application of predictive models is validation in food matrices: this involves the comparison of model predictions with additional data coming from literature sources or by means of inoculating the target organism in a given food (supposedly within the range of conditions covered by the model) and evaluating the observations with model predictions to judge if they are biased. Throughout this brief, a specific section about model validation is proposed, with special attention to validation procedures, indexes used, and reliability of models when applied to food matrices.

In this section, differences between structural composition between artificial media and foods are presented.

Traditionally, when comparing model predictions in broth media with observations in foods, one can assume that results will be fail-safe, that is, the predicted growth in liquid media is much faster than that observed in food. Several factors are attributable: food matrix (which in most cases is semisolid or solid),

Fig. 2.3 Representation of the most common spatial distributions of microbial populations according to the type of matrix in which they are present



indigenous flora, or additional environmental factors present in the food and not included in the model.

Growth of microorganisms in a liquid aqueous phase in foods is typically planktonic and can be accompanied by motility allowing taxis to preferred regions of the food. Transport of nutrients to the bacteria and of metabolites away from them results in a locally uniform environment until considerable accumulation of microbial biomass and metabolites cause bulk chemical changes (such as a decline in pH). It is this microarchitecture in food that is mimicked in microbiological experiments by the use of broth culture medium (Wilson et al. 2002).

However, foods are not typically homogeneous. The structure of foods creates local physical or chemical environments that clearly affect spatial distribution of microorganisms. Consequently, microbial growth or survival is influenced. Microorganisms occupy the aqueous phase of foods, and structural features of this phase cause an effect on microbial behavior such as constraints of mechanical distribution of water, redistribution of organic acids, and food preservatives (Hills et al. 1997).

Predictions based on data obtained from broth systems can be successfully applied to microbial growth and survival in foods. In many cases, microorganisms grow more slowly in a given structure food than in broths: this is the case when performing a challenge testing, in which lower growth rates (and longer lag phases) are observed than are proposed by predictive models (Pin et al. 1999). In structured (heterogeneous) foods, microbial growth can strongly depend on the position in the food and the assumption of perfect mixing can thus not be accepted. In consequence, space must be considered (Dens and Van Impe 2000).

This concept is related to the microbial distribution of microorganisms. Figure 2.3 represents the distribution of a microbial population in a specific matrix (International Life Sciences Institute 2010). Normally, in liquid media random distribution is obtained, indicating that there is equal chance for any individual cell to occupy any specific position. Presence of organisms can be considered independent (Fig. 2.3a). Regular distribution is observed when high cell densities are encountered in the food and these cells are neither clumped nor aggregated (Fig. 2.3b), as when contamination occurs in equipment or utensils when they are insufficiently cleaned. Finally, in solid matrices colonies are normally aggregated, forming clusters (biofilms): the presence of organisms can be considered dependent.

Once a food product becomes contaminated, microbial growth can transform the initial homogeneous distribution into a more clustered one. When cells are growing inside the product, the spatial distribution is more similar to that presented in Fig. 2.3c, mainly as a result of the physical constraints of the food matrix.

Regarding inactivation treatments, it is demonstrated that application of heat may have a different impact on microbial death depending on the considered food matrix. If thermal diffusivity of the product is lower, some contaminants can survive if the heat has not sufficiently conducted to the interior of the food product. This formation of the so-called cold spots may affect the spatial location of microorganisms.

Thus, the use of model foods that mimic real food structures entails significant advantages from a practical point of view, such as better control, ease of operation, and repeatability of analyses (Antwi et al. 2007; Noriega et al. 2008).

2.3 Data Generation

Data generation must be based on the optimization of the number of data together with the implied cost of their acquisition. Generally, when building a growth and survival model it is known that data should be collected throughout the whole analytical period of time, about 100 kinetic curves being needed to make the model significant (McDonald and Sun 1999). Gibson et al. (1987) concluded that 15 points per kinetic curve are necessary, 20 being the optimum number. Less than 10 points per curve makes the adjustment not fully representative of microbial behavior, thus increasing uncertainty. Poschet and Van Impe (1999) established that this uncertainty on dependent variables increases when fewer points are collected, but, above a certain limit, this value remains stable.

Similarly, distribution of collected points within the experimental design is crucial for estimation of dependent variables: this is achieved in such a way that the representativeness of the model increases, at the same time reducing variance of the estimated parameters.

There are currently different methods to collect data, but the classical one has been widely used in predictive microbiology, that is, plate count techniques. This method has been used to monitor microbial growth although it presents some drawbacks. First, possible underestimation could occur because of the presence of clumped cells. Second, plate count is a slow, labor-intensive, and costly method. Further, it does not provide immediate results, so that model development becomes more difficult, especially when a high number of data is required. However, to a certain extent, plate counts can be done automatically by the use of automated platers such as the spiral plater and automatic colony readers.

Traditionally, models performed in broth systems are based on the modification of artificial culture media with preservatives, such as organic acids, sodium chloride, or sodium nitrite, or on lowering pH with the addition of chlorhydric acid. Afterward, these modified media are inoculated with the specific microorganism, and growth and survival are monitored over a certain period of time.

When concerned with food matrices, one way to assess microbial behavior is to perform a challenge test, that is, to inoculate the target microorganism in a specific food and to evaluate if the food supports growth and survival under a limited set of conditions.

The next section explains in more detail the experimental procedure to carry out a general challenge test. Of course, there are many variants and uses of this method, in accordance with the final objective to be fulfilled. This explanation summarizes the most generic steps to achieve a challenge test.

2.3.1 Traditional Methods: Challenge Testing

Microbiological challenge testing has been and continues to be a useful tool for determining the ability of a food to support the growth of spoilage organisms or pathogens (US Food and Drug Administration 2009). In predictive microbiology, it is needed to evaluate the behavior of a particular strain (or a cocktail of different strains) to subsequently calculate kinetic parameters. Microbiological challenge tests also play an important role in the validation of processes that are intended to deliver some degree of lethality against a target organism or group of target organisms (for example, a 5 log reduction of *Escherichia coli* O157:H7 for fermented meats). Selection of microorganisms to use in challenge testing and/or modeling depends on the knowledge gained through commercial experience and/or on epidemiological data that indicate that the food under consideration or similar foods may be hazardous because of pathogen growth.

An appropriately designed microbiological challenge test will validate that a specific process is in compliance with the predetermined performance standard. The design, implementation, and assessment of microbiological challenge studies form a complex task that depends on factors related to how the product is formulated, manufactured, packaged, distributed, prepared, and consumed.

Failure to account for specific product and environmental factors in the design of the test could result in flawed conclusions.

Microbiological challenge studies can be used in specific cases for the determination of the potential shelf life of certain refrigerated or ambient-stored foods. The determination of whether challenge studies are appropriate or useful must consider such factors as the likelihood of the product to support growth of spoilage organisms or pathogens or include knowledge of the previous history of the product.

When conducting a microbiological challenge study, a number of factors must be considered: (a) the selection of appropriate pathogens or surrogates, (b) the inoculation level, (c) the method of inoculation, (d) the duration of the study, (e) formulation factors and storage conditions, and (f) sample analyses. These are described next.

(a) Selection of appropriate strains or surrogates

The ideal organisms for challenge testing are those that have been previously isolated from similar formulations. Additionally, pathogens from known food-borne outbreaks should be included to ensure the formulation is robust enough to inhibit those organisms as well.

For certain applications, surrogate microorganisms may be used in challenge studies in place of specific pathogens. For example, introducing pathogens into a processing facility is not feasible; therefore, it is desirable to use surrogate microorganisms in those cases. An ideal surrogate is a strain of the target pathogen that retains all other characteristics except its virulence.

In any case, it is important to incubate the microorganisms in standardized conditions, preferably similar to those encountered in the food.

(b) Inoculation level

The inoculum level used in the microbiological challenge study depends on whether the objective of the study is to determine product stability and shelf life or to validate a step in the process designed to reduce microbial numbers. Typically, an inoculum level between 10^2 and 10^3 colony-forming units (cfu)/g of product is used to ascertain the microbiological stability of a formulation. If the inoculum level is too low, microorganisms could not grow in the food product because of the increased lag phase, so that one can assume in certain cases that food formulation assures food safety when it is not low. In contrast, at high inoculum levels, microbial growth could be overestimated. For studying lethality processes, higher levels of microorganisms are needed (generally more than 10^6 cfu/g).

(c) Method of inoculation

The method of inoculation is another extremely important consideration when conducting a microbiological challenge study. Every effort must be made not to change the critical parameters of the product formulation undergoing challenge. A variety of inoculation methods can be used depending upon the type of product being challenged. In aqueous liquid matrices such as sauces and gravies with high a_w (>0.96), the challenge inoculum may be directly inoculated into the product with mixing, using a minimal amount of sterile water or buffer as a carrier. Use of a diluent adjusted to the approximate a_w of the product using the humectant present in the food minimizes the potential for erroneous results in intermediate a_w foods. In studies where moisture level is one of the experimental variables, the inoculum may be suspended in the water or liquid used to adjust the moisture level of the formulation. Products or components with $a_w < 0.92$ may be inoculated using the atomizer method with a minimal volume of carrier water or buffer. Again, the product should always be checked to ensure that the final product a_w or moisture level has not been changed. A short postinoculation drying period may be needed for some products before final packaging. A minimum volume of sample should be inoculated so that a minimum of three replicates per sampling time is available throughout the challenge study. In some cases, such as in certain revalidation studies and for uninoculated control samples, fewer replicates may be used.

(d) Duration of the study

The microbiological challenge test should be conducted for at least the whole shelf life period of the food product. Some regulatory agencies recommend extending the duration of the study a margin beyond the desired shelf life

because it is important to determine what would happen if users held the product beyond its intended shelf life and then consumed it.

The frequency of analysis depends on the environmental conditions under which the food is subjected. It may be desirable to test more frequently (for example, daily or multiple times per day) early in the challenge study (that is, for the first few days or week) and then reduce the frequency of testing to longer intervals.

(e) Formulation factors and storage conditions

When evaluating a formulation, it is important to understand the range of key factors that control its microbiological stability. It is, therefore, important to test each key variable singly or in combination in the formulation under worst-case conditions. Experimental temperature should be similar to real processing, distribution, and sale conditions. In a last step, the use of temperature shift might be recommended, such as storing the food product at one specific temperature for a portion of its shelf life, after which time the product may be subjected to elevated temperatures.

(f) Sample analysis

In challenge tests, it is recommended to analyze at least three replicates per analytical point, although more replicates would be needed when requiring more accuracy in the results. The culture media to be used will depend on the type of microorganism to be controlled, but if the food product contains high concentration levels of competitive flora, it would be better to use selective media. Similarly, if the targeted microorganism is a toxin producer, toxin concentration should be measured during the study period. In parallel, control samples (uninoculated) may be analyzed in the same way as inoculated samples to evaluate the effect of the food flora on the analytical period of time.

2.3.2 *Rapid Methods*

(a) Viable counts

Viable counts are commonly obtained by spread-plate and pour-plate techniques and therefore are linked to the classical microbiological methods that are considered to be reference methods, even though these can have certain limitations (Rasch 2004). For instance, a clump of cells falling onto culture agar would give rise a single colony, impeding estimation of the actual number of individual cells when clumps are present in the medium. Hence, microbiologists refer to results as colony-forming units (cfu) (McMeekin et al. 1993a). Although automation has been also applied to plate count methods (e.g., the spiral plating method) reducing sample preparation time (e.g., decimal dilutions, human resources, etc.), this methodology still implies long waiting times for enabling bacterial growth in culture media (24–48 h). Predictive models can be developed based on data obtained in liquid and solid media or on food matrices. When models are developed on food, more

additional steps such as a homogenization step or filtering are required, making the analytical process more laborious. Also, the medium where data are being generated can affect the precision and accuracy of plate counts, which should be considered when predictive models are built, although it is generally accepted that the repeatability of enumeration data may only be precise to about $\pm 0.5 \log \text{ cfu}$ (Mossel 1995).

(b) Turbidimetry

Bacterial kinetic modeling is mainly based on colony counting (traditional method) and absorbance measures. It is well known that cellular concentration in a liquid medium can be related to the optical density (OD) of the growth medium. OD, or absorbance, is a measure of the light that is absorbed by a cellular suspension. The chief characteristic of OD is that OD of a bacterial suspension increases proportionally with the increment of bacterial concentration. OD of a cellular suspension can be also related to transmittance and turbidity of the medium, important optical properties related to bacterial concentration. Other factors affect OD such as the refractive index of the bacterial strain and its shape and size. The main advantages of OD are rapidity, simplicity, and noninvasiveness, which make the technique quite suitable for modeling purposes. Automation in measuring OD has led to sophisticated photometers such as Bioscreen C, which are able to perform multiple measurements (200 wells) at specific time intervals while maintaining a fixed incubation temperature. Nevertheless, important limitations exist that should be considered when OD is used as the enumeration technique for modeling bacterial growth. Linearity may be one of the most important drawbacks because the linear relationship described by Beer-Lambert only holds for approximately a tenfold increase in cell numbers:

$$\log \left(\frac{I_{\text{incident}}}{I_{\text{transmitted}}} \right) = \text{absorbance} = -x c l \quad (2.1)$$

here I_{incident} is the intensity of light entering the medium and $I_{\text{transmitted}}$ is the intensity of light exiting the medium, x is a constant dependent on the medium and microorganism, c is microorganism concentration, and l is the distance the light travels through in the medium (i.e., light path).

In some cases, nonlinearity of OD response has been corrected using an empirical function derived from specific experiments in which bacterial suspensions with concentration levels outside the range of linearity are diluted and absorbance is measured. The relationship found between initial absorbance and absorbance after dilution is analyzed to derive a correction function for nonlinear OD data (Dalggaard et al. 1994).

Another important drawback associated with measuring OD is its relatively high detection limit, which is often above $6 \log \text{ cfu/ml}$, meaning that a growth model should be based on high initial inoculum levels. The impossibility of differentiating between living and dead cells limits its application to growth models.

In spite of all these limitations, predictive models based on OD data are very often reported in the scientific literature, probably because fewer experimental resources are needed to assay the multiple environmental conditions needed for building secondary models. Moreover, kinetic parameters such as growth rate can be estimated only based on OD measures because this parameter expresses a rate of change with respect to time. Several studies have successfully modeled these parameters based on OD data for different microorganisms and environmental conditions (Dalgaard et al. 1994; Begot et al. 1996; Augustin et al. 1999). However, some studies have proved that models based on OD data can underestimate maximum growth rate, recommending the use of the detection time approach for better estimation of this parameter (Lindqvist 2006; Lianou and Koutsoumanis 2011). The detection time (DT) approach consists of performing several decimal dilutions of the initial inoculum and estimating the DT based on OD data. Then, DTs are used to fit the following equation, which provides an estimation of maximum growth rate (Cuppers and Smelt 1993; Lianou and Koutsoumanis 2011):

$$\log(N_i) = k - \mu_{\max}DT_i \quad (2.2)$$

where N_i is the inoculum size corresponding to different decimal dilutions of the initial inocula, μ_{\max} is the maximum growth rate, and k is a constant.

The use of calibration curves relating OD and bacterial concentration also produces reliable estimates of kinetic parameters and is an alternative to using OD directly. Calibration equations estimated from experimental data are used to transform OD values to count data. Then, the estimated counts can be used to estimate kinetic parameters (Dalgaard and Koutsoumanis 2001). Precautions should be taken when environmental stresses are applied during bacterial growth because they can affect OD measures (i.e., bacterial shape and size); therefore, calibration curves should be done for each specific growth condition (Valero et al. 2006).

The development of growth/non-growth models is mainly based on use of the OD technique because some of the limitations of the OD technique are not given for this type of study in which no growth rate is observed but only if growth takes place. That condition is experimentally determined based on recording a significant change of OD in the microorganism suspension that is related to growth (Salter et al. 2000; Skandamis et al. 2007; Valero et al. 2009, 2010).

(c) Flow cytometry

Flow cytometry is a rapid technique based on labeling cells in suspension with fluorochrome molecules and passing them, in a liquid stream, through a micro-capillary equipped with an electronic detection apparatus. The characteristics of light scattering, light excitation, and emission of fluorochrome from cells are collected to provide information on physiological state, size, shape, and integrity of the analyzed cells. Moreover, the technique can be used to enumerate target microorganisms, showing a good correlation with colony-counting methods (Sørensen and Jakobsen 1996; Endo et al. 2001; Holm et al. 2004).

The combination of both applications, that is, cell enumeration capacity and cell physiological characterization, makes this technique an excellent method to study and model microbial population heterogeneity, which is particularly relevant under stress conditions (Fernandes et al. 2011). In spite of its promising application in predictive microbiology, few studies have been carried out based on data obtained by flow cytometry (Sørensen and Jakobsen 1996; Ferrer et al. 2009). It is likely that further development of omic models, based on a molecular level approach, and new technological advances in cytometry will boost the application of flow cytometry in predictive microbiology studies in future years.

(d) Microscopy and image analysis

This method offers some advantages when compared to plate counting methods and enumeration methods based on optical density. One of the most important advantages is that direct observation on food matrices or artificial media enables obtaining a lower limit of quantification, improving accuracy and the precision of results. In addition, based on the biochemical properties of cells and the use of specific fluorochromes, this technique can provide information on the physiological state of bacteria on the surface (alive/dead, sterase activity, etc.) (Bredholt et al. 1999). More recently, microscopy has been applied to investigate and model individual cell lag times based on observation of systems containing isolated cells (Métris et al. 2005; Niven et al. 2006; Stringer et al. 2011; Gougouli and Koutsoumanis 2012). In general, these systems consist of a surface inoculated with the test microorganism, which can be agar or a microscope slide placed within a tailor-made chamber or a device enabling control of environmental conditions such as temperature or atmosphere (Métris et al. 2005; Niven et al. 2006). A photographic camera is usually coupled with the microscope to capture images of cellular division at certain intervals of time. Moreover, specific software such as ImageJ (Abramoff et al. 2004) should be applied to analyze images taken by the camera, obtaining counts and estimating lag time.

Image analysis can be also applied to estimate kinetic parameters based on radial growth of colonies of bacteria (Dykes 1999; Guillier et al. 2006). However, this methodology is most preferred to model kinetic parameters (i.e., growth rate and lag time) of molds, monitoring the radial growth of mycelium (Rosso and Robinson 2001; Baert et al. 2007; Garcia et al. 2010). Predictive model studies have involved different fungal species such as *Botrytis cynerea*, *Penicillium expansum*, and *Aspergillus carbonarius* (Dantigny et al. 2007; Judet-Correia et al. 2010). In specific cases, image analysis supported by suitable software has been also used to evaluate mycelium growth (Judet-Correia et al. 2010). Automation of this process via image-analyzing systems would further facilitate the application of this method to generate more reliable predictive models.

(e) Electrochemical methods: impedance and conductance.

This technique is based on the fact that bacteria during growth produce positively and negatively charged chemical compounds that modify the impedance of growth medium (Rasch 2004). The time at which a significant change

of impedance in the growth medium is detected is the so-called detection time (DT), which is inversely proportional to the logarithm of the initial concentration level of the microorganism (Jasson et al. 2010). Also, conductance and capacitance of growth medium can be used to enumerate bacteria in culture media and foods (Lanzanova et al. 1993; Noble 1999; Koutsoumanis and Nychas 2000). Besides their application for microorganism enumerations, impedance data can be directly used to derive kinetic parameters (McMeekin et al. 1993a). Some examples of this have been successfully developed for *Salmonella enteritidis*, acid lactic bacteria, and *Yersinia enterocolitica*, in which kinetic parameters have been derived from fitting primary growth models (e.g., Gompertz model) to conductance or impedance data and using DT with a similar mathematical approach to that used in optical density methods (Lanzanova et al. 1993; Lindberg and Borch 1994; Fehllhaber and Krüger, 1998).

Predictive Microbiology in Foods

Perez-Rodriguez, F.; Valero, A.

2013, VI, 128 p. 21 illus., 11 illus. in color., Softcover

ISBN: 978-1-4614-5519-6